Enhancement of calcium concentration in *Zantedeschia* plants

by

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ABSTRACT

As part of a research programme developing the use of enhanced calcium (Ca) concentraton of plant tissue as a means of control of bacterial soft rot in hybrid *Zantedeschia,* changes in Ca concentration were monitored using two methods of application. Gypsum application at 9 kg/m^3 and a pre-plant vacuum application of 7.5% calcium chloride ($CaCl_{2)}$ solution were used to follow changes in calcium (Ca) concentration and its forms in tuber peel and matured leaves of hybrid *Zantedescia* plants.

Throughout the period of growth, gypsum application increased approximately 2.0 mg/g Ca concentration of tuber peel tissue and matured leaves. The concentration in tuber peel tissue was increased to a maximum of 17.42 mg/g at 84 days after planting, then, declined to the concentration recorded at planting by 140 days. In contrast, the calcium concentration of the matured leaves increased continuously throughout the growing period. Plant available Ca in the gypsum amended medium was approximately three fold greater compared to that of the control (13.08 and 4.33mg/g at 14 days after planting or 15.53 and 4.96 mg/g at 98 days after palnting), and neither treatment showed any consistent trend of change over time. The decline in Ca concentration in tuber peel tissue coincided with the period of rapid tuber growth. Hence, it was suggested that this increase in tuber growth may have diluted the Ca concentration throughout the period of tuber enlargement. As evident by the

continued accumulation of Ca within leaves, a further factor contributing to the decline in Ca concentration of the tuber peel may have been the limited ability of plants to regulate Ca distribution between the high (leaves) and low (tubers) transpiring tissues. It was concluded that the application of gypsum is able to enhance the Ca concentration of the plant tissue. It was also concluded that there were no relationship between the trend of plant tissue calcium concentration and the plant available Ca in the growing medium.

Pre-plant vacuum infiltration of 7.5% $CaCl_2$ increased the total Ca concentration of the tuber peel in addition to the different forms of Ca (i.e., soluble Ca, calcium oxalate and the Ca bound in the cell wall). However the increase was not maintained for the whole duration of growth. The concentration of all forms of Ca was increased to a maximum at post vacuum (PV) sampling and then subsequently declined before planting (BP). Once planted, and throughout the duration of growth, tubers vacuum infiltrated with 7.5% $CaCl_2$ did differences in the total Ca concentration or in the different forms of Ca were evident compared with those vacuum infiltrated with 0% $CaCl_2$ or the non-vacuum treated tubers. Similarly with the matured leaves, the concentration of the total Ca and the different forms of Ca, did not result in any differences between treatments.

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1.0 INTRODUCTION

1.1 *Zantedeschia*

The total world consumption of cut flowers in 1991 was estimated at N.Z. $6.08 billion (Watson 1990). Of this figure, over 80% was grown and sold locally. A demand for new and different flower crops exists (NZTDB, 1992). The latest trend is to tropical or "exotic" looking flowers such as *Hedychium coranarium* Koen (white ginger), *Heliconia humilis* Lam (lobster claws or false bird of paradise), *Anthurium andraeanum* Rubrum (tail flower or wax flower), *Zantedeschia spp*, *Anigozanthus manglessii Maund* (kangaroo paw) and various Proteaceae.

Zantedeschia is a perennial herb belonging to the *Araceae* (Letty, 1973). This family characteristically produces an inflorescence consisting of fleshy spadix supporting the true flowers, subtended by an often showy spathe. The entire spadix and spathe complex is commonly known as the flower.

Zantedeschia has recently been identified as a highly promising crop, and a comprehensive development plan has drawn up to facilitate further growth of the industry (Kepner *et al.*, 1990).

Zantedeschia is a crop grown in New Zealand for cut flower and tuber production. Cut flower exports of this crop have grown at an average of 65% per annum from $1.27 million in 1989 to $3.54 in 1992 (Anon 1992). The latest export receipts are $5.5 million for flowers and $2 million for tubers (Moody, 1995).Most of the *Zantedeschia* flower production is exported to the Pacific Rim markets. Japan remains the major flower market, taking 60%, followed by North America, Europe and Asia. Tubers are also exported for the flowering pot plant market.

1.2 Bacterial soft rot *(Erwinia corotovora var corotovora)*

Many bacteria are present in our environment which can cause diseases in plants, animals or humans. It seems plausible that such bacteria have co-evolved with their hosts, acquiring traits that allow them to colonize host tissues and produce symptoms by triggering deleterious physiological responses or by destroying pre-formed structural components (Perombelon & Kelman, 1980). An example of the latter symptom is the elicitation of soft rot in a variety of plant tissues by a microbial consortium containing several *Erwinia* species as the primary component of decay (Perombelon and Kelman, 1980). These bacteria produce an array of degradative enzymes that act on middle lamella and plant cell wall polysaccharides and proteins, weakening or solubilizing them, and ultimately causing cell separation and death.

Bacterial soft rot, primarily caused by *Erwinia corotovora* causes, severe losses in

Zantedeschia produced in New Zealand. Losses were estimated as being between $0.5 million in 1991 to $2 million in 1992 (Moody, 1995). *Erwinia* bacterium produce quantities of macerating enzymes that induce cellular electrolyte leakage and cell death (Tribe, 1955; Mount *et al.*, 1970; Garibalde & Bateman, 1971). In advanced stages, the tuber becomes macerated and leaves collapse, effectively killing the whole plant. This bacterium naturally resides in the soil, and, being mobile, may move in association with soil micro-fauna, larvae of certain flies, nematodes, and cultural practices such as soil cultivation and irrigation (Chantanao & Jensen, 1969).

Enzymes commonly found in cultures of bacterial soft rot like *Erwinia spp* or in rotted (i.e., macerated) tissues are pectinase, cellulase, protease, and phospholipases (Bateman and Millar, 1966; Chatterjee and Vidaver, 1986; Collmer and Keen, 1986). Theoretical considerations alone imply that these enzymes, by acting on such structural components as pectin, cellulose, wall proteins, and membrane phospholipid, could inflict physiological and physical stress to which host tissues may ultimately succumb.

Contamination and increased risk of decay commonly occur following wounding and inoculation, with decayed tuber tissue frequently occurring during mechanical harvesting, bin loading and grading with potatoes (Perombelon & Kelman, 1980). The greatest hazard occurs when tubers are moved in water flumes and during washing in large vats, particularly if drying facilities after washing and prior to shipment are inadequate. In temperate regions, a higher proportion of potato seed tubers are often

contaminated (De Boer & Kelman, 1975; Nielsen, 1978; Perombelon, 1973; Perombelon, 1974). Moreover the pathogen is present, although in low number, in a high proportion of the stem of apparently healthy plants when the mother tuber has senesced (Perombelon & Kelman, 1980). Yet the disease incidence is usually low (less than 2%) under favourable conditions. This result was in contrast by the study of Loh *et al* (1992) in *Zantedeschia*, they reported that between 20 to 40% plant loss before the unfurling of the first leaf.

Erwinia is primarily a vascular pathogen when inoculated directly into stems in potatoes (Hellner & Dowson, 1953), and progressive decay of the stem tissue follows movement up the stem in the vascular system. In the case of *Zantdeschia*, *Erwinia* bacteria causes decay in the tuber and follows collapse of the plants. Histopathological studies of black leg of potato caused by *Erwinia carotovora* (Artschwager, 1920) and bacterial wilt of carnation caused by *Erwinia chrysanthemi* (Wolt & Nielson, 1969) also support this view. Therefore, factors that would induce the mother tuber to rot, and increase the number of bacteria that invade the stem, would be likely to also favour expression of more serious disease.

Initiation of rotting in potato tubers occurs when: anaerobic conditions prevail, free water covers the tuber surface, the temperature is above the minimum required for growth of the pathogen (25°C), and physiological factors are evident that favour infection (Cromarty & Easton, 1973; Kelman *et al.*, 1978). In addition, rotting is

more rapid under low O_2 concentration than in air (De Boer & Kelman, 1978; Leach, 1930; Lipton, 1967; Lund & Nicholls, 1970; Lund & Wyatt, 1972; Perombelon & Lowe, 1975). This effect has been attributed to a lowering of the tuber resistance to infection (Wigginton, 1974).

Other researchers, (Scholey *et al.*,1968), support the low O_2 findings as they have suggested that decay is also favoured by high levels of CO_2. However, at a high relative humidity, non inoculated, sound, but naturally contaminated tubers, cannot be induced to rot under anaerobic conditions, with or without CO_2, unless there is free water on the tuber surface (Perombelon & Lowe, 1975). Findings of previous studies therefore, show that an anaerobically induced reduced tuber resistance alone, cannot be cited as being the primary cause rotting. Since rotting can occur when bacteria are injected into the tuber under anaerobic conditions, the bacteria in the lenticels of sound tubers cannot be in contact with living tuber cells which could provides a food base when adversely affected by a lack of O_2 (De Boer & Kelman, 1978; Perombelon & Kelman, 1980).

The role of a film of water in the initiation of tuber decay is two fold. First, it may lead to an increase in turgidity of tuber tissue, which is usually under water stress (Gandar & Tanner, 1976) and will, therefore, absorb water at a rate dependent on the degree of suberization of the periderm. Susceptibility of tuber has been shown to be related to water potential (Kelman *et al.*,1978; Perombelon & Lowe, 1975); the more

turgid the tuber is, the more susceptible it is to decay. Second, and of primary importance, a continuous water film on the tuber surface results in rapid depletion of O_2 within the tuber. If a film of water is present, and water is absorbed, turgidity increases in cells adjacent to the lenticel, and the thin suberized layer of cells of the lenticel is broken (Perombelon & Lowe, 1975). In addition, oxygen deficiency affects cell membrane integrity and solutes leak from turgid cells. A continuous liquid phase is established between the cortex and the lenticels. Under these circumstances bacteria in the lenticels can penetrate the cortex tissue. The increased availability of nutrients, as well as apparent reduction of the tuber resistance under aerobic conditions, fosters rapid growth of the soft rot bacteria, allowing a soft rot lesion to be established.

At present, growers follow preventive methods in controlling the bacterial soft rot disease in *Zantedeschia.* Infected plants are immediately disposed of, and the soil and equipment sterilized to minimize the spread of bacteria. Generally, growers used bactericides or bacteriostats by dipping tubers in the solution before planting out, and after lifting. Many of these compounds act as a surface sterilant, and while this method is preventative in nature current rates of plant loss indicate a low rate of success. Controlling this disease is still a major problem in *Zantedeschia*, therefore, examining alternatives strategies to control this pathogen are needed.

1.3. Calcium and its role in disease resistance

1.3.1. Physiological function of calcium

Essentially, there are four biological functions of Ca that may be associated with the development of the disorders: a) effects on membrane stability, permeability to ion transport and synthesis, b) effects on enzyme activity, c) effects on cell wall rigidity and d) interactions between Ca and phytohormones (Burstorm, 1968; Christiansen & Foy 1979; Jones & Lunt,1967; Bangerth, 1979).

1.3.1.1 Calcium and membrane stability

Examples illustrating the importance of Ca for the stabilization of membranes are : a) selective ion uptake is mediated by Ca and this has been found to be localized in the plasmalemma (Epstien, 1961; Jones & Lunt, 1967), b) leakiness of the cell is affected by the concentration of Ca surrounding these cells (Jones & Lunt, 1967), c) studies using electron microscopy of Ca for the stabilization of membrane (Hecht-Bucholz, 1979; Marinos, 1962). Bangerth (1979), in his study illustrated that even membranes that have become highly disorganized can be restored by the addition of Ca. The action of Ca on the membrane can be seen as a continuous interaction with other ions. Some cations, depending on their concentration, can replace Ca from its binding site in the membranes. Only manganese and strontium, however, can displace Ca without

causing a great increase in leakiness and loss of compartmentation (Van Steveninck, 1965; Garrard & Humpreys, 1967; Siegel, 1970). Consequently strontium sprays have been found to reduce blossom end rot in tomatoes, internal breakdown in apples and black heart in celery (Takatori *et al*, 1961; Wills *et al*, 1965; Bangerth, 1973).

In most plant tissues, only the concentrations of magnesium, potassium, and hydrogen are such that they are potentially antagonistic to the effect of Ca (Bangerth, 1974). However, their ability to stabilize the membrane is limited, and, after replacing Ca they can greatly increase permeability. This is particularly true for hydrogen, and, because the concentration is increased at higher respiration rates, Ca, by reducing respiration, may prevent its own displacement (Marschner *et al*, 1966).

1.3.1.2 Calcium and cell wall rigidity

Considerable evidence indicates that the formation of calcium pectate increased the rigidity of the cell wall (Tagawa & Bonner, 1957; Rasmussen, 1966; Cormark, 1965). While the process of calcification increases the walls to polygalacturonidase (Bateman & Lumsden, 1965), a more complex relationship between cell rigidity, elongation and Ca is discussed as indicated by the detailed and extensive studies of Burstrom (1952, 1954, and 1957). He concluded that root cell growth occurs in two stages: a) an increase in plasticity and elasticity of the cell wall, and b) the biosynthesis and laying down of new cell wall material. The first stage is enhanced by auxin but antagonized

by Ca, whereas the relationship is reversed in the second stage.

1.3.1.3 Interaction between calcium and phytohormones

Like the phytohormone ethylene (C_2H_4) Ca deficiency, induces an enhancement of membrane permeability, respiration, ripening and senescence (Simon, 1978). In addition, other interactions seem to exist as manifested by C_2H_4 production being stimulated in Ca-deficient tissue (Faust & Shear, 1969), while the enzyme system for ethylene synthesis is obviously located in a cell wall-cell membrane complex (Mattoo & Leberman, 1977); where the very first Ca-deficiency symptoms can be demonstrated by electron microscopy (Hecht-Buchholz, 1979). It has been suggested that C_2H_4 might therefore be intimately involved in the development of the necrosis in the final stage of almost all Ca-deficiency disorders (Bangerth, 1979). This interaction between Ca and C_2H_4 is widely unexplored but nevertheless is a promising field for further research, not only with respect to Ca deficiency disorders, but also with respect to ripening of fruits, and leaf and flower senescence, which can be hastened by applications or C_2H_4 (Bangerth, 1963; Poovaiah & Leopold, 1973; Sharples & Johnson, 1977).

In the past, Ca-auxin relations in cell extension growth have been extensively studied and it was reported that these relations may be significant in the development of Ca-deficiency disorders (Burstrom, 1968; Crisp *et al*, 1976). These authors suggested that

one of the causal agents in the development of lettuce tip burn might be the presence of supra-optimal levels of the auxin IAA. High levels of auxin can arise because the enzyme IAA oxidase can be inactivated by chlorogenic acid, and the lettuce cultivar most susceptible to tip burn had indeed the highest concentration of this polyphenol (Collier *et al.*, 1979). Other effects of Ca on auxin mediated processes, e.g. its influence on auxin binding, or an acidification of auxin tissue, are less obviously related to Ca-deficiency disorders.

Physiological stresses that increase membrane permeability of plant tissue enhance disease development, with exudate leakage serving as a nutrient for the pathogen (Sol, 1965). Addition of Ca to plant tissue alters plant senescence (Poovaiah *et al.*, 1978; Ferguson, 1984) and limits permeability (Simon, 1978). Additionally, a number of plant diseases are inhibited by the addition of Ca, inhibition being associated with the ability of Ca to strengthen the cell wall and inhibit degradation by pectinolytic enzymes (Corden, 1965; Liptay & Dierendock, 1987).

1.3.2 Relationship between calcium and disease resistance

The relationship between calcium cation (Ca^{+2}) and the cell wall has been shown to play a key role in diseases resistance. Calcium ions are bound to the pectin in the cell wall (Demarty *et al.*, 1984). Few pectin are free of neutral sugars, notably rhamnose, and are composed of polygalacturonic acid residues into which rhamnose is inserted

(Preston, 1979). The rhamnose insertion puts a marked kink in this chain. The resulting bunched configuration of the polygalacturonic chain allows spaces for the insertion of a series of cations, all of which may be filled because the binding of one ion causes a chain alignment that facilitates the binding of the next (Grant *et al.*, 1973). The formation of cation cross bridges between pectic acids or between pectic acids, and other polysaccharides with acid groups, may make the cell wall less accessible to pectolytic enzymes produced by pathogens that cause decay (Tepfer and Taylor, 1981).

Calcium may also affect pectolytic enzymes directly. Calcium inhibits polygalacturonase activity at low concentrations (Buescher *et al.*, 1979), but such low concentrations stimulate pectate lyase activity. In contrast Ca concentrations reduced the reaction rate of pectate lyase (Pratt and McIntyre, 1972). There is a high affinity of the carboxylic groups for Ca^{+2}, and the resulting effect on physiological or pathological processes is greater then for other cations routinely encountered in plant tissues. In addition, the middle lamella exists as a gel and Ca is very efficient in promoting gelling in a pectic solution (Tepfer and Taylor, 1981). Conway (1989) supported the conclusion that the reduction in decay caused by *Pseudomonas expansum* is due, in part at least, to a decrease in maceration of cell walls by polygalacturonase. This reduction in maceration resulted from an improved structural integrity caused by an increase in Ca content. Thus, by increasing the amount of Ca in plant tissue, the level of defense in plant tissue to enzymatic tissue maceration is increased, and decay and resulting plant loss is reduced.

1.3.3 Translocation and distribution of calcium in plants

The transport of calcium an do other ions in the xylem was thought to be due to mass movement in the water of the transpirational stream (Craft *et al.*,1949). This was supported by evidence of accumulation in specific organs according to the transpirational rate (Hylmo, 1953; Stebbins & Dewey, 1972). Following the work of Bell and Biddulph (1963), an ion-exchange hypothesis is now generally accepted. Calcium ions are adsorbed on the negative sites in the xylem cylinder and move upward in the transpirational stream by a series of exchange reactions. Bell and Biddulph postulated further that metabolic removal of Ca from the exchange columns into, for example, the oxalate crystal system was necessary for the continual supply of these ions to a particular plant part. The accumulation of calcium as insoluble and immobile oxalate in senescing foliage, occurs as fruits and roots develop (Carulos, 1975).

The dependence of Ca on the transpirational stream is associated with faster movement leading to a greater distance travelled before being reabsorbed (Biddulph *et al.*, 1959). Calcium absorbed by herbaceous plants is rapidly immobilized, and redistribution into new growth is minimal (Biddulph *et al.*, 1959; Millikan & Hanger, 1966).

During periods of rapid growth, new tissues may not receive an adequate Ca supply, and localized deficiency occurs (Maynard & Baker, 1972). Fruits and vegetables with

symptoms of Ca-related disorders have a lower Ca content than other tissues (Marschner, 1974). Leaves are continuously supplied with Ca through the xylem. Once in the leaves, Ca tends to remain there, accumulating as leaves age. On the other hand, fruits such as apple only receive an initial supply of Ca via the xylem and as transpirational rates fall off, levels in developing fruit fall.

The immobility of Ca would account for Ca disorders which occur in rapidly growing vegetable crops as a result of localized deficiency. Some success has been achieved with foliar Ca application with higher concentrations of Ca and divalent cations, together with the use of a chelator (Biddulph *et al.*,1958, Millikan & Hanger, 1965, and 1966).

1.3.4 Calcium content and uptake

The Ca content of soil commonly ranges from 65 to 85% of its total capacity (Chapman, 1966). Leaves of dicotyledonous plants contain from 0.5 to 5.5 mg/g on a dry weight basis. In *Zantedeshia* the mature blades and petioles contain an approximately of 0.7mg/g on a dry weight basis (Clark & Boldingh, 1985).

An increasing number of economically important physiological disorders and diseases have been associated with inadequate Ca content. Examples of these are: bitter pit in apples (Edgington *et al.*, 1961), blossom end rot (Gubbels & Carolus, 1971), black

heart of celery (Geraldson, 1954), internal browning of Brussels sprouts (Maynard & Barker, 1972), Maynard & Hangar, 1966), tip burn of lettuce, cavity spot and cracking of carrots (Mason *et al.*, 1961), and soft rot in tuber of potato and *Zantedeschia* tubers (McGuire & Kelman, 1984; Loh *et al.*, 1992).

Increased Ca content in tissue of various plants has been correlated with increased resistance to disease (Bateman & Lumsden, 1965; Bateman & Millar, 1966; Forster & Echandi, 1975; Platero & Tejerina, 1976). It is now recognized that Ca plays a complex role in plant physiology and disease resistance. In particular, studies with potato and *Zantedeschia* have shown an inverse relationship between severity of bacterial soft rot caused by Erwinia corotovora and the Ca concentration of the tuber tissue (McGuire & Kelman, 1984; Loh *et al.*, 1992).

Loh *et al* (1992) illustrated that Ca concentration of *Zantedeschia* tuber peel can be increased through gypsum amended media and vacuum infiltration. They reported, that vacuum infiltration of non cured tubers resulted in a linear increase in the Ca content of tuber peel tissue. Using 7.5% $CaCl_2$ raised the peel tissue Ca concentration four fold, from 0.55% to 2.32% per unit dry weight. However, while, the increase in Ca concentration of the tuber peel tissue following gypsum increased the tuber peel tissue were smaller but they were significant.

Within the soil, Ca movement to the roots depend more on the transpiration rate of the

plant than on root elongation and interception. After reaching the root surface, Ca moves across the root cortex either by diffusion or more likely by displacement exchange in the free space (Barber & Ozanne, 1970; Bangerth, 1979). Before entering the stele and the xylem vessel, ions and water move through the endodermis. The suberized casparian strip of endodermal cells, however, effectively blocks transport through the apoplast and thereby forces water and ion transport to proceed through the symplast. Because calcium transport is limited, its transport into the stele and xylem occurs preferably at the root tip and temporarily at the site of branch root formation, where the development of the casparian strip appears to lag behind endodermal cell division (Dunbroff *et al.*, 1971; Ferguson, 1979).

Respiration inhibitors and low temperatures have been found to depress Ca uptake (Lauchli, 1972) especially at low Ca concentration in nutrient solution. However, not all species respond in this way (Shone *et al.*, 1973) as compared to most other nutrient ions. Uptake of Ca^{2+} is still considered to be mainly passive (Higinbotham, 1973; Kirby, 1979). Interaction with other ions strongly influences Ca^{2+} uptake. Competition between non specific cations notably by K^+, Mg^{2+}, and NH_4^+, can substantially depress Ca^{2+} uptake, but their depressive effect depends on their concentration in the soil solution Wilcox *et al.*, 1973; Kirby, 1979). Stimulated uptake by other ions such as NO_3^{1-} or PO_4^{2-} is also a non specific process (Jakobsen, 1979; Kirby & Knight, 1977). In the root, as in the shoot, Ca moves only in an upward direction, and not to the root tip.

1.3.5 Calcium treatment methods

Various methods of increasing the Ca concentration of storage organs have been investigated, such as gypsum application in the growing medium, dipping in solution and vacuum infiltration. Efforts made to enhance the Ca content of potato and *Zantedeschia* by growing plants in growing medium amended with Ca were successful (McGuire and Kelman, 1984; Loh *et al.*, 1992 ; Adams *et al.*, 1993). Gypsum was used since it does not influence the pH of the growing medium as do other Ca sources (e.g. dolomite or agricultural lime). With *Zantedeschia*, Loh *et al.* (1992) reported that 9 kg/m^3 gypsum applied to a peat based medium provided the highest concentration on the tuber peel tissue but reduced the percentage of plant loss compared with that of the control. However, the increase in Ca content in the plant tissue is insufficient for plant growth due to many complex environmental and physiological interaction which effect Ca uptake and distribution (Bangerth, 1979).

Foliar sprays can increase the Ca content, but the magnitude of the increase can vary from year to year depending on the growing conditions (Glenn *et al.*, 1985). By dipping storage organs directly in a Ca solution, the Ca content was increased (Bangerth *et al.*, 1972). Mason *et al* (1974) found that through adding food thickeners to Ca dip solutions a further increase in Ca uptake occurred. However, active infiltration procedures, such as vacuum or pressure, that force solution into storage organs would seem to be the an efficient way of increasing Ca content (Scott & Wills,

1975; Poovaiah, 1979; Bangerth *et al.*, 1979; Poovaiah, 1980; Arteca, 1982; Drake & Spayd, 1983; McGuire & Kelman, 1984; Poliyath *et al.*, 1984; Loh *et al.*, 1992).

From previous studies, the critical concentration of $CaCl_2$ for vacuum infiltration depends on the plant's tolerance, with a 7.5% concentration reported for *Zantedeschia* tubers Loh *et al.*, 1992), 2% for potato tubers (McGuire & Kelman, 1984), and 16% for apple fruits (Poovaiah *et al.*, 1978). Loh *et al.* (1992) reported that vacuum infiltration dramatically raised the tuber peel Ca concentration of the *Zantedeschia* by a factor of almost 4. However, in contrast to the reduction in disease achieved when Ca concentration was increased by application of gypsum tubers did not subsequently show any reduction in severity towards *Erwinia* soft rot after vacuum infiltration. Hence . Hence, Loh *et al.*, (1992) suggested that this may have been due to the changes of calcium concentration in the plant tissue of *Zantedeschia* throughout the whole duration of growth following the gypsum application (9 kg/m^3) and vacuum infiltration (7.5% $CaCl_2$).

1.3.6 Calcium content of *Zantedeschia* plants

Clark and Boldingh (1985) determined the Ca concentration of dormant tubers of *Zantedeschia elliottiana* to be 5.4 mg/g. At planting it was 4.3 mg/g and at its maximum accumulated reached 15.9 mg/g. During field cultivation in the summer, the greatest nutrient uptake was in the first 6 to 12 weeks following planting. In addition,

seasonal changes in concentration were evident in different parts of the plant. In the tuber, the concentration in the new growth of the plant (tuber peel) still remained the same but on the six week following planting, the concentration increased to a maximum level of 12.0 mg/g dry matter and gradually decreased 12 weeks after planting onward. In contrast, the Ca concentration of the roots rose sharply in the first six weeks and then subsequently remained constant for the rest of the season. Ca concentration both in the leaf blade and petioles increased continuously during the season, while the flower calcium concentration ranged from 4.4 to 5.7 mg/g dry matter.

1.4. Project overview

The aim of this project was to investigate the use of gypsum and vacuum infiltration of $CaCl_2$ solution on the calcium concentration in *Zantedeschia* plants.

1.4.1 Gypsum application

Loh *et al.* (1992) reported that calcium concentration of the tuber peel of Zantedeschia was successfully increased using gypsum amended media and 9 kg/m^3 gypsum provided the highest concentration. In this study, it was attempted to grow *Zantedeschia* in gypsum amended medium with 9 kg/m^3 with a duration of 140 days after planting. Calcium concentration of both the tuber peel tissue and matured leaves and the plant available Ca in the growing medium will be determine throughout the

whole duration of growth of *Zantedeschia*. In addition, we will also determine if there is a relationship between the plant available Ca in the growing medium and the calcium concentration of both tuber peel tissue and matured leaves. Another parameter that will be interpreted within the duration of the study was the electrical conductivity (EC) and the pH of the growing medium throughout the normal growing period of *Zantedeschia*.

1.4.2 Vacuum infiltration

Technique in increasing calcium concentration following vacuum infiltration was previously conducted, in apples to combat bitter pit diseases, potato and calla (*Zantedeschia*) tubers (Bangerth *et al*, 1972; Scott & Wills, 1979; McGuire & Kelman, 1984; Loh *et al.*, 1992). Loh *et al.* (1992) found out in their study in *Zantedeschia* that 7.5% was the critical limit on the plant tolerance. They also found out that 7.5% $CaCl_2$ increased approximately 4 fold. In this study, we will attempt to grow *Zantedeschia* plants after a pre-plant application of vacuum infiltration at 7.5% $CaCl_2$ with a duration of 140 days after planting. The total Ca and the different forms of Ca (soluble Ca, calcium oxalate and the bound Ca in the cell wall) will be determine throughout the whole duration of growth on both the tuber peel tissue and the matured leaves. We will also investigate if there is a relationship between the soluble Ca and total Ca in both the tuber peel tissue and matured leaves. With the outcome of this project, growers can incorporate this practice into their conventional cultural practices.

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2. Calcium status of *Zantedeschia* plant tissue and growing medium, following gypsum application

2.1 Introduction

An increase in Ca concentration of plant can control pathogen development through the direct inhibition of polygalacturonase enzymes produced by bacteria (Huber, 1981; Ko & Kao, 1989; Lyon, 1989). Furthermore, gypsum influence physical or physiological components that could influence tolerance in plants (Tzeng *et al.*, 1986). For example, results of an increase in the netting of potato tubers surfaces which may increase tolerance for invasion by bacterial soft rot. One of the methods of increasing Ca concentration in plant tissue such as tubers of potato and *Zantedeschia* is through gypsum (calcium sulphate ($CaSO_4.2H_20$) application to the growing medium (McGuire & Kelman, 1984; Platero & Tejerina, 1976; Loh *et al.*, 1992). Simmonds *et al.* (1990) reported that gypsum was effective in increasing plant available Ca in the soil.

Calcium concentration in *Zantedeshia* whole tuber tissue reaches a maximum about 3 months after planting. Subsequent to this period a decline in the Ca concentration can be observed until it reaches the same as that at planting (Clark & Boldingh, 1990). In contrast, Ca concentration of the leaves continuously increased throughout the growing period. Therefore, tuber peel tissue and matured leaves will be analyzed following gypsum application to confirm this findings. If confirmed, it may support the hypothesis of Kirby and Pilbeam (1984) in their study with kiwifruit, that Ca

concentration is being regulated by the limited ability of the plant to distribute Ca between the high and low transpiring tissues, leaves and fruits, respectively.

Loh *et al.* (1992) illustrated that following gypsum application Ca concentration increases in *Zantedsechia* tuber peel but falls back to that of early season concentration. He suggested that, release characteristics of gypsum in the growing media will be monitored. In addition, using different rates of application ranging from 3 to 12 kg/m^3 gypsum, they illustrated that gypsum at 9 kg/m^3 provided the highest Ca concentration in tuber peel tissue.

Most of the Ca on the roots is held on exchange sites in free space (Huett & Menary, 1979). The first phase of aluminum uptake involves adsorption exchange with Ca. This was reflected in substantially reduced Ca levels in roots for transport to shoots (Huett & Menary, 1980). Further movement of Ca through the cortex of the roots is the apoplast which is restricted at the endodermis by the casparian strip.

There is evidence that Ca absorption by plants in the growing medium can be categorised as non-metabolic and metabolic calcium (Moore *et al.*, 1961; Maas, 1969). Since calcium may move into the root and xylem vessels against a concentration gradient, it has been concluded that energy mediated processes are involved. However, Ca has been found to move into and through root tissue down an electrochemical-potential gradient and passively accumulated against concentration gradients

(Bowling,1966 ; Bowling, 1973).

Calcium uptake by roots is influenced both by the root system and by its environment (Atkinson & Wilson, 1980). The total volume of the root system, root density, the periodicity of both growth and activity in relation to plant demand, and distribution of the system in the soil or growing medium will influence Ca absorption from soil or growing medium solution. Therefore, growing medium characteristics such as EC and pH will be monitored throughout the growing period.

Once Ca is deposited in a specific tissue, a large percentage of Ca becomes quite immobile and little or no re-translocation takes place within the plant (Biddulph *et al.* 1958). As a consequence of the lack of redistribution, organs with a high metabolic rate such as growing fruits or shoots, must depend on a continuous supply of Ca through root uptake (Millikan & Hanger, 1966). The immobility of Ca would account for Ca disorders which occur in rapidly growing organ as a result of localized deficiency.

2.2 Aim and Objectives

The aim of this experiment was to investigate the changes in Ca concentration of plant tissue and growing medium resulting from the application of gypsum at planting.

The objectives of this experiment was to determine:

a) any changes in Ca concentration of plant tissue during the growing season,

b) any changes in plant available Ca of growing medium throughout the growing season,

c) the relationship between the plant available Ca in the growing medium and the Ca concentration of the plant tissue.

2.3 Materials and Methods

2.3.1 Plant Material

Commercially sourced tubers of the *Zantedeschia rehmanii* (Engl.)-like selection 'Galaxy' (2.72 0.07 cm diameter and 7.96 0.54 g fresh weight) were used in this experiment. Tubers were approximately 6 months of age from exflasking tissue culture. Once received, tubers were washed in water and submerged in a 1% sodium hypochlorite solution for 30 minutes. Treatment with sodium hypochlorite was intended to remove any latent microbial infection on the tuber surface. This was followed by a soak in Rovral^{R} (1.5 g/l iprodione a.i.) and Previcur^{R} (1.5 g/l propamocarb a.i.) for 30 minutes for control of *Rhizoctonia* and *Phytium*. The tubers were subsequently cured for 12 days in a controlled environment room (20 2 °C and 80 2% relative humidity) before planting.

2.3.2 Treatments and experimental design

Treatments comprised either the presence or absence of a supplemental source of Ca to the growing medium (Appendix A). This was achieved by the addition of either 9 or 0 kg/m^3 gypsum at the time of planting. The bark based growing medium also contained a base level of slow release fertilizers (Appendix A). A randomized complete block design was used with four blocks, ten sequential harvest and four

plants in each experimental unit. The blocking design accounted for variations in the initial size of the tubers, as well as light and temperature strata within the greenhouse.

2.3.3 Planting and cultivation

Tubers were planted in 1.3 litre, black, polythene bags to a depth of 5-6 cm. This depth of planting was intended to avoid the subsequent exposure and resulting desiccation of roots above the growing medium. Refilling of pots with growing medium was employed as required, together with the planting depth thus, preventing root damage that might enhance the attack of bacterial soft rot (*Erwinia carotovora*). Damaged plant parts facilitate the entrance of soft rot pathogens and are susceptible to diseases.

Plants were grown in an unshaded glasshouse (minimum 15°C, vented at 20°C), and watered by capillary matting on drained benches. The irrigation was controlled by time clock, but aimed to keep the capillary matting at a continuous level of saturation. Once a week plants were overhead watered to reduce the build up of excess soluble salts.

Standard pest and disease control measures were employed as required. Plants exhibiting symptoms of soft rot throughout the pre-plant handling period and cultivation were removed.

2.3.4 Collection of data

At fortnightly intervals all plants designated for harvest were sampled and the following parameters recorded:

1. Dry weight of:
    a) tuber,
    b) tuber peel,
    c) matured leaves (Leaves that are fully expanded),
2. Leaf area (matured leaves only),
3. Calcium concentration of:
    a) tuber peel,
    b) matured leaves,
    c) growing medium (Plant available calcium),
4. Growing medium:
    a) EC
    b) pH

2.3.5 Plant tissue and growing medium sampling, and Ca analysis

Immediately upon harvest, matured leaves and tubers were separated carefully and the tubers were washed, roots were removed, and dried with the used of towel. Tubers were immediately peeled with a standard vegetable peeler, yielding a peel 4 mm thick

containing the periderm and about 10 layers of cortical cells (Loh *et al.*, 1992). The apical region of the tuber was avoided to minimize error in tuber peel tissue sample. collection.

For each plant the mature leaves per plant were pooled to represent one sample. In addition, for each plant harvested 200 g of growing medium was sampled of.

All plant tissue and growing medium samples were oven dried at 80°C for 48 hours and then grounded to a fine powder, either in a ring grinder (Glenn Creston Hammer Mill) or with a pestle and mortar. Prior to mineral analysis the ground material was dried again for 24 hours at 60°C, and then cooled in the desiccator for 30 minutes.

The mineral analysis of plant tissue followed the procedure reported by Loh *et al.* (1992) provided by the Soil Department of Massey University. Dried plant material (100 mg) was extracted with 4 ml of 80% nitric acid for 4 hours at 150°C using a digestion block. When the solution was clear, the temperature was raised to 250°C for 3 hours and boiled to dryness. Before the dried material cooled completely, 50 ml of a solution was taken up from 2,500 ml solution made up of 100 ml strontium/caesium at 25,000 ppm, 250 ml of 0.2M HCl and deionized water. Fifteen (15) ml aliquots were stood for 60 minutes and absorbance at 422.7 nm read using a GBC 904 AA, spectrophotometer.

The preparation of 25,000 ppm strontium/caesium solution was by dissolving 30.19 g strontium and 15.84 g caesium in 250 ml deionized water while preparation of 0.2 M HCl was by placing 44 ml of 35.4% assay HCl in deionized water to made up 2,500 ml solution.

The method of Ca analysis of the growing medium followed that reported by Hughes and Gilkes (1984). Dried growing medium (200 mg) was placed in the container together with 5 ml of deionised water. Samples were then added subsequently with 15 mls of combined solution of 2 M NaCl and 2 M trietanolamine buffered at pH 8.1. Samples were shaken vigorously at 300 rpm for 60 minutes and by being centrifuggated at 100 g for 10 minutes. The supernatant was collected, stood for 60 minutes and the absorbance was read using a GBC 904 AA spectrophotometer at a wavelength of 422.7 nm. In addition, the solution of 2 M NaCl and 2 M trietanolamine buffered to 8.1 extract the plant available Ca in the growing medium.

2.3.7 Determination of EC and pH of the growing medium

The method of determining the EC and pH of the growing medium followed that of Handreck and Black (1992). Growing medium (100 ml) together with 150 ml of distilled water were shaken by hand twice, for 50 times, with an interval of 15 minutes. The samples were left standing for 15 minutes, before the EC and pH were read using a Jenway 410 conductivity and Jenway 510 pH meter, respectively.

2.3.8 Statistical analysis

Analysis of variance for all data were computed using PROC GLM of SAS for a randomized complete block model (SAS Inst. Inc., Cary, N.C., USA, 1980). Least significant means and least significant differences were used to compare treatments on sequential harvest throughout the whole duration of growth of *Zantedeschia.*

2.4 Results

2.4.1 Plant available calcium in the growing medium

Apart from the measurement made at planting, both treatments showed minimal changes in the plant available Ca concentration throughout the growing period (Figure 1). Throughout this period of sampling the treatment amended with 9 kg/m^3 gypsum, resulted in approximately a three fold greater concentration of plant available Ca in the growing medium (13.98 mg/g) with that of the control (5.15 mg/g) ($P \leq 0.05$) (Appendix B).

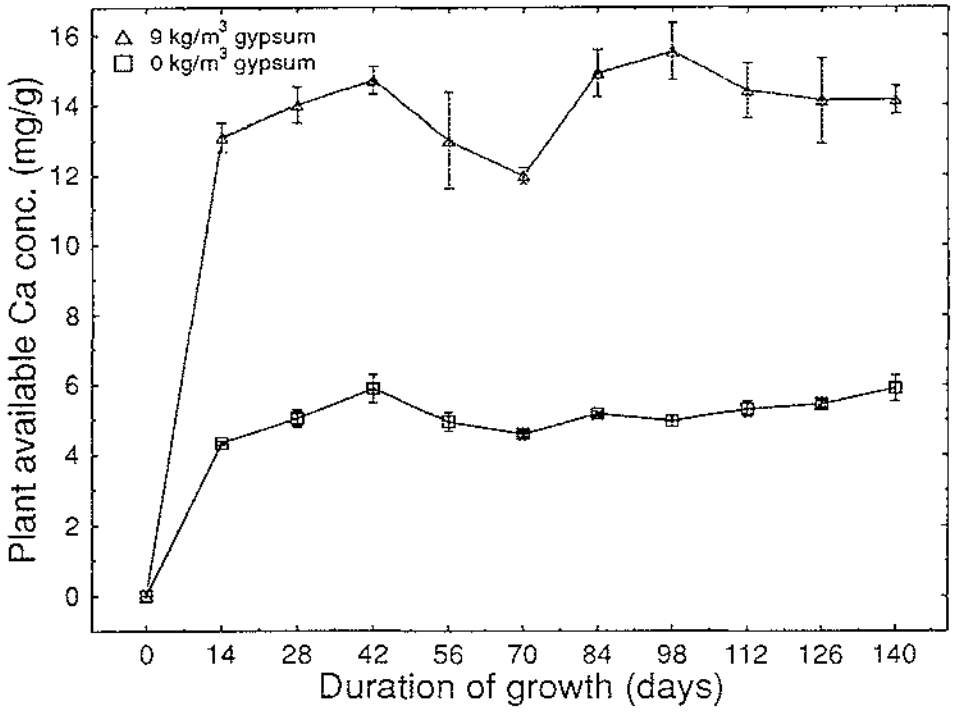

Figure 1. Plant available calcium of the growing medium, throughout the duration of growth, resulting from the application of either 0 or 9 kg/m^3 gypsum at planting. Mean values se., n = 4.

2.4.2 Tuber peel calcium concentration

Subsequent to planting, tuber peel Ca concentration was consistently greater, throughout the period of growth, in plants grown in gypsum amended medium compared with that in the control (Figure 2.) From 14 days after planting up to the last harvest (140 days) tuber peel Ca concentration from plants in gypsum amended medium was approximately 2 mg/g greater compared with that from the control. However significant differences between treatments in tuber peel Ca concentration were only evident at 28, 56, 84 and 140 days after planting ($P \leq 0.05$) (Appendix C).

Concentrations from both treatments followed the same pattern of change which increased continuously from planting up to 84 days after planting, and subsequently declined up to the last harvest. Tuber peel Ca concentration in gypsum amended medium reached its maximum at 84 days with 17.42 mg/g compared with that in control with 16.25 mg/g and on the last harvest, the tuber peel Ca concentration were 8.38 and 6.97 mg/g at gypsum amended medium and the control, respectively.

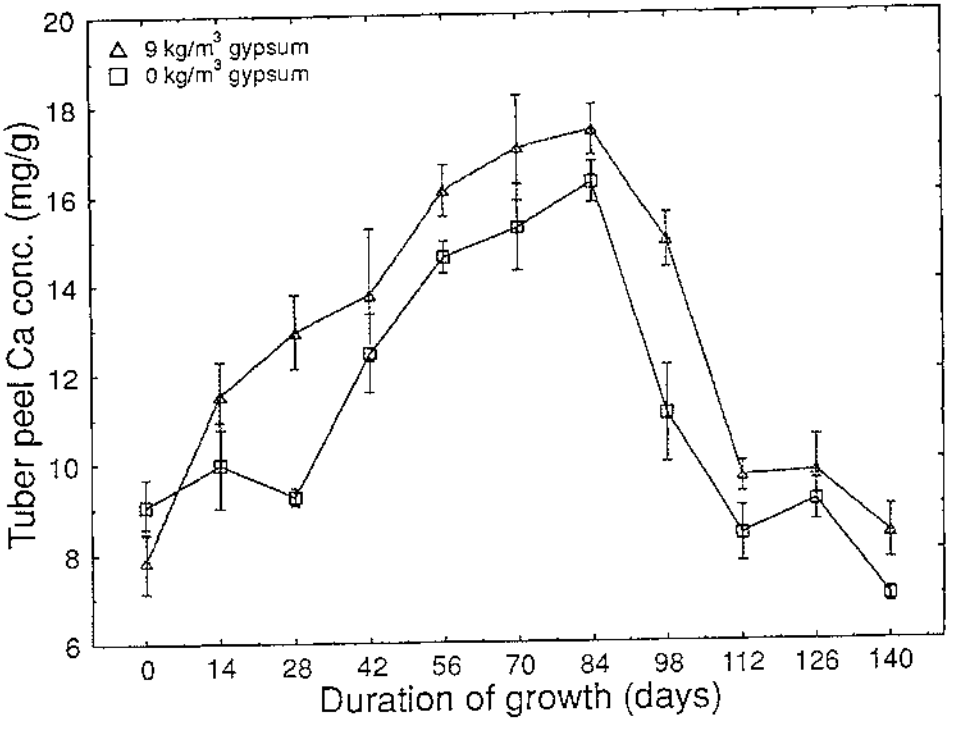

Figure 2. Total calcium concentration of tuber peel tissue for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 and 9 kg/m^3 gypsum at planting. Mean values se., n = 4.

2.4.3 Calcium concentration of matured leaves

Mature leaves Ca concentration was consistently greater, throughout the period of growth, in plants grown in gypsum amended medium compared with that in the control (Figure 3). From the first sequential harvest (56 days) up to the last harvest (140 days) mature leaves Ca concentration from plants in gypsum amended medium was approximately 2 mg/g greater with that from the control. However significant differences were only evident at 70,112 and 126 days after planting ($P \leq 0.05$) (Appendix D).

Concentration from both treatments followed the same pattern of change with the duration of growth. The Ca concentration increased continuously from planting up to the last harvest. Mature leaves Ca concentration in gypsum amended medium at 56 days was 10.76 mg/g compared with that in the control with 8.14 mg/g and reached its maximum at 126 days with 14.97 in gypsum amended medium while with that in the control the maximum concentration was achieved at 140 days after planting.

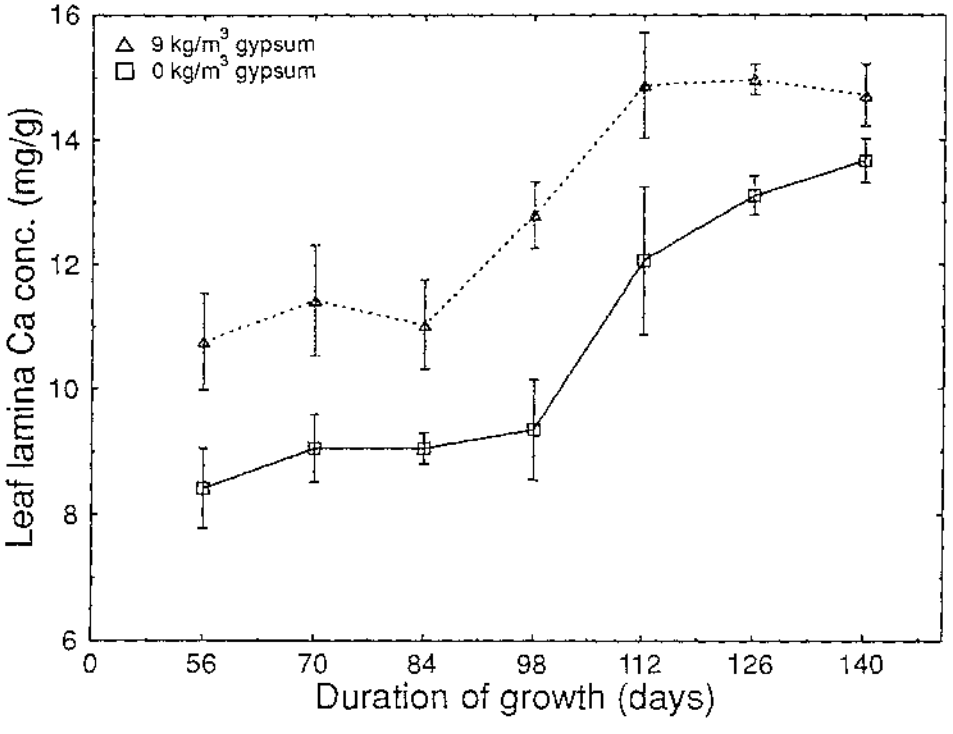

Figure 3. Total calcium concentration of mature leaves for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 and 9 kg/m^3 gypsum after planting.
Mean values se., n = 4.

2.4.4 Tuber dry weight

Throughout the period of observation, there were no differences in the tuber dry weight between the two treatments ($P \leq 0.05$). Similarly, changes in the tuber dry weight for both treatments followed the same pattern (Figure 4). Tuber dry weight declined up to 70 days after planting and subsequently increased to the final harvest (140 days after planting) (Appendix E).

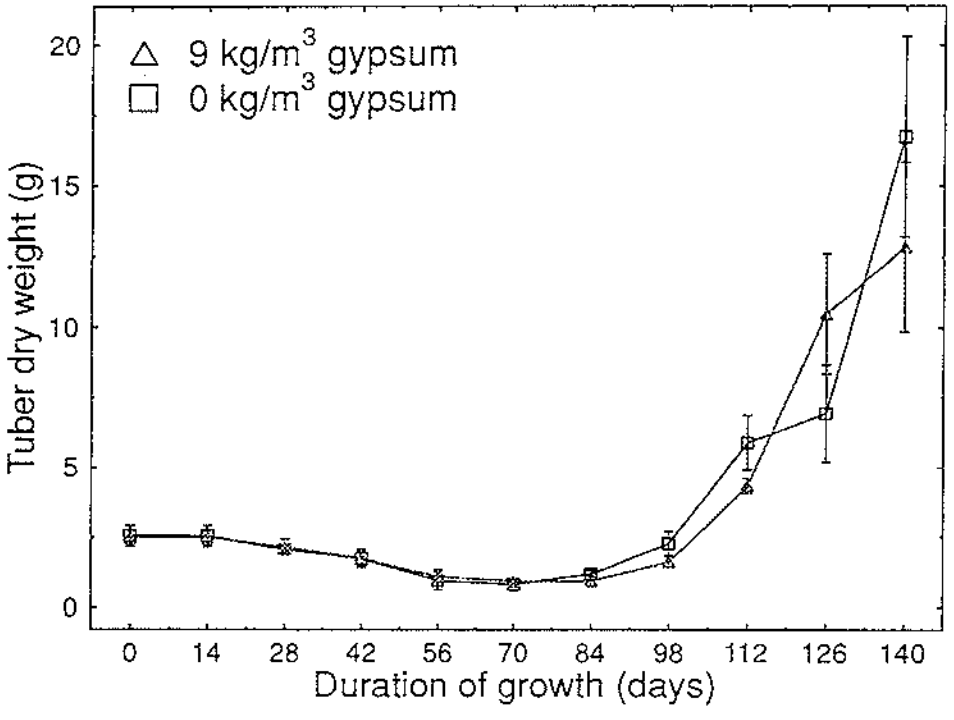

Figure 4. Tuber dry weight for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 or 9 kg/m^3 gypsum at planting. Mean values e., n = 4.

2.4.5 Leaf area of matured leaves

Matured leaves were not evident until 56 days after planting. Leaf area increased throughout the period of observation in both treatments (Figure 5). While there was an indication of a possible reduction in leaf area beyond 126 days for the plants grown in gypsum amended medium, there were no significant differences between treatments at any time ($P \leq 0.05$) (Appendix F).

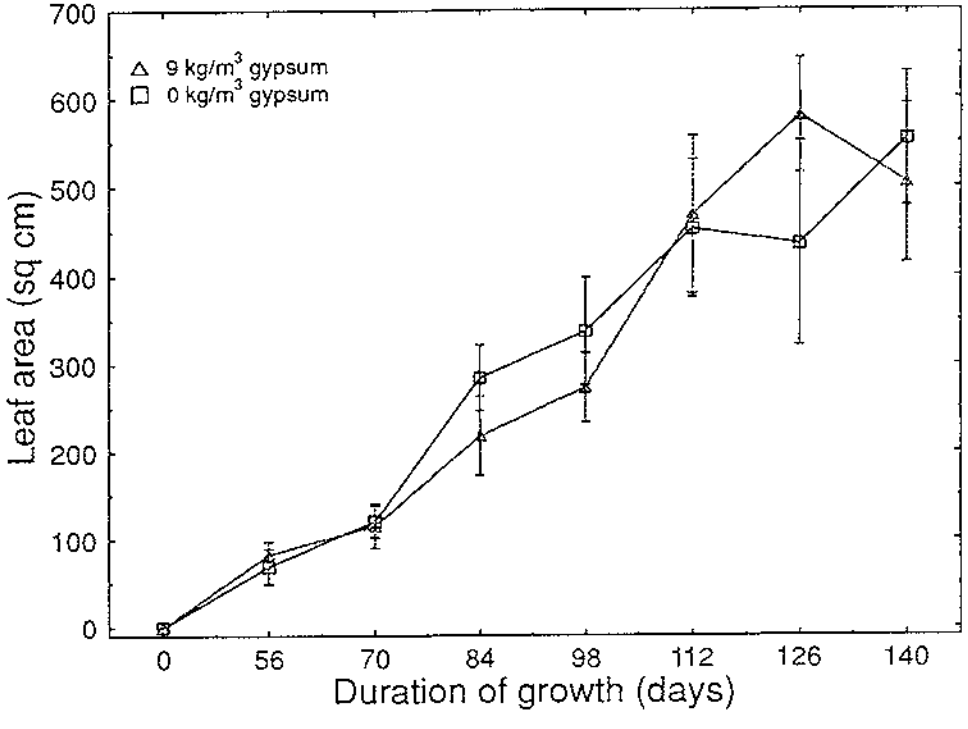

Figure 5. Total plant leaf area of matured leaves for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 or 9 kg/m^3 gypsum at planting. Mean values se., n = 4.

2.4.6 Growing medium pH and EC

The growing medium EC was consistently greater, throughout the period of growth, in the gypsum amended medium compared with that in the control (Figure 6). From planting (0 day) up to the last harvest (140 days) EC in gypsum amended medium consistently greater than that achieved with the control by a magnitude ranging 0.5 to 2.5 S/cm. Significant differences between treatments in EC were evident at all harvest time ($P \leq 0.01$) (Appendix G).

Changes in EC of the growing medium followed the same pattern of changes for both treatments. At 14 days after planting an aberrant data were observed, this could be attributed to possible wrong calibration of the equipment and/or by the effect by overhead irrigation done prior to the collection of samples.

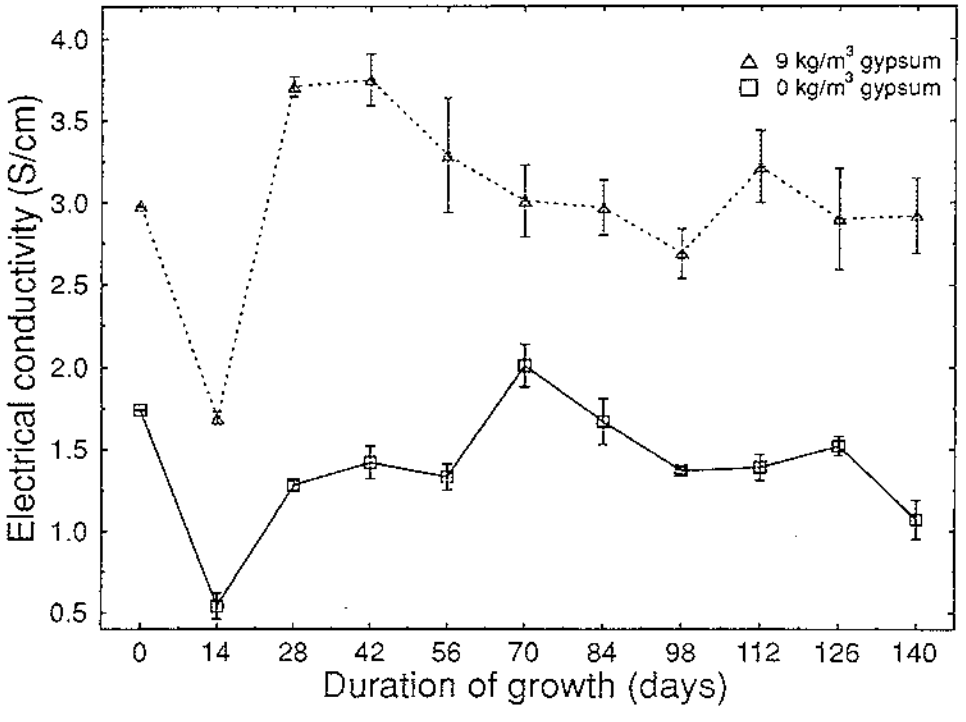

Figure 6. Electrical conductivity of the growing medium for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 and 9 kg/m^3 at planting. Mean values se., n = 4.

The pH of the growing medium was consistently greater throughout the period of growth in plants grown in the control compared with that in the gypsum amended medium except on 0 and 70 days after planting. Except at 0 and 70 days after planting up to the last harvest (140 days) pH in the control was approximately 0.2 greater compared with that from the gypsum amended medium. However, significant differences between treatments in pH were only evident at 28 and 56 days after planting ($P \leq 0.05$) (appendix H).

Like the EC, changes in the pH of the growing medium also followed the same pattern of change for both treatments. Gypsum does not increased the pH but rather decreased the pH since pH of the control medium was higher compared with that of gypsum amended medium. At 14 days after planting the pH of the control was 6.35 compared with 6.09 achieved at gypsum amended medium. The pH in control medium and the gypsum amended medium reached its maximum at 84 days with 5.66 and 5.55, respectively.

Apart from a potentially aberrant pH at 84 days after planting. A possible cause of potentially aberrant result achieved at 84 days after planting is due to the old buffer solution used (pH 4 and pH 7), or either the change of pH meter equipment.

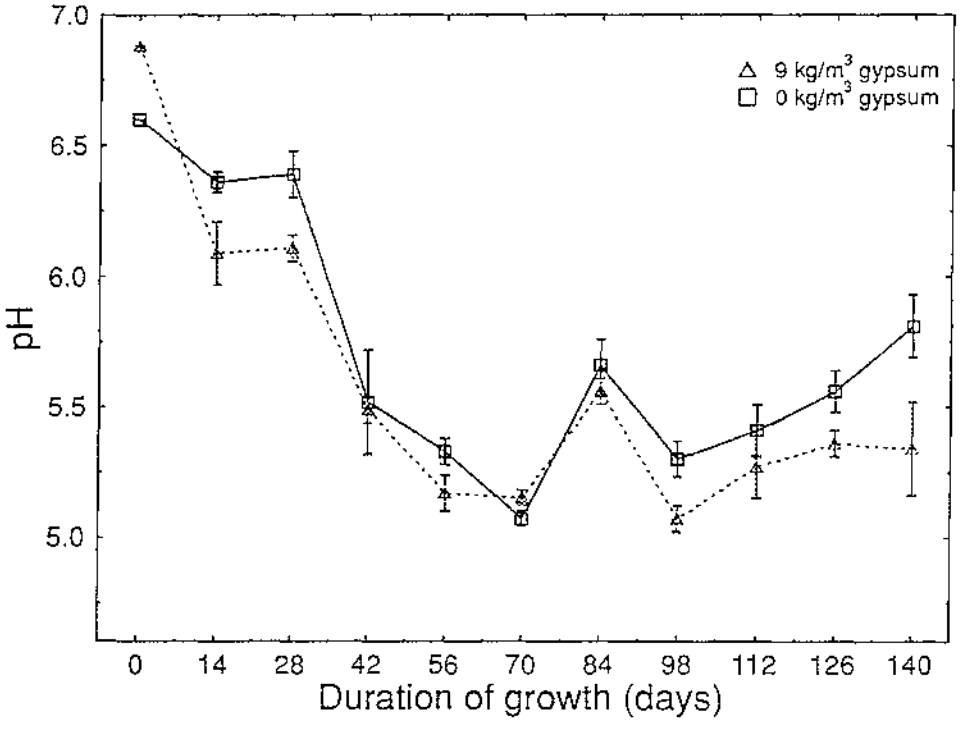

Figure 7. pH of the growing medium for *Zantedeschia* 'Galaxy', throughout the duration of growth, resulting from the application of either 0 and 9 kg/m^3 gypsum at planting. Mean values se., n = 4.

2.5 Discussion

As reported previously, the Ca concentration of tuber changed as growth progressed (Clark & Boldingh, 1990). In the current experiment, the Ca concentration of tuber peel reached a maximum of 17.42 mg/g at 84 days after planting and subsequently declined to that achieved at planting (7.86 mg/g). Calcium concentration of the tuber peel on gypsum amended medium achieved was 17.42 mg/g which was higher than that achieved by Clark and Boldingh (1990) in the tuber which was (12.5 mg/g). Similarly, Loh *et al.*(1992) illustrated that after 19 weeks of growth, gypsum amended medium (9 kg/m^3) raised % peel Ca per unit dry weight to 0.71% compared with 0.54% of the control plants. This difference in Ca concentration in plants is under genetic control (Horak & Kienzel, 1971). Therefore, cultivars, and of different tissue in the plants varies in the Ca concentration.

The decline in Ca concentration of the tuber peel tissue (Figure 2) was not due to a decline in the plant available Ca concentration in the growing medium (Figure 1). In fact, plant available Ca concentration in the gypsum amended medium has an average of 13.98 mg/g applied with 9 kg/m^3 and has a minimal changes throughout the growing period (Figure 1). The decline in Ca concentration in tuber peel was due to the continuous increased in the Ca concentration of the mature leaves throughout the growing period (Figure 3). Faust (1989) illustrated that even though the growing medium contained high levels of Ca, and was taken up by the plants, Ca was

preferentially distributed to the leaves. In addition, the decline in the tuber peel Ca concentration (Figure 2) coincided with the rapid increase in tuber growth at 84 days after planting (Figure 4). Once the rate of tuber growth increased, the accumulated Ca was diluted throughout the enlarging tubers. This result is similar to that of Ferguson *et al.* (1987) with apple, and, Kirby and Pilbeam (1984) with kiwifruit where they illustrated that those tissues in the cortical flesh of the fruit contain the lowest Ca concentration, and also undergoes the greatest rate of expansion. This decline in tuber peel Ca concentration was also due to the limited ability of the plant to regulate the Ca concentration distribution.

Calcium concentration in mature leaves was indirectly affected by the continuous increase of the leaf area (Figure 5). Adams and Ho (1992) illustrated that the continuous increase in leaf area, increased transpiration and consequently increased the accumulation of Ca. It was supported with the results of Biddulph *et al.* (1958) and Millekan and Hanger (1966) where they found out that when Ca reached the leaves the Ca concentration become immobile or little are translocated with the plants. And so, to confirm or prove this hypothesis, another study was conducted to measure the different forms of Ca.

The result of this study illustrated that gypsum amended medium (9 kg/m^3) does not provide any differences on the tuber dry weight, leaf area and pH of the growing medium. Hence in the EC of the gypsum amended medium increased by a magnitude

of 0.5 to 2.5 S/cm compared with that of the control. In fact with the study of Loh *et al.*, (1992) they illustrated that this rate of application plants were more tolerant to bacterial soft rot compared with that from the control but higher than that at 3 kg/m^3. It was also suggested that this could due to the potentially detrimental EC levels observed which affect the health of the plants.

The results of this study illustrated that despite of high plant available Ca in the gypsum amended medium throughout the growing period, at the beginning and the end of the growing season (140 days) the Ca concentration in the tuber peel tissue was low compared with that achieved at 84 days. These periods of low Ca concentration in the tuber peel tissue coincide with the collapse of tubers infected by bacterial soft rot observed by many growers (personal communication). If an enhanced Ca concentration is to be achieved throughout the growing season, further study of other techniques will be required to raise the tuber peel tissue Ca concentration high enough to compensate for the low Ca concentration in the early and late period of the growing season. This can be done by vacuum infiltrating Ca solutions into the tubers before planting or by strip application of gypsum.

2.6 Conclusion

From this experiment it was concluded that :

1. A single application of gypsum (9 kg/m^3) is sufficient to provide plant available Ca in the growing medium.

2. Amended medium with 9 kg/m^3 gypsum increased the plant available Ca approximately three fold.

3. The decline in Ca concentration of the tuber peel tissue in the latter part of the growing period was not due to the decline in the plant available Ca in the growing medium.

4. Tuber peel and mature leaves Ca concentration in gypsum amended medium (9 kg/m^3) increased approximately 2 mg/g in the dry weight basis.

5. The continuous increase in Ca concentration of the mature leaves throughout the growing period was not due to the continuous increase in the plant available Ca in the growing medium.

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3 Forms of calcium in *Zantedeschia* throughout the growing season following vacuum infiltration

3.1 Introduction

Applying solutions containing Ca directly to a storage organ is one method of increasing tissue Ca concentration. Dipping apples in calcium chloride ($CaCl_{2)}$ solutions can increase tissue Ca concentration (Bangerth *et al.*, 1972). Adding food thickeners such as Keltrol to Ca dip solution can further increase Ca uptake in apples (Mason *et al.*, 1975). However, active infiltration procedures, such as vacuum or pressure infiltration, that force solution to enter in tissue and filling up the air space with solution in fruit were more effective than dipping for controlling bitter pit (Scott & Wills, 1979). In addition, vacuum infiltration also increased the Ca concentration in potato and *Zantedeschia* tubers (McGuire & Kelman, 1984; Loh *et al.*, 1992).

Vacuum infiltration of tubers such potato and *Zantedeschia,* and fruits of apples, was affected by the epidermis (Tzeng *et al.*, 1990; Conway *et al.*, 1992; Loh *et al.*, 1992). Tubers of potato cultivars harvested late in the season had a higher amount of Ca than those harvested earlier (Tzeng *et al.*,1990). This is best explained by the study of Conway *et al.*,(1992) in the case of apple fruits. Results showed that if the fruits were harvested and infiltrated in the early phase of maturity, insufficient $CaCl_2$ solution is taken into the fruit and does little to inhibit decay. If the fruit is harvested in the later phase of maturity, more Ca is taken up than is needed for optimum decay control, and severe fruit injury may result.

Using *Zantedeschia* tubers Loh *et al.* (1992) illustrated that the tolerance limit must was between 7.5% to 10% $CaCl_2$, and they suggested that 7.5% $CaCl_2$ concentration be used as the upper limit for future vacuum infiltration work. In addition, they found out that cured tubers (i.e. outer periderm layer have been replaced with a thick periderm) were more resistant to the movement of the solution (Tzeng *et al.*, 1990). Therefore, optimise solution uptake vacuum infiltration should ideally be conducted just after lifting of the tubers; i.e. prior to curing.

In the preceding experiment the total Ca concentration of organs of the *Zantedeschia rehmanii* (Engl)-like selection 'Galaxy' and plant available Ca in the growing medium were determined following gypsum application (refer Chapter 2). Results illustrated that there is a need of another technique to raise tuber peel tissue Ca concentration high enough to compensate the low Ca concentration on the early and later part of the growing season of *Zantedeschia.*

Several investigations sought to predict the development of Ca disorders based on the total Ca concentration of leaf or fruit tissue (Holand, 1980; Shear, 1975; Turner *et al.*, 1977). However, total Ca concentration may not be the best indication of the physiological status of the plant (Himelrick, 1981). Ferguson *et al.* (1980) in his study suggested that the principal forms of Ca could be identified by extraction in successive solvents. Soluble Ca was extracted using 80% acetic acid, calcium oxalate through the

use of 0.25 M HCl and the bound Ca in the cell wall was completely extracted using 80% HNO_3.

Soluble Ca is known to affect various properties of biological membranes (Manery, 1966) and may be associated with protein or be present as Ca phosphotides in the plant membrane. In addition, soluble Ca is also associated with the activation and for stabilization of certain enzymes (Mandels & Reese, 1957; Doods & Ellis; Chrispeels & Varner, 1967; Hewitt & Smith, 1975).

Little is known about the function of calcium oxalate in the plant though there has been an speculation (Franceschi & Horner, 1980). They hypothesized that calcium oxalate is the end product of metabolism, and, in excess, may be toxic to the plants. As such, it is sometimes thought of as an excretory product (Frank, 1972). Thus it has been suggested that Ca oxalate crystals and crystal forming idioblasts are a means of isolating this product from total Ca, and the calcium oxalate crystals idioblast have been classified as excretory idioblast (Foster, 1956). Similarly, the crystals have been viewed as function to remove excess Ca from the system. Evidences showing that calcium oxalate may function as a storage form of Ca or oxalate (Rao & Mohar, 1972), they suggested that calcium oxalate can be broken down into different form of Ca be utilized by the plants.

Calcium bound in the cell wall is essential for the formation of the middle lamella

(i.e, calcium pectate). Calcium pectate acts as a cementing agent in the cell wall thus increasing the plasticity of the cell wall (Jones & Lunt, 1967). In addition, some studies also provided considerable evidence that the formation of calcium pectate increased rigidity of the cell wall (Tagawa & Bonner, 1957; Cormack, 1965). Furthermore, calcium pectate enhance the process of calcification which increases the resistance of tissue to infection probably by increasing the resistance of the cell wall to bacterial polygalacturonase (Bateman & Lumsden, 1965). Therefore, with the information listed above with the function of different forms of Ca in plants study will be conducted in *Zantedeschia*. Since *Zantedeschia* can be easily infected with bacterial soft rot and at present no chemical control was recommended, increasing Ca concentration particularly the specific form will be of great help to provide tolerance to this disease, more so, growers will benefit more.

3.2 Aim and objectives

The aim of the experiment was to investigate the effect of different forms of calcium (Soluble Ca, calcium oxalate and calcium bound in the cell wall) in *Zantedeschia* plant tissues through vacuum infiltration.

The objectives of this experiment were to determine the following through vacuum infiltration:

a) any changes in soluble Ca concentration of plant tissue during the growing

season

b) any changes in Ca oxalate concentration of plant tissue during the growing season

c) any changes in bound Ca in the cell wall concentration in plant tissue during the growing season

d) any relationship between the soluble Ca, calcium oxalate and bound Ca with respect to total Ca concentration of the tuber peel and mature leaves

3.3 Materials and Methods

3.3.1 Plant material

Tubers of the *Zantedeschia pentlandii* (Engl)-like selection 'Black Magic' (2.4 0.12 cm diameter and 5.2 0.16 g fresh weight) were used in this experiment. Tubers were sourced from a commercial supplier following approximately 6 months growth from tissue culture exflasking. After receipt, tubers were hand-cleaned while roots and leaves were carefully removed. The tubers were divided into three designated treatments and then followed by a pre-plant application of vacuum infiltration to avoid problems arising from the cured tubers. Tubers were vacuum infiltrated in their respective treatment for 30 minutes at 600 Hg. To avoid effect on the vacuum infiltration of different $CaCl_2$ solutions tubers were dipped in 1% sodium hypochlorite for 30 minutes. Treatment with sodium hypochlorite was intended to remove latent microbial infection on the tuber surface. Tubers were subsequently cured for one week at 20 1 °C and 90 1.5% relative humidity.

3.2.2 Treatments and experimental design

Randomised complete block design was based on 2 variables tested (vacuumed infiltration and non-vacuumed infiltration application). This is not a full factorial since there were no combination between the two variables. The pre plant application of

vacuum infiltration used were 7.5% $CaCl_2$ and 0% (water). The non vacuum infiltration, i.e.dry tubers was the control treatment. For each set of treatments, there were eight blocks, eight sequential harvest for tubers, four sequential harvest for matured leaves, and five plants per experimental unit. The blocking design was accounted for initial tuber size, as well as the light and temperature strata within the greenhouse.

3.3.3 Planting and cultivation

With the exception of the preparation of the growing medium, planting and cultivation were as described in Chapter 2 (refer Section 2.3.3). For the preparation of the growing medium the control treatment in the previous experiment was used, i.e. 0 kg /m^3 gypsum applied at planting (Appendix A).

3.3.4 Collection of data

Prior to planting, three sequential harvests were conducted; i.e. before treatment (BT), just after the tubers were hand cleaned, followed by post treatment application; post vacuum (PV), and lastly, after curing for one week; before planting (BP).

Once planted, sequential harvests followed at 28 day intervals. At each harvest the following parameters were recorded:

Concentration of different forms of Ca in the tuber peel tissue and in matured leaves viz:

a) soluble Ca

b) calcium oxalate

c) bound Ca in the cell wall

d) total Ca

3.3.5 Plant tissue sampling, preparation, and calcium analysis

Plant tissue harvested were the tuber peel tissue and the matured leaves. Plant tissue preparation was conducted as described in Chapter 2 (refer Section 2.3.5). However, as outlined below, the procedure for Ca analysis followed that of Ferguson *et al.* (1980). This procedure permitted fractionation of the different forms of Ca in the plant tissue (i.e., soluble Ca, calcium oxalate and calcium bound in the cell wall).

Dried powder of plant tissue (100 mg) was extracted with 3.5 ml of 80% acetic acid by vigorously shaking (300 rpm) for 30 minutes followed by 10 minutes centrifugation (50 g). The supernatant was removed and the extraction procedure repeated. The supernatant from both 80% acetic extractions were combined. The same sample was then twice extracted with 5 ml of 0.25 M HCl, as described for the acetic acid extractions, and the supernatant from both 0.25 M HCl extraction were combined. The residue was transferred directly in the digestion blocks for digestion with HNO_3.

Supernatant samples (5ml) from both the 80% acetic acid and 0.25 M HCl extractions were also digested in HNO_3. Four ml of 80% nitric acid was added in the supernatant or residue set using a digestion block set at 150°C for 4 hours. When the solution was clear, the temperature was raised to 250°C for 3 hours and boiled to dryness. Before the dried material cooled completely, 50 ml of a solution was taken from 2,500 ml solution made up of 100 ml strontium/caesium at 25,000 ppm, 250 ml 0.2 M HCl, and deionised water. Preparation of 25,000 ppm strontium/caesium solution and 0.2 M HCl was described in previous study (refer Section 2.3.5). Fifteen (ml) aliquots were stood for 60 minutes and absorbance at 422.7 nm read using a GBC 904 AA, spectrophotometer.

3.4 Results

3.4.1 Soluble calcium concentration of the tuber peel

The concentration of soluble Ca in the vacuum infiltrated tubers with 7.5% $CaCl_2$ was higher after the application of vacuum infiltration (PV) and just prior before planting (BP) compared with that of the Vacuum infiltrated with 0% $CaCl_2$ and the control (Figure 8a). Subsequent to the application of vacuum infiltration with 7.5% $CaCl_2$ increased the initial Ca concentration from 0.43 mg/g to 4.73 mg/g and declined just before planting (3.31 mg/g). Differences between the vacuum infiltrated with 7.5% $CaCl_2$ and the vacuum infiltrated with 0% $CaCl_2$ and the control were evident ($P \leq 0.001$).

Subsequent to planting, soluble Ca concentration was almost the same in concentration, throughout the period of growth, in the tubers vacuum infiltrated with 7.5% $CaCL_2$, 0% $CaCl_2$ and the control. Differences between the treatments and the control in the soluble Ca concentration were not evident ($P \leq 0.05$) (Appendix I).

Once planted, concentration of the soluble Ca from the treatments and the control followed the same pattern of change with the duration of growth. The soluble Ca concentration has a minimal change from 28 days after planting up to the last harvest (140 days) except an increase in tubers vacuum infiltrated with 7.5% $CaCl_2$ which

increase its maximum of 0.83 mg/g at 84 days.

3.4.2 Calcium oxalate concentration of the tuber peel

Before treatment up to 84 days after planting, the concentration of calcium oxalate in the tuber peel, following the application of vacuum infiltration with 7.5% $CaCl_2$ was greater with that of the vacuum infiltrated with 0% $CaCL_2$ and the control (Figure 8b). From time before treatment up to the time just before planting, differences between the treatments and the control in the calcium oxalate concentration in the tuber peel tissue were evident ($P \leq 0.01$). The calcium oxalate concentration from the tuber vacuum infiltrated with 7.5% $CaCl_2$, 0% $CaCl_2$, and the control reached its maximum concentration of 6.81, 4.33, and 2.89 mg/g (Appendix J).

Once planted, throughout the period of growth, calcium oxalate concentration of the vacuum infiltrated tubers and the control increased its maximum and subsequently declined up to the last harvest. In tubers vacuum infiltrated with 7.5% $CaCl_2$ its maximum was achieved at 56 days after planting with 5.38 mg/g while the tubers infiltrated with 0% $CaCl_2$ and the control reached its maximum at 84 days after planting with 4.33 and 4.22 mg/g. However, significance difference between vacuum infiltrated and the control was only evident at 56

days after planting ($P \leq 0.05$). At 84 days after planting up to the last harvest (140 days) a subsequent declined was observed in the vacuum infiltrated tubers and the control with a magnitude ranging 0.6 to 1.4 mg/g in every harvest.

3.4.3 Bound calcium in the cell wall concentration of the tuber peel

Before treatment up to just before planting, all treatments and the control followed the same general pattern of change which increased continuously from before treatment up to the time just before planting, and subsequently declined up to 28 days after planting Figure 8c). Once planted, throughout the period of growth, a continuous increased up to the last harvest (140 days). A greater increase in bound Ca concentration in tubers vacuum infiltrated with 7.5% $CaCl_2$ was recorded at 56 days after planting compared with that of the tubers infiltrated with 0% $CaCl_2$ and the control. At this stage, tuber vacuum infiltrated with 7.5% $CaCl_2$ increase was approximately 1.5 fold and 2 fold greater compared with that in 0% $CaCl_2$ and the control tuber, respectively. Differences between the treatments and the control in the bound Ca from before treatment up to the time just before planting were evident at $P \leq 0.05$. In contrast, once planted, throughout the period of growth, bound Ca concentrations between treatments was only evident at 56 days after planting ($P \leq 0.05$) (Appendix K).

3.4.4 Total calcium concentration of the tuber peel

Changes in the total Ca concentration in the tuber peel tissue followed the same pattern for the tubers vacuum infiltrated with 0% $CaCl_2$ and the control, while different changes was recorded for tubers vacuum infiltrated with 7.5% $CaCl_2$. Like the bound Ca in the cell wall, tuber vacuum infiltrated with 7.5% $CaCl_2$ was consistently greater during PV and BP as compared with the two treatments. At time of PV and BP, total Ca concentration of tubers vacuum infiltrated with 7.5% $CaCl_2$ was 2 fold compared with that in tuber vacuum infiltrated with 0% $CaCl_2$ and the control tubers, respectively. It is worth noting that heterogeneity in the results of the total Ca concentration were recorded during these stages for the vacuum infiltrated and the control as indicated by the s.e. values of 2.84 and 2.00, respectively. Differences on total Ca concentration among treatments are statistically significant from PV to BP at $P \leq 0.05$. In contrast, no evident differences among treatments were recorded after planting ($P \leq 0.05$).

3.4.5 Soluble calcium concentration of the mature leaves

Changes in the soluble Ca concentration in matured leaves for both the vacuum infiltrated with 0% $CaCl_2$ and the non-vacuum tubers followed the same pattern of change. Soluble Ca concentration of both treatments decreased from initial Ca concentration to 56 days after planting, and subsequently increased up to the last

harvest (140 days). In contrast, soluble Ca concentration in matured leaves from vacuum infiltrated tubers with 7.5% $CaCl_2$ increased continuously throughout the period of growth (Figure 8d). However, differences between treatments in soluble Ca concentration were not evident at all harvest time ($P \leq 0.05$) (Appendix M). The soluble Ca concentration in tubers vacuum infiltrated with 7.5% $CaCl_2$ increased continuously from the initial Ca concentration at 56 days with 0.35 mg/g up to 0.76 mg/g at the last harvest. In contrast, the initial soluble Ca concentration from tubers vacuum infiltrated with 0% $CaCl_2$ and the control were 0.42 and 0.54 mg/g, respectively and decreased at 84 days after planting to 0.39 and 0.35mg/g, respectively. From this period, the soluble Ca concentration in tubers vacuum infiltrated with 0% $CaCl_2$ and the control increased continuously and reached to 0.95, and 0.85 mg/g, respectively at the last harvest (140 days).

3.4.6 Calcium oxalate concentration of the mature leaves

Like in soluble Ca concentration, both the vacuum infiltrated with 0% $CaCl_2$ and the control tubers followed the same pattern of change. Calcium oxalate concentration increased continuously from 56 days after planting up to 112 days after planting, and subsequently declined. In contrast, calcium oxalate concentration from vacuum infiltrated with 7.5% $CaCl_2$ reaches its maximum at 84 days, decreased slightly and remain almost constant (Figure 8f). However, differences between treatments in matured leaves calcium oxalate concentrations were only evident at 84 days after

planting ($P \leq 0.01$) (Appendix N). Maximum concentration of calcium oxalate for both the vacuum infiltrated with 0% $CaCl_2$ and the non-vacuum tuber was 6.24 mg/g at 112 days after planting; while 5.76 mg/g at 84 days after planting in vacuum infiltrated with 7.5% CaCl2 solution was achieved.

3.4.7 Bound calcium concentration of the mature leaves

Throughout the period of observation, bound Ca concentration of matured leaves in all treatments increased continuously from 56 days after planting up to 140 days after planting (Figure 8g). However, differences between treatments in matured leaves bound Ca concentration in the cell wall were only evident at 140 days after planting ($P \leq 0.05$) (Appendix O). From the first harvest (56 days after planting), bound Ca concentration of the tubers vacuum infiltrated with 7.5% $CaCl_2$ was 4.14 mg/g and increased continuously up to 16.47 mg/g at the last harvest (140 days). In the case of plants vacuum infiltrated with 0% $CaCl_2$ and the control the initial bound Ca concentration were 5.41 and 4.42 respectively and reached its maximum with 25.93 and 12.12 respectively at the last harvest.

3.4.8 Total calcium concentration of the matured leaves

Like in Ca bound in the cell wall, total Ca concentration in the mature leaves continuously increased from initial harvest (56 days after planting) up to the last

harvest (140 days after planting) (Figure 8h). However, differences between treatments in total Ca concentration in matured leaves were only evident at 84 days after planting ($P \leq 0.01$) (Appendix P). In tubers vacuum infiltrated with 7.5% $CaCl_2$ the total concentration at 84 days after planting was 14.86 mg/g which was greater compared with that of the total Ca concentration in tubers vacuum infiltrated with 0% $CaCl_2$ and the control with 12.10 and 13.58 mg/g, respectively. Concentration of total Ca in matured leaves in vacuum infiltrated with 7.5% $CaCl_2$ at 56 days after planting was 9.35 mg/g and continuously increased up to 22.85 mg/g at the last harvest. In the case of the tubers vacuum infiltrated with 0% $CaCl_2$ the total Ca concentration at 56 days after planting was 9.14 and 9.35 mg/g respectively and reached their maximum at last harvest with 18.57 and 23.06 mg/g, respectively.

Tuber peel Mature leaves

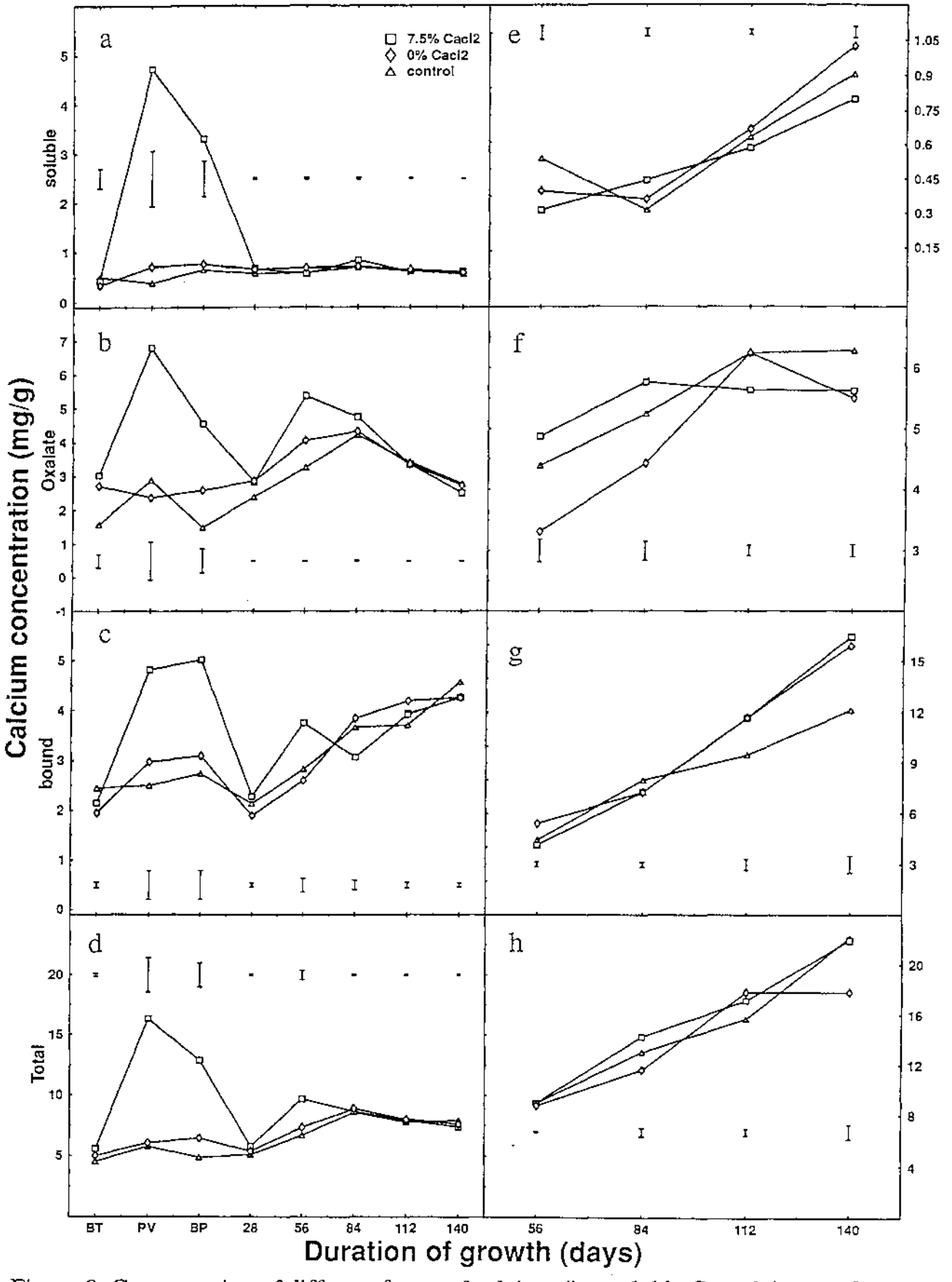

Figure 8. Concentration of different forms of calcium (i.e. soluble Ca, calcium oxalate, and bound calcium) in the tuber peel and matured leaves resulting from the infiltration of either 0%, 7.5% $CaCl_2$ and control. BT - before treatment, PV - post-vacuum, BP - before planting n = 5.

3.5 Discussion

Vacuum infiltration of 7.5% $CaCl_2$ increased the concentration of all forms of Ca in the tuber peel (i.e. soluble Ca, calcium oxalate, bound Ca in the cell wall, and the total Ca) (Figure 8a, 8b, 8c, 8d). This is consistent with previous reports with potatoes, apples and *Zantedeschia* (McGuire & Kelman, 1984; Conway *et al.*, 1993; Loh *et al.*,1992) which found that vacuum infiltration as an effective means of enhancing tuber Ca concentration. Vacuum infiltration at 7.5% $CaCl_2$ solution does not provide evident increase in all forms of Ca (i.e. soluble Ca, calcium oxalate, bound Ca in the cell wall and total Ca) in the tuber peel tissue just after planting. The Ca concentration was assumed that after pre-plant application of vacuum infiltration, the accumulated Ca initially localized in the periderm was progressively moved into the medulla. This result was supported by the study of Arteca (1982) in potato where active assimilation process involved in potato tubers following vacuum infiltration.

Same pattern of change were observed in soluble Ca and the total Ca both in the tuber peel tissue (Figure 8a, 8d) and matured leaves (Figure 8e, 8h). Soluble Ca in the tuber peel of *Zantedeschia* was found to equate to approximately 10% of the total Ca, from planting up to the last harvest. This is in contrast with the fruit of apple and kiwifruit where it comprised 76.0% and 76.1% of the total Ca concentration, respectively (Ferguson *et al.*,1980).

Once planted, vacuum infiltration increased two fold on the total Ca concentration which was almost similar with that of the previous study (refer Chapter 2). In the case of Clark & Boldingh (1985) study, they illustrated that accumulation of Ca at its maximum increased almost 3 fold while in Loh *et al.* (1992) study in vacuum infiltration an increased of approximately four fold.

Soluble Ca concentration in matured leaves in *Zantedeschia* in this experiment was approximately 4% of the total Ca. This finding was supported by the study of Clark *et al.* (1986) in kiwifruit leaves, but in contrast with the study of Ferguson *et al.* (1980) in apple leaf. This differences in Ca concentration in plants is under genetic control (Horak & Kienzl, 1971). Result of this study suggested that further work has to be recommended to be undertaken with same $CaCl_2$ solution and other cultivars of *Zantedeschia.*

3.6. Conclusion

From this experiment it was concluded that,

1 Different forms of Ca in the tuber of *Zantedeschia* (i.e., soluble Ca, calcium oxalate, bound Ca in the cell wall, and total Ca) was increased following vacuum infiltration with 7.5% $CaCl_2$ but does not maintain in the whole duration of growth.

2 Vacuum infiltration does not provide any changes in concentration of different forms of Ca (i.e., soluble Ca, calcium oxalate, bound Ca in the cell wall, and total Ca) matured leaves.

3 The continuous increase of tuber peel Ca bound in the cell wall concentration is not due to the increase of the total Ca concentration.

4 The decline in the calcium oxalate of the mature leaves is not due to the decrease of total Ca concentration.

5 The decline in the soluble Ca in mature leaves at 84 days was not due to the decline in the total Ca concentration.

3.7. References

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Appendix A

Rates of fertilizer applied to treatment growing media

Fertilizers	Treatments	
	Control	9 kg/m^3 gypsum
Agricultural lime	3.0 kg/m^3	3.0 kg/m^3
Dolomite	3.0 kg/m^3	3.0 kg/m^3
Iron Sulfate	0.5 kg/m^3	0.5 kg/m^3
Osmocote 15-4.8-10.8	1.5 kg/m^3	1.5 kg/m^3
Osmocote 16-3.5-10	3.0 kg/m^3	3.0 kg/m^3
IBDU	1.0 kg/m^3	1.0 kg/m^3
Gypsum ($CaSO_4.2H_2O$	0 kg/m^3	9.0 kg/m^3

Appendix B

Influence of gypsum application on the available calcium concentration (mg/g) in the growing media over time.

Treatment	Duration of growth (days)									
	14	28	42	56	70	84	98	112	126	140
9 kg/m^3 gypsum	13.08***	14.01***	14.71***	12.99*	11.98****	14.91***	15.53***	14.41***	14.13**	14.14***
0 kg/m^3 gypsum	4.33	5.04	5.88	4.94	4.59	5.17	4.96	5.31	5.45	5.90
s.e.	3.09	3.17	3.12	2.85	2.61	3.44	3.74	3.22	3.07	2.91

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix C

Influence of gypsum application on the calcium concentration (mg/g) of the tuber peel tissue over time.

Treatment	Duration of growth (days)										
	0	14	28	42	56	70	84	98	112	126	140
9 kg/m^3 gypsum	7.86^{ns}	11.52^{ns}	12.95*	13.76^{ns}	16.09*	17.03^{ns}	17.42*	14.94^{ns}	9.64^{ns}	9.75^{ns}	8.38*
0 kg/m^3 gypsum	9.06	9.96	9.26	12.46	14.59	15.25	16.25	11.05	8.38	9.11	6.97
s.e.	0.42	0.55	1.31	0.46	0.53	0.63	0.41	1.38	0.45	0.23	0.50

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix D

Influence of gypsum application on calcium concentration (mg/g) of the matured leaves over time.

Treatment	Duration of growth (days)						
	56	70	84	98	112	126	140
9 kg/m^3 gypsum	10.76^{ns}	11.42**	11.03^{ns}	12.80^{ns}	14.89*	14.97*	14.72^{ns}
0 kg/m^3 gypsum	8.14	9.06	9.06	9.34	12.07	13.11	13.67
s.e.	0.83	0.84	0.70	1.22	0.10	0.66	0.37

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix E

Influence of gypsum application on tuber dry weight (g) over time.

Treatment	Duration of growth (days)										
	0	14	28	42	56	70	84	98	112	126	140
9 kg/m^3 gypsum	2.5^{ns}	2.5^{ns}	2.2^{ns}	1.7^{ns}	0.9^{ns}	0.9^{ns}	1.6^{ns}	1.6^{ns}	4.3^{ns}	10.5^{ns}	12.8^{ns}
O kg/m^3 gypsum	2.6	2.5	2.1	1.7	1.0	0.8	1.2	2.3	5.9	6.9	16.8
s.e.	0.21	0.02	0.04	0.00	0.49	0.39	0.88	0.23	0.54	1.26	1.38

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix F

Influence of gypsum application on leaf area (cm^2)
of matured leaves over time

Treatment	Duration of growth (days)						
	56	70	84	98	112	126	140
9 kg/m^3 gypsum	83.07^{ns}	115.49^{ns}	218.01^{ns}	272.98^{ns}	469.06^{ns}	580.00^{ns}	504.03^{ns}
0 kg/m^3 gypsum	69.59	120.67	284.55	337.23	453.24	435.90	553.43
s.e.	4.77	1.83	23.52	22.72	5.57	50.95	17.47

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix G

Influence of gypsum application on the EC of the growing medium over time.

Treatment	Duration of growth (days)										
	0	14	28	42	56	70	84	98	112	126	140
9 kg/m^3 gypsum	3.08***	1.68****	3.71****	3.75***	3.29**	3.01**	2.97**	2.69***	3.22**	2.90**	2.92**
0 kg/m^3 gypsum	1.71	0.54	1.27	1.42	1.33	2.01	1.67	1.37	1.39	1.52	1.07
s.e.	1.25	0.58	1.22	1.17	0.98	0.50	0.65	0.66	0.92	0.69	0.93

^{ns,*,**,***} non significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=4

Appendix H

Influence of gypsum application on the pH of the growing medium over time.

Treatment	Duration of growth (days)										
	0	14	24	42	56	70	84	98	112	126	140
9 kg/m^3 gypsum	6.89^{ns}	6.09^{ns}	6.10*	5.49^{ns}	5.16*	5.15^{ns}	5.55^{ns}	5.07^{ns}	5.26^{ns}	5.36^{ns}	5.34^{ns}
0 kg/m^3 gypsum	6.66	6.35	6.39	5.15	5.33	5.07	5.66	5.30	5.40	5.55	5.18
s.e.	0.10	0.10	0.10	0.01	0.06	0.03	0.04	0.08	0.50	0.07	0.17

^{ns,*,**,***} non significant or significant at $P \leq 0.05$, 0.01, 0.001, respectively n=4

Appendix I

Soluble calcium concentration (mg/g) of tuber peel following vacuum infiltration

Treatment	Duration of growth (days)							
	Before Treatment (BT)	Post Vacuum (PV)	Before Planting (BP)	28	56	84	112	140
7.5% $CaCl_2$	0.43 ^{ns}	4.73***	3.31***	0.67^{ns}	0.57^{ns}	0.83^{ns}	0.61^{ns}	0.61^{ns}
0% $CaCl_2$	0.34	0.71	0.76	0.66	0.69	0.71	0.65	0.60
Control	0.50	0.39	0.65	0.57	0.59	0.70	0.62	0.56
s.e.	0.04	1.14	0.71	0.03	0.03	0.03	0.01	0.01

^{ns,*,**,***} no significant, significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix J

Calcium oxalate concentration (mg/g) of the tuber peel following vacuum infiltration

Treatment	Duration of growth (days)							
	Before Treatment (BT)	Post Vacuum (PV)	Before Planting (BP)	28	56	84	112	140
7.5% $CaCl_2$	3.03	6.81^{***}	4.56^{**}	2.83^{ns}	5.38^{**}	4.75^{*}	3.35^{ns}	2.51^{ns}
0% $CaCl_2$	2.72	2.37	2.59	2.86	4.06	4.33	3.35	2.73
Control	1.58	2.89	1.49	2.39	3.26	4.22	3.42	2.78
s.e.	0.36	1.14	0.73	0.12	0.50	0.13	0.06	0.07

^{ns,*,**,***} not significant, significant at $P \leq 0.05$, 0.01, 0,001, respectively n=8

Appendix K

Bound calcium concentration (mg/g) of tuber peel following vacuum infiltration

Treatment	Duration of growth (days)							
	Before Treatment (BT)	Post Vacuum (PV)	Before Planting (BP)	28	56	84	112	140
7.5% $CaCl_2$	$(2.15)^{W\ ns}$	4.82^{**}	5.02^{**}	2.28^{ns}	3.76^{ns}	3.07^{ns}	3.93^{ns}	4.26^{ns}
0% $CaCl_2$	(1.95)	2.98	3.10	1.89	2.60	3.85	4.19	4.26
Control	(2.45)	2.50	2.74	2.13	2.83	3.67	3.72	4.57
s.e.	0.12	0.58	0.58	0.09	0.29	0.19	0.11	0.08

^{ns,*,**,***} not significant, significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix L

Total calcium concentration (mg/g) of tuber peel following vacuum infiltration

Treatment	Duration of growth (days)							
	Before Treatment (BT)	Post Vacuum (PV)	Before Planting (BP)	28	56	84	112	140
7.5% $CaCl_2$	5.61	16.35^{***}	12.89^{***}	5.76^{ns}	9.71^{ns}	8.66^{ns}	7.89^{ns}	7.38^{ns}
0% $CaCl_2$	5.02	6.06	6.44	5.37	7.34	8.88	8.01	7.60
Control	4.53	5.78	4.87	5.09	6.68	8.59	7.76	7.91
s.e.	0.25	2.84	2.00	0.16	0.75	0.07	0.06	0.13

$^{ns, *, **, ***}$ not significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix M

Soluble calcium concentration (mg/g) of mature leaves following vacuum infiltration

Treatment	Duration of growth (days)			
	56	84	112	140
7.5% $CaCl_2$	0.35^{ns}	0.46^{ns}	0.58^{ns}	0.76^{ns}
0% $CaCl_2$	0.42	0.39	0.65	0.95
Control	0.54	0.35	0.62	0.85
s.e.	0.05	0.03	0.02	0.04

$^{ns, *, **, ***}$ not significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix N

Calcium oxalate concentration (mg/g) of mature leaves following vacuum infiltration

Treatment	Duration of growth (days)			
	56	84	112	140
7.5% $CaCl_2$	4.87^{ns}	5.76^{**}	5.63^{ns}	5.62^{ns}
0% $CaCl_2$	3.31	4.43	6.24	5.50
Control	4.39	5.24	6.24	6.28
s.e.	0.38	0.32	0.17	0.20

$^{ns, *, **, ***}$ not significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix O

Bound calcium concentration (mg/g) of mature leaves following vacuum infiltration

Treatment	Duration of growth (days)			
	56	84	112	140
7.5% $CaCl_2$	4.14^{ns}	7.28^{ns}	11.67^{ns}	16.47^{**}
0% $CaCl_2$	5.41	7.28	11.69	15.93
Control	4.42	7.99	9.49	12.12
s.e.	0.31	0.32	0.60	1.12

$^{ns, *, **, ***}$ not significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8

Appendix P

Total calcium concentration (mg/g) of matured leaves following vacuum infiltration

Treatment	Duration of growth (days)			
	56	84	112	140
7.5% $CaCl_2$	9.35^{ns}	14.86^{**}	17.88^{ns}	22.85^{ns}
0% $CaCl_2$	9.14	12.10	18.58	18.57
Control	9.35	13.58	16.34	23.06
s.e.	0.06	0.65	0.54	1.19

$^{ns, *, **, ***}$ not significant or significant at $P \leq 0.05, 0.01, 0.001$, respectively n=8