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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.