EFFECT OF PLANT GROWTH REGULATORS ON THE POSTHARVEST HANDLING OF TUBEROSE (*Polianthes tuberosa* L.) CUT FLOWERS

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BY

DANIEL KIPROP CHEBET

CROP SCIENCE DEPARTMENT

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

DECLARATION

This thesis is my original work and has not been presented
for a degree in any university

Chebtei   28th October 1999

DANIEL KIPROP CHEBET   DATE

This thesis has been submitted for examination with our approval
as the university supervisors

MHutch   28th OCT 1999

Dr. M. J. HUTCHINSON   DATE

                                  9th November 1999

Dr. V. E. EMONGOR   DATE

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ACKNOWLEDGEMENTS

DEDICATION

THIS THESIS IS DEDICATED TO MY PARENTS

ENOCK CHERUTICH CHEBET

and

CHRISTINE SOTE CHEBET

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LIST OF ABBREVIATIONS

ABA	-	Abscisic acid
ACC	-	1- Aminocyclopropane -1- carboxylic acid
ATP	-	Adenosine triphosphate
AVG	-	Aminoethoxyvinylglycine
BA	-	Benzyladenine
CEPA	-	Chloroethylphosphonic acid
DNA	-	Deoxyribonucleic acid
EFE	-	Ethylene Forming Enzyme
GA	-	Gibberellic acid
HQS	-	8-Hydroxyquinoline sulphate
IPA	-	Isopentenyl adenosine
NAA	-	Naphtalene acetic acid
NaOCl	-	Sodium hypochlorite
RNA	-	Ribonucleic acid
STS	-	Silver thiosulphate

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ABSTRACT

The study was conducted to investigate the influence of Accell™ (containing benzyladenine and gibberellic acid at ratio 10:1), benzyladenine, gibberellic acid, sucrose, silver thiosulphate and Florissant 500™ on the longevity and percentage of floret opening, rate of water uptake, transpiration water loss and dry weight changes and the rate of accumulation and subsequent decline in sucrose and reducing sugars of tuberose (*Polianthes tuberosa* L.) cut-flower

Accell™ at 25 and 50 ppm increased the vase-life by 3 and 2 days and increased floret opening by 11% and 9% respectively.. However, Accell™ at 75 and 100 ppm did not affect the vase-life and floret opening of tuberose cut-flowers. There was no significant difference in vase-life and floret opening between Accell™ and the equivalent BA concentrations.

Pulsing tuberose cut-flowers in 10% sucrose for 24 hours before holding in solution containing 0.5% NaOCl and 150 ppm citric acid increased the vase-life by 4 days and floret opening by 13%. Similarly, pulsing in 10% sucrose for 24 hours before holding in 25, 50, 75 and 100 ppm Accell™ increased the vase-life by 7, 6, 4 and 2 days and floret opening by 22%, 20%, 16% and 10%, respectively. However, pulsing tuberose cut-flowers in 10% sucrose increased the efficiency of only the 25 and 50 ppm Accell™ as the net increase in vase-life and floret opening was higher, in relation to the sucrose treatment alone.

Pretreatment of tuberose cut-flowers with 2.0 mM STS for 1 hour increased the vase-life by 7 days and floret opening by 26%, while Florissant 500™ and gibberellic acid ($GA_{4,7}$) at 2.5, 7.5 and 10 ppm had no effect on vase-life and floret opening of tuberose cut-flowers.

The rate of water uptake and transpiration water loss of tuberose cut-flowers was increased by 25, 50 and 75 ppm Accell™ treatments. There was no significant difference in the rate of water uptake and transpiration water loss between Accell™ and the equivalent BA concentrations. 10% sucrose pulse increased water uptake and initially decreased transpiration but later had no effect on transpiration. Similarly, STS pretreatment increased water uptake but had no effect on transpiration. However, Accell™ at 100 ppm, $GA_{4,7}$ at 2.5, 7.5 and 100 ppm and Florissant 500™ had no effect on water uptake and water loss.

Pulsing tuberose cut-flowers in 10% sucrose increased the dry weight as well as the accumulation of reducing sugar in the petals and slowed the rate of depletion of the substrates. Holding tuberose cut-flowers in 25 and 50 ppm Accell™ and pretreatment with STS also delayed

the rate of reduction in dry weight and reducing sugar while the rate of reduction in tuberose cut-flower held in 75 and 100 ppm Accell,[TM] Florissant 500[TM] and $GA_{4,7}$ was similar to that of untreated cut-flowers. All the preservatives used however did not affect the amount of sucrose accumulating in the petals of the florets.

The results of this study indicated that holding tuberose cut-flower in Accell[TM] at 25 and 50 ppm or BA equivalents, or pulsing in 10% sucrose and 2.0 mM STS promoted longevity and floret opening while Accell[TM] at 75 and 100 ppm, $GA_{4,7}$ at 2.5, 7.5 and 10.0 ppm and Florissant 500[TM] had no effect.

1.0 INTRODUCTION

1.1 General overview of the horticulture industry in Kenya.

Kenya's horticultural industry has gained rapid growth over the years, to become one of the leading foreign exchange earners (HCDA, 1997). The foreign exchange earnings have increased in value from Kshs. 10.3 million in 1970 to Ksh. 7.7 billion in 1996 and increased in weight from 1,477,682 Kgs. in 1970 to 84,823,640 Kgs. in 1996 (HCDA, 1996). There has also been a rapid expansion in cut-flower exports, increasing in value from Ksh 502 million to Ksh. 4.4 billion and increasing in volume from 8,613,000 Kgs. in 1987 to 35,212,000 Kgs. in 1996 (HCDA, 1996). The floriculture exports thus represented 57% in value and 41% in volume of all horticultural exports in 1996 (HCDA, 1996). Apart from earning foreign exchange to the country, the industry generates employment and increases the per capita income of the farmers (HCDA, 1996)

Initial flower production in Kenya started with spray carnation and statice (HCDA, 1996). However, recent industry expansion has led to production of other high value crops including *Alstroemeria*, buplearum, carthamus, eryngium, gypsophila, hypericum, lilies, lisianthus mollucela, roses, solidago, and tuberose, among other cut-flowers (HCDA, 1996). Kenya's primary markets for cut-flower exports include the United Kingdom (30%), Holland (28.9%), France (16.1%), and Germany (10.2%) among others (HCDA, 1995). Despite a strong presence in these markets, Kenya faces stiff competition from Israel, South Africa, Zimbabwe, Thailand and Spain (HCDA, 1995).

Other problems facing Kenya's floriculture industry include: poor quality seed, inadequate planting materials or lack of new varieties, lack of appropriate technology, lack of cold storage facilities, lack of proper marketing channels, poor cultural practices, diseases and pests, inadequate irrigation facilities, low soil fertility, non-availability of trained personnel, low priority and inconsistent research and poor post harvest handling (Wabule *et al.*, 1991).

1.2 Tuberose botany

Tuberose (*Polianthes tuberosa* L.) belongs to the family Agavaceae (Bailey, 1961). The plant was cultivated in Mexico before the Spanish conquest of 1522 (Bailey, 1961). It was then used

medicinally to treat fever, tumours, diarrhoea and rash (Trueblood, 1973). Presently, tuberose is used as a cut-flower and for the extraction of essential oils derived from the flowers, which are used in India and France for the production of perfumes (Heywood, 1982).

Tuberose plant is a herbaceous perennial with short, thick rhizomes and thickened roots (Bailey, 1961). It grows to a height of 0.5 to 1.0 m (Bailey, 1961). The leaves are clasping, grasslike and mostly basal and are 4-6 cm in length and 1.5 cm wide (Bailey, 1961). The spike has 8-12 leaves (Bailey, 1961). Spikes have single, semidouble or double florets, which are all white in colour (Sambandamurthi and Appavu, 1980). The florets open progressively upwards within the racemose spike and are waxy and brittle in texture (Bailey, 1961). The perianth is also white, narrowly funnel shaped and curved (Bailey, 1961). Stamens are affixed at the middle of the tube and the ovary is 3-celled, free at the apex while the stigmas are oval (Bailey, 1961). The spikes are usually harvested when the lowest one or two florets are open (Watako, 1992).

1.3   Importance of the study.

Commercial production of tuberose around the world has recently increased tremendously since it can be grown in the field under minimal management practices (Armitage and Laushman, 1990). Further expansion may however, be hampered by the poor postharvest life of the tuberose inflorescence (Naidu and Reid, 1989). Poor postharvest handling account for 30-40% of the total losses incurred in cut flowers (HCDA, 1997). The principal postharvest problem in tuberose is the premature yellowing, then browning and abscission and/or abortion of the distal florets and short vase-life of the remaining inflorescence. This makes the flower to be of low value, relative to other flowers, as evidenced by a volume of 48,617 kilograms exported in Kenya in 1995 over a total of 29,376.862 kilograms of cut-flowers exported at the same period (HCDA, 1995). Indeed, Muhuhu (1991) on a paper outlining on the areas of floriculture to be considered for future research in Kenya recommends an intensive study to establish the best preservatives to be used in tuberose postharvest handling to counteract the short vase-life of the crop.

The short vase-life of tuberose cut-flower could be caused by unfavourable water balance or substrate limitations. Senescence could also be accelerated by a high sensitivity to ethylene or a rapid depletion of antagonists of ethylene synthesis and/or action. Previous attempts to achieve a complete (100%) flower opening and consequently longer vase-life of tuberose cut-flower with

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sucrose and silver thiosulphate (STS) (Naidu and Reid 1989; Watako 1992) have been unsuccessful. The present study was therefore an attempt to enhance the postharvest handling of tuberose cut-flowers using other plant growth regulators especially cytokinins which are currently receiving much attention as playing a bigger role in the postharvest physiology of cut-flowers than originally anticipated (Hutchinson, 1996).

1.4   Objectives of the study

The overall objective of the study was to determine the effect of various plant growth regulators on the postharvest physiology of tuberose (*Polianthes tuberosa* L.) cut-flower and to investigate their possible mode(s) of action. The specific objectives were to determine the effect of benzyladenine, sucrose, silver thiosulphate, gibberellic acid (GA_{4-7}) and Florissant 500^{TM} on: -

i) Vase-life and flower bud opening of tuberose cut-flower
ii) Water uptake, transpiration water loss and dry weight changes of tuberose cut-flower
(iii) Sucrose levels of tuberose cut-flowers.
(iv) Reducing sugar levels of tuberose cut-flowers.

2.0 LITERATURE REVIEW

2.1 Flower Senescence

Senescence refers to those processes that follow horticultural or physiological maturity and leads to death of tissues (Watada *et al.*, 1984). The process of senescence is genetically programmed, involving the regulated expression of specific genes and is regulated by plant hormones (Halevy and Mayak, 1979) but can be hastened by stress factors such as water stress (Borochov *et al.*, 1976).

Flower senescence entails processes leading to cell disorganisation. It is characterised by a decline in RNA (Matile and Winkenbach, 1971), proteins (Borochov *et al.*, 1976; Hobson and Nichols, 1977), phospholipids and other macromolecules (Beutelmann and Kende, 1977), enhanced respiration (Baker, 1983); increased activity of enzymes (Baumgartner, *et al.*, 1975); ethylene production (Burg, 1962; Lieberman *et al.*, 1974; Nichols, 1968); increased microviscosity and permeability of membranes leading to ion leakage and water loss (Borochov *et al.*, 1978; Van Meeteren, 1979).

Much effort has been put to delineate the physiological processes involved in senescence. Plant hormones have been proposed to play key roles in the many processes associated with senescence (Halevy and Mayak, 1979). Out of the group of known plant growth hormones (auxins, ethylene, cytokinins, gibberellic acid and abscisic acid) ethylene has received the greatest attention such that ethylene is sometimes refered to as the 'senescence hormone'.

Recent advances in science have however, indicated that the action of the various hormones are interrelated and so the possible role of other hormones in such a key role as senescence has become a possible reality (Hutchinson, 1996). The group of plant hormones currently receiving attention as possibly playing key roles during senescence are gibberellins and cytokinins.

2.2 Role of Gibberellins on cut-flower senescence

Various researchers have reported different effects of gibberellins (GAs) on senescence of cut-flowers. Gibberellic acid treatment resulted in a slight increase in longevity in carnations (Goszczynska and Nowak, 1979). Similarly, Garrod and Harris (1978) reported that application of

200 ppm GA₃ to isolated carnation petals growing on agar or in liquid medium delayed senescence. In *Matthiola incana* spikes, Aarts (1957) reported that 1 ppm GA₃ extended longevity with and without sucrose. Treatment with GAs was also reported to inhibit chlorophyll loss and increased the vase-life of lilies (Nowak and Mynett, 1985). However, little or no effect, or even harmful effect of GAs have also been reported.

Little or no effect on longevity was reported by treatment of carnation flowers in 0.1 to 200 ppm GA₃ solutions (Nichols, 1968; Wong *et al.*, 1989). In opening solutions, GA₃ at 100 to 400 ppm promoted opening but decreased longevity and caused discolouration of flowers (Cywinska-Smother *et al.*, 1978). In chrysanthemum, 1-9 ppm GA₄₇ and silver nitrate with or without sucrose and GA₄₇ alone was not efficient in extending the vase-life (Garibaldi and Dearmborgia, 1988). In orchids, GA₃ at higher concentrations induced the post-pollination phenomenon, which is characterised by stigmatic closure, swelling and loss of the curvature of the column, wilting of the perianth, deformation of the calli and anthocyanin production (Arditti *et al.*, 1971). Gibberellic acid treatment was also not beneficial in gladiolus (Nunes 1989), *Liatris spicata* (Perez *et al.*, 1985), and hybrid *Limonium* (Doi and Reid, 1995). The mode of action of GAs during postharvest physiology of cut-flowers remains unclear, but among other speculations, one is that plant phytohormones are closely interrelated and so GAs could be mediating it's own response or those of other phytohormones (Hutchinson, *et al.*, 1997).

The influence of GAs on senescence of tuberose cut-flower has not been investigated

2.3 Role of cytokinins on flower senescence

Cytokinins are defined as substituted adenine compounds that promote cell division in tobacco pith, carrot phloem or similar assay systems grown on an optimally defined medium (Salisbury and Ross, 196). Cytokinins have a side chain rich in carbon and hydrogen, which is attached to the nitrogen protruding from the top of the purine ring (Salisbury and Ross, 1986). Cytokinins can either exist in the free base form or as a nucleoside, in which ribose group is attached to the nitrogen atom at position 9 (Salisbury and Ross, 1986). Nucleosides can also be converted to nucleotides in which sulphate is esterified to the 5-carbon of ribose (Salisbury and Ross, 1986). It is likely that cytokinins are widespread, if not universal, in the plant kingdom, but very little is known of their functions, except in angiosperms, some conifers and mosses (Salisbury and Ross, 1986).

Kinetin, a very active compound formed by the partial breakdown of aged or autoclaved herring sperm DNA was the first cytokinin to be discovered (Miller, 1954). However, it has not been found in plants but related cytokinins are found in most plants.

Using tissue culture techniques in coconut milk, Steward *et al.* (1974), found several cytokinins that enhance cell division in carrot tissues. The most active of these cytokinins were shown by Letham (1974) to be compounds previously given the names zeatin and zeatin riboside. Zeatin had first been identified by Miller (1954) who used the milky endosperm of corn (*Zea mays*) as a source. Since then, other cytokinins with adenine-like structure similar to kinetin and zeatin have been identified, including dihydrozeatin, 2-ip isopentenyl adenine (IPA) and benzyladenine (Salisbury and Ross, 1986). Although benzyladenine is a synthetic cytokinin, it is believed that it might be present in some species because its nucleoside derivative, 6-benzyladenine riboside was found to occur in anise (*Pimpinella anisum*) cells (Ernst *et al.*, 1983).

Cytokinins have been found to play diverse roles in plant physiology. Cytokinins promote lateral bud development in dicots, increase cell expansion in dicot cotyledons and leaves, promote chloroplast development and chlorophyll synthesis, increase nutrient sink activities and delay senescence in plant tissues (Salisbury and Ross, 1986).

Exogenous application of cytokinins delays senescence in various flowers. A short-term (2 min at 225 ppm or 12 hrs. at 22.5 ppm) treatment of carnation stems with BA increased the vase-life by about 30% compared to untreated flowers (Heide and Oydvin, 1969). Similarly, Goszczynska and Nowak (1979) demonstrated that pulsing carnation buds with BA before cold storage caused a slight acceleration of bud opening. Application of BA also increased the petal area and increased the number of primary petals produced (Jeffcoat, 1977). Kinetin delayed senescence in carnation flowers with leaf and stem tissue removed and also in intact flowers (Eisinger, 1977). Further evidence of cytokinin enhancement of the vase-life of carnation cut-flowers was provided by Cook *et al*, (1985), Cywinska-Smother *et al.*, (1978), Kelly *et al.*, (1985), Mayak and Dilley (1976a), Paulin and Muloway (1979), Staden and Bosse (1989), Upfold and Staden (1990) and Wong *et al.*, (1989).

In iris, Wang and Baker (1979) showed that 0.1 mM isopentenyl adenosine (iPA) increased the vase-life by about 24%. Iris cut-flowers also failed to open after dry storage but treatment with kinetin induced opening (Swart, 1986). Similarly, injection of BA (De Munk and Gijzenberg, 1977) and zeatin (Vonk *et al.*, 1986) into iris flower buds prevented floral blasting induced by insufficient

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light

In daffodils, Ballantyne, (1966) reported that BA retarded senescence in flowers that had been stored for two weeks but was ineffective in fresh cut-flowers. In *Alstroemeria*, zeatin riboside and 100 mg/l BA increased the time to 50% shedding by 22% relative to the control (Dai and Paull, 1991). In roses, holding both leafy and leafless flowers continously in 20 or 60 mg/l kinetin delayed fading (Mayak and Halevy, 1974). In *Leucospermum*, Napier *et al.*, (1986) reported that flower quality was improved by 200 mg/l BA while in gladiolus, cytokinin stimulated bud opening and delayed senescence (Wong *et al.*, 1989).

The possibility of cytokinins actually playing a regulatory role in cut-flower senescence has been given by the analysis of the change in the endogenous levels of cytokinins during senescence. The endogenous levels of cytokinins in rose petals decreased as the flower aged (Mayak and Halevy, 1970). Likewise, the endogenous cytokinin content of a long-lived variety of roses was higher than in a short-lived variety (Mayak and Halevy, 1970). However, exogenous application of BA to buds of the short-lived variety delayed its senescence (Mayak and Halevy, 1970). Similarly, the development of *Gerbera* plants both on the plant and on the vase was accompanied by a decrease in the cytokinin activities of petal extracts from day zero (commercial stage of harvest) (Van Meeteren and Van Gelder, 1980). Evidence for the reduction of the endogenous cytokinins have also been presented by Even-Chen *et al.*, (1978), Hewett and Wareing (1973) and Oritani and Yoshida (1973). However, the possible theories on the actual role of cytokinins in regulating cut-flower senescence have not been established. The cytokinin enhancement of cut-flower vase-life could be attributed to the promotion of a favourable water balance, increment in the flower nutrient status or to the relationship with other plant hormones, especially ethylene.

2.3.1 Role of water relations in cut-flower senescence

The opening and development of a flower is an active growth process which only occurs when the flower cells are in a fully turgid state. Turgidity is achieved when water is retained in the flower tissue This occurs when the rate of water uptake exceeds transpiration loss.

After flowers are cut and placed in water, they exhibit changes in fresh weight. Typically, cut-flowers initially increase and subsequently decrease in fresh weight (Rogers, 1973). Water uptake and water loss may fluctuate cyclically with an overall declining trend (De Stigter, 1980; Mayak *et*

al, 1974) Whenever the amount of transpiration exceeds uptake, water deficit occurs. The main reason for the deficit is the resistance to water flow which develops in the stem. This resistance to water flow can be attributed to several factors.

Micro-organisms are considered to be one of the main causes of reduced water uptake by cut flowers. Microbial growth paralleled the increase in stem resistance to water flow (Larsen and Florich, 1969) and germicides which controlled microbial growth partially decreased the resistance to water flow (Burdett, 1970; Marousky, 1969; Van Meeteren, 1978). An increase in water deficit was also observed when bacterial filtrate devoid of bacteria was added to the holding solution (Accati *et al.*, 1981; Mayak and Accati-Garibaldi, 1979) indicating that bacteria affects longevity not only by plugging the vessels but also by secreting metabolites which directly affect water balance. Stem blockage however, is not wholly dependant on microbes as cut roses held in sterile water also had reduced rates of water uptake (Marousky, 1969).

Water deficit in cut-flowers could also be attributed to vascular blockages induced from harvesting injury (Burdett, 1970; Durkin and Kuc, 1969; Marousky, 1971). The vascular occlusions were induced from oxidative processes and were described as gummy substances (Aarts, 1957), pectinaceous or carbohydrate in nature (Burdett, 1970; Parups and Molnar, 1972) or to be composed of breakdown products of cell walls (Rasmussen and Carpenter, 1974).

Vascular occlusions could also be due to ethylene-stimulated production of gums at the cut surfaces. The cut surface of rose stems has been found to produce considerable amount of ethylene (Van Doorn *et al.*, 1980). Cutting of *Anthurium* stems during harvest or recutting later has been found to induce the production of ethylene (Paull and Goo, 1985). Similarly, ethephon treatment of *Prunus cesarus* resulted in the blockage of the xylem vessels (Olien and Bukovac, 1982). Likewise, a substantial production of wound ethylene has been reported in cut maidenhair stripe segments and a marked increase in vase-life was achieved by holding the tissues in solutions containing Amino oxyacetic acid (AOA) or cobalt ions, which inhibit ethylene biosynthesis in plant tissues (Fujino *et al.*, 1981). Ethylene-induced vascular plugs have also been reported in *Ricinus communis* (Vandermolen *et al.*, 1983).

Water deficit in cut-flowers could also be attributed to the disruption of water columns by air embolism (Crafts, 1968). Air entering the base of cut stems has been recognised as a major factor hindering the rehydration of flowers and Durkin (1979) has shown that removing air from water by vacuum increases water flow rates through rose stem sections.

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Stem blockage may however not be the only cause of water deficit in cut-flowers. Detailed anatomical studies reveal quite variable levels of xylem element blockage from less than 4% (Rasmussen and Carpenter, 1974) to 11% (Burdett, 1970) to 20% (Lineberger and Steponkus 1974) in senescent rose stems.

The development of water deficit in cut-flowers could also be caused by a reduction in the water holding capacity of the flower tissues due to physiological changes associated with senescence at the cellular level (Van Meeteren, 1978, 1979). In *Tradescantia reflexa*, the first visible feature demonstrating the initiation of fading of the flowers is the infiltration of the cell sap into the intercellular spaces (Horie, 1962). An increased leakage of ions and water from the cells with ageing due to an increase in membrane permeability has also been reported in carnations (Nichols, 1968), *Gerbera* (Van Meeteren, 1978) and roses (Sacalis, 1975).

The increase in membrane permeability could be stimulated by ethylene. Exposure of cut carnation flowers to a short-term (12 hrs) water stress caused an increase in 1-Aminocyclopropane-1-carboxylic acid (ACC) content and ethylene synthase activity followed by ethylene production (Apelbaum *et al*, 1981; Borol7chov *et al*., 1982). Similarly, exposure to 2ul/l ethylene caused an increase in ion leakage from the cells, which coincided with a drop in water uptake (Mayak *et al*., 1977).

Since cytokinins seem to play a key role in senescence, attempts to delineate the possible roles of cytokinins in water relations has yielded various results.

2.3.1.1 Effect of cytokinins on the water balance of cut-flowers

Various researchers have reported that cytokinins play a role in delaying flower senescence by promoting a favourable water balance in cut-flowers. In roses, Mayak and Halevy (1974) reported that kinetin enhanced fresh weight increase in all flower parts and delayed the subsequent reduction in fresh weight by one day. Furthermore, kinetin promoted growth and expansion of the rose petals and maintained the petal turgidity for an extended period of time (Mayak and Halevy, 1974) Application of BA to chrysanthemum flower buds at an early stage of development also increased the fresh weight (Jeffcoat, 1977). Carnation flowers treated with BA did not show a change in fresh weight over a 10-day period while control flowers lost over 60% of their initial weight (Cook *et al*., 1985). The promotion of increased fresh weight by cytokinins was attributed to maintenance of the

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cell integrity through membrane stabilisation (Cook *et al.*, 1985). This is further supported by the findings of Van Meeteren (1978) in *Gerbera* that BA treatment retarded the decrease in the petal water content and also the increase in ion leakage and thus delayed senescence. Kinetin was also reported to increase carnation flowers fresh weight and transpiration and Paulin and Muloway (1979) suggested that since water uptake follows transpiration, increase in transpiration increases the flower fresh weight.

Cytokinins have also been reported to cause stomatal opening in oat leaves (Salisbury and Ross, 1986) and roses (Mayak and Halevy, 1974). Cytokinins may thus slow senescence by allowing carbon dioxide to enter the tissues. Carbon dioxide (CO_2) inhibits the strong promotive action of ethylene on senescence by competing for the ethylene receptor sites (Burg and Burg, 1966). Indeed, Smith and Parker (1966), reported that a concentration of CO_2 as low as 0.4% prevented the deleterious effects of 50 ppb ethylene on carnation flowers. Similarly, Nichols, (1968) reported that 2% to 5% CO_2 counteracted the effects of 0.2 ppm ethylene in promoting premature senescence of carnations.

Cytokinins could also promote a favourable water balance by preventing the ethylene-induced vascular occlusions on the stems thus enabling water uptake to proceed uninterrupted. Cytokinins have been reported to delay ethylene production in carnations (Cook *et al.*, 1985; Mor *et al.*, 1983, Paulin and Muloway, 1985) and *Ipomoea tricolor* (Kende and Hanson, 1976).

The influence of cytokinins on the water balance of other floral crops such as tuberose cut-flowers has not been investigated.

2.3.2 Role of sucrose on flower senescence

Flower tissues accumulate high levels of carbohydrates during their development on the parent plant (Nichols, 1973). This stops at harvest and the rate at which the accumulated sugar is metabolised is one of the factors that determine the cut flower vase-life (Rogers, 1973). One of the critical roles played by sugars in delaying senescence is the maintenance of respiration.

The respiratory behaviour of a developing rose flower from the period of growth to senescence was studied and a decline in respiration during the early stages of flower bud growth, followed by a sharp rise during the period of rapid petal expansion to peak at full bloom was observed (Siegelman *et al.*, 1958). After full bloom, the respiratory rate tended to fall and later, there was a second

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dramatic increase over a relatively short period followed by a final decline (Siegelman et al., 1958). The sharp respiratory peak at full bloom seems to be a common feature in flowers as it has been reported in brodiae (Han et al., 1990), in the outer florets of chrysanthemum (Nakamura and Ito, 1975) and gladiolus (Ferreira et al., 1986). This increase in respiration during the period of rapid petal expansion is attributed to the breakdown of sugars to supply energy and carbon skeletons required for bud opening (Doi and Reid, 1995). The subsequent decline in respiration after flower opening occurs as a result of the depletion of respiratory substrates (Nichols, 1973). Supplying cut-flowers with exogenous sugars therefore maintains the pool of respiratory substrates especially in the petals, thus promoting respiration and extending longevity (Coorts, 1975; Rogers, 1973).

Supplementary sucrose application has been used to promote bud opening and increase the vase-life in a number of flowers. Cut roses that lasted 5 days in water often lasted as long as 14 days in sucrose solution (Sacalis, 1973). The vase-life of carnation CV 'Samantha' cut at bud stage was upto 10 days in 6-8% sucrose and only 5 days in water (Amarintei and Burzo, 1981). Sucrose also promoted opening and delayed senescence in roses (Mayak and Halevy, 1970), standard and spray carnations (Borochov et al., 1975; Kofranek and Halevy, 1972); Gerbera (Borochov et al., 1975), brodiae (Han et al., 1990), Anthurium (Paull and Goo, 1982), Strelitzia (Halevy et al., 1978), Lily-of-the-Nile (Mor et al., 1984), gladiolus (Mayak et al., 1973), freesia (Woodson, 1987), Limonium (Doi and Reid, 1995), gypsophila (Downs et al., 1988, Farnharm, 1975) and tuberose (Naidu and Reid, 1989; Watako, 1992). However, sucrose did not improve the vase-life of Alstroemeria cut-flower as the petals of all cut-flowers of 'Carmen', 'Marina' and 'Pink Perfection' cultivars were shed at the same time as the controls (Chepkairor, 1986). Sucrose treatments applied to narcissus also caused a small improvement in vase-life but resulted in substantial growth of the ovary (Nichols, 1973).

The principle sugars of carnation corolla are reducing sugars and sucrose (Nichols, 1973). However, the ratio of the concentration of sugars depended on the stage at which the flower was cut In narcissus cv 'Actaea' cut at the proper stage of flower development ('goose neck' stage when the bud is reflexed at right angles to the stem) reducing sugars increased to a maximum, roughly coincident with full flower opening and then decreased until half of the maximum amount remained at wilting (Nichols, 1973). Sucrose, the predominant sugar in the corolla of the narcissus buds, disappeared as reducing sugar increased (Nichols, 1973) indicating that inversion of sucrose to reducing sugars may have occurred. Indeed, petal tissues were found to contain high levels of

invertase (Hawker *et al.*, 1976).

The residual pool of reducing sugars at incipient wilting suggests that wilting may not entirely be caused by the depletion of respiratory substrates. Apart from narcissus, substantial amount of sugars at wilting was also reported in senescing carnations (Weinstein, 1957) and roses (Kaltaker and Steponkus, 1974; Sacalis and Chin, 1977). The gradual decline in respiration and decrease in respiration efficiency of rose petals was suggested to result from the progressive inability of the mitochondria to utilise the substrate due to a change in the mitochondria integrity affecting its functional capacity (Kaltaler and Steponkus, 1974). Hence Kaltaler and Steponkus (1974) concluded that the main effect of applied sugar in extending longevity could be to maintain mitochondria structure and functions. However, the effect of sugar on mitochondria may not be a specific effect and may stem from the sugars' general protective effect on membrane integrity (Santarius, 1973). Apart from maintaining respiration and membrane integrity, sugar effect on cut-flower vase-life has been attributed to the maintenance of a favourable water balance.

Gladiolus shoots have been observed to absorb sucrose in increasing quantity with increasing sucrose concentration of up to 40% in the holding solution (Bravdo *et al.*, 1974). Transfer of the gladiolus shoots from the sugar solution to water resulted in increased water uptake, which was dependent upon the amount of sucrose previously absorbed. In tuberose, Watako (1992) reported that sucrose caused an increase in fresh weight and water uptake in relation to untreated flowers. Similarly, Naidu and Reid (1989) reported that a 15% sugar-containing preservative or pretreatment for 15-20 hours with 20% sucrose caused a high solution uptake in tuberose cut-flowers and increased the percentage of open florets and vase-life. Available literature suggest several mechanisms may be involved in sucrose improving water balance.

Sucrose, at 2% to 4% concentration in the holding solution reduced the stomata aperture in the leaves of cut-flowers (Aarts, 1957), thus reducing water loss and improving water retention and solute uptake capacity (De Stigter, 1980; Marousky 1969, 1971). However, the reduction of the stomatal aperture seem not to account for the great increase in water uptake in gladiolus (Bravdo *et al.*, 1974).

The increase in the water uptake by sucrose-treated flowers could also be attributed to the sucrose effect on the osmotic potential of the petal tissues. Initially, the water deficit in sucrose-treated rose flowers was higher than that of control rose cut-flowers held in water (Borochov *et al.*, 1976) since less water was absorbed, presumably due to the lower water potential of the sugar

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solution. At a later stage, most probably after the supplied sugar had reached the flower head, an improvement in water balance was observed (Borochov *et al.*, 1976). Sucrose supplied at concentrations of 5% to 40% as a short-term treatment accumulated in the cells and increased the osmotic potential of the rose petals (Halevy, 1976) thus improving their ability to absorb and retain water and maintain turgidity.

Sugars have also been reported to delay senescence by delaying the onset of ethylene production. A high sucrose concentration in holding solutions (upto 16%) markedly delayed the onset of autocatalytic ethylene production in carnations (Dilley and Carpenter, 1975). Flowers supplemented with sucrose also senesced less rapidly when treated with ethylene than untreated flowers (Mayak and Dilley, 1976b).

In vivo experiments have shown that the activity of ethylene-forming-enzyme (EFE) is affected by the osmotic shock created by a sugar osmoticum (Apelbaum *et al.*, 1981). Sugars also act as scavengers of free radicals (Asada and Kiso, 1973). The catalysis of the conversion of ACC to ethylene depends on the presence of free radicals (McRae *et al.*, 1982). Thus, if sugars are present, the activity of the free radicals and EFE activity would be reduced. Sucrose effect on ethylene production is also linked with CO_2 production since increase in sucrose supply increases the CO_2 production (Aarts, 1957; Dilley and Carpenter, 1975). Carbon dioxide is a competitive inhibitor of ethylene action (Burg and Burg, 1966; Smith and Parker, 1966).

2.3.2.1 Interaction between cytokinins and sugars

The increase in the vase-life of cut-flowers by an interaction of cytokinins and sugars has been widely reported. The vase-life of carnation cut-flowers pretreated with 225 ppm BA for 2 min. or 22.5 ppm BA for 12 hrs. was increased by 30% compared to untreated flowers (Heide and Oydvin, 1969). Addition of 5% sucrose and acetic acid to lower the pH to 3.5 resulted in even greater increase in vase-life than BA alone (Heide and Oydvin, 1969). A 8-day increase in vase-life was also observed in carnation flowers treated with 5 mg/l BA, 5% sucrose and 300 mg/l 8-hydroxyquinoline sulphate (HQS), in relation to flowers treated with 5% sucrose and 300 mg/l 8-HQS (Cook *et al.*, 1985). The longevity of carnations was also enhanced by solutions containing 50 mg/l kinetin and 5-10% sucrose, compared to kinetin and sucrose alone (Mayak and Dilley, 1976a).

The enhancement of vase-life by a combination of sucrose and cytokinins is attributed to an increase in the movement of assimilates into the flower. Injection of high concentration of BA into bulbous plants reduced the growth of the daughter bulbs, accelerated the exhaustion of the mother bulb while strengthening the sink activity of the flower buds (De Mung and Gijzenburg, 1977). In cut-flowers, enhanced movement of assimilates to the flower buds has been reported in a solution of BA and 4% sucrose (Jeffcoat, 1977; Paulin and Muloway, 1979).

Cytokinins may also increase the vase-life by slowing down the reduction in dry weight, which occurs as the flower senesces (Mayak and Halevy, 1974). This is achieved by stabilising respiration thus slowing down the rate of depletion of substrates. Benzyladenine inhibited respiration and retarded senescence in *Anthurium* (Shirakawa *et al.*, 1964), carnation and chrysanthemum flowers (Maclean and Dedolph, 1962).

Cytokinins may also increase the vase-life by maintaining the protein and RNA levels (Ballantyne, 1966). However, the cytokinin enhancement of the dry weight of flowers was attributed to counteracting the ethylene effect on flower development and transport of carbohydrates to the flower buds (Halevy, 1987). Indeed, carnation flowers treated with BA in addition to sucrose and HQC maintained low levels of ACC accumulation and ethylene production (Cook *et al.*, 1985) and when the BA treated flowers eventually senesced, it was not marked by a significant increase in ethylene production.

2.3.3 Role of ethylene on cut-flower senescence

Ethylene has been demonstrated to have diverse effects on plant tissues. Ethylene has been reported to promote flower bud initiation, stimulate flower development, inhibit flower induction or induce abortion of flower buds (Mayak and Halevy, 1980).

Ethylene-induced flower bud abortion was first reported by Zimmerman *et al.* (1931) in roses, lilies, narcissus and tulips. Since then, ethylene-induced flower bud abortion has been reported in lilium 'Enchantment' (Swart, 1980), roses (Goszczynska and Reid, 1985; Reid *et al.*, 1989), tulips (Kinet *et al.*, 1985), Freesia (Spikman, 1987) and iris (Nowak and Rudnicki, 1990). Ethylene also accelerates floret yellowing and/or abscission in snapdragon (Farnharm *et al.*, 1980), delphinium, euphorbia 'scarlet plume' and sweet pea (Nowak and Rudnicki, 1990) and roses (Lukaszewska *et al.*, 1990). Ethylene also cause epinasty, fading and wilting of sepal tips in orchids (Akamize, 1936;

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Nowak and Rudnicki, 1990) and in-rolling of carnation petals (Nichols, 1968).

Other studies indicated that water soluble ethylene releasing compounds such as 2 chloroethylphosphoric acid (CEPA) induced flower bud abortion in many species, including roses and lilium (Zieslin and Halevy, 1976). Ethylene also reduces the number of flowers produced in the inflorescence of various lilium cultivars (Simmonds and Cummings, 1977). Some carnation buds failed to open after 20 - 24 weeks of storage and others had a short vase-life but continuous evacuation of ethylene through hypobaric pressure enabled all buds to open (Goszczynska and Rudnicki, 1982). Similarly, ethephon treatment in carnations inhibited the transport of ^{14}C-metabolites to the flower buds (Moe, 1979). These results indicate that the natural balance between growth substances controls flower bud development and led Halevy (1987) to conclude that ethylene weakens while cytokinins and GAs strengthens the sink activity of the flower buds

Ethylene induces senescence in plants through its effects on various physiological and biochemical processes. Ethylene induces cell membrane disintegration, especially in the tonoplasts, leading to uncontrolled mixing of vacuole content with cytoplasm, where the ethylene generating system is located (Kende and Baumgartner, 1974; Matile and Winkenbach, 1971). Other effects include increased respiratory activity, increased cell permeability, loss of cell compartmentalisation and alteration of auxin transport and metabolism (Pratt and Goeschl, 1964). Ethylene also promotes the translocation and accumulation of sugars and inorganic materials to the ovary accompanied by the loss of fresh weight and dry weight of the petals (Nichols, 1976).

The auto-stimulation of ethylene production occurring in senescence will be initiated once the sensitivity to ethylene has changed to respond to existing ethylene. The sensitivity to ethylene can result from an intricate, complex interaction between factors such as osmotic potential of petal tissues (Mayak et al, 1978), carbohydrate reserves (Mayak and Dilley, 1976a) and plant hormones such as abscisic acid (Mayak and Dilley, 1976b; Sacher, 1973). The responding system may also be affected by environmental factors such as temperature (Maxie et al., 1973) and water stress (Parups and chan, 1973).

In tuberose, conflicting information on the role of ethylene on flower bud opening and vase-life have been reported Tuberose florets were reported to produce very little ethylene and were not affected by exposure to exogenous ethylene (Naidu and Reid, 1989). This is in contrast with the findings of Khondakar and Muzandur (1985) who reported reduced floret and bud abscission and a

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4-day increase in the vase-life of flowers treated with 3% sucrose, 0.03% hydroxyquinoline citrate and 0.01% $AgNO_3$. An improvement of floret opening and vase-life of cut tuberose florets was also reported by Watako (1992), who attributed this to the development of young buds at the apex of the inflorescence after treatment with STS.

Various groups of compounds have been developed to counteract the effects of ethylene. Some are ethylene synthesis inhibitors, others are ethylene action inhibitors, others moderate ethylene sensitivity while others are a combination of one of the above with synthetic plant growth regulators

2.3.3.1 The use of Silver compounds

The use of Ag as a relatively inexpensive procedure to extend the vase-life of cut-flowers has been widely adopted by the cut-flower industry. Silver nitrate and silver acetate are the two common silver compounds used in preservative formulations (Aarts, 1957). Silver nitrate however, has several disadvantages: it reacts with chlorine present in tapwater to form an insoluble AgCl and is also photo-oxidised by light to form insoluble compounds which precipitate (Baker, 1983). Silver ions in the form of $AgNO_3$ also move slowly up the flower stem from the preservative solution (Kofranek and Paull, 1974; Veen and Van de Geijn, 1978) Whereas silver ion in the form of STS moves at a rate of 1 m/hr in carnation stems, silver ion from $AgNO_3$ moves at 3 cm/day (Veen and Van de Geijn, 1978). Thus, unlike $AgNO_3$, treatment of flowers with STS has been found to sufficiently improve the vase-life of many cut-flowers.

Basal pulse of Lily-of-the-Nile (*Agapanthus orientalis*) cut-flower with STS resulted in a 60% to 120% increase in the vase-life and a substantial reduction in bud abscission (upto 20% of control) (Mor *et al.*, 1984). A short term (20 minutes) treatment of miniature carnations with 4 mM STS or overnight treatment with 1 mM STS extended the vase-life as much as continuous use of preservative 'Everbloom' which contains sucrose, weak acid and certain other components (Reid *et al.*, 1980). Lilium 'Enchantment' vase-life was also improved and more florets reached full bloom by STS pretreatment (Swart, 1980). In *Alstroemeria*, petals from all cut-flowers pretreated for one hour with STS then held in deionised water were shed 8, 7 and 6 days later than the control in 'Carmen', 'Pink Perfection' and 'Marina', respectively (Chepkairor, 1986). Five minutes to 24 hour base treatment with STS (1 to 4 mM) was also effective in *Anthurium* (Paull and Goo, 1985),

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carnations (Reid *et al.*, 1978; Veen and van de Geijn, 1978), gypsophila (Downs *et al.*, 1988), lilies (Nowak and Mynett, 1985), sweet peas (Mor *et al.*, 1984) and tuberose (Watako, 1984). Silver thiosulphate had no effect on roses when applied alone but prevented the damage induced by application of ethephon (De Stigter, 1980). The extension of the vase-life by STS treatment could be attributed to several factors.

Silver ion (Ag) was found to be a potent and specific inhibitor of ethylene action in flower tissues (Beyer, 1976). Pretreatment of snapdragon with STS sufficient to provide 2.5 mM Ag per spike prevented floret abscission caused either by ethylene or by the addition of ethephon to the vase solution (Farnharm *et al.*, 1980). Silver thiosulphate also reduced ethylene sensitivity and extended floret longevity beyond that of the controls in *Pelargonium domesticum* (Deneke *et al*, 1990). Silver thiosulphate also prevented normal and ethylene-induced abscission in *Delphinium* and sweet peas (Shillo *et al.*, 1980). A good response of spray carnations to STS pretreatment was reported (Reid *et al.*, 1980) but Systema (1981) found some spray carnation cultivars to be less responsive to STS than were standard carnations. Silver thiosulphate however, had no effect in roses when applied alone but prevented the damage induced by ethephon (De Stigter, 1980).

Apart from ethylene effect, silver ions could also delay senescence in flowers by maintaining a favourable water balance. Silver nitrate pulsing has been demonstrated to reduce the rate of decline of water uptake in *Anthurium* (Paull and Goo, 1985). In maidenhair fern, Fujino and Reid, (1983), reported that Ag improved water relations through an increase in the inflorescence fresh weight after 4 days in the vase. Similarly, 30% to 50% increase in narcissus vase-life through the maintenance of water uptake was reported when STS was used in combination with sucrose or 8-HQS (Piskornik, 1981, 1985).

In cut-flowers, silver ions could be acting as a biocide thus eliminating microbial blockage of the xylem vessels (Aarts, 1957; Kofranek and Paull, 1974) thus improving water uptake. Silver ions could also interfere with wound ethylene binding sites (Paull and Goo, 1985; Sisler, 1982) thus preventing physiological blockage of cut-flower stems. However, the continued use of STS hangs in the balance because of the heavy metal pollution and the associated implications on the environment (Hutchinson *et al.*, 1997; Serek *et al.*, 1994). 1-methyl cyclopropane (1-MCP) is a new and novel gaseous compound receiving increasing attention as an alternative to STS (Serek *et al.*, 1994).

2.3.3.2 The use of Aminoethoxyvinylglycine (AVG)

Aminoethoxyvinylglycine at 7×10^{-5}M was found to be effective in extending longevity in carnations, producing increases of 100% or more in vase-life (Baker *et al.*, 1977) The vase-life of snapdragon, chrysanthemum, iris and narcissus have also been extended by AVG treatment (Wang and Baker, 1979, Wang *et al.*, 1977). The mode of action of AVG is through the inhibition of ACC synthase (Adams and Yang, 1979). However, AVG has been found to be very toxic in certain tissues and not to always block ethylene biosynthesis (Biddington, 1992; Songstad *et al.*, 1989 cited by Hutchinson *et al.*, 1997).

2.3.3.3 The use of Aminooxyacetic acid (AOA)

Aminooxyacetic acid is a readily available inhibitor of ACC synthase (Yu *et al.*, 1979). At concentration of 0.5 mM AOA is effective in extending the vase-life of carnation as much as AVG at 0.1 mM (Broun and Mayak, 1981; Fujino *et al.*, 1981; Wang and Baker, 1979).

2.3.3.4 Cytokinins

Various researchers have studied the influence of cytokinins on ethylene evolution by flowers. In *Ipomoea tricolor*, Kende and Hanson (1976) reported that BA delayed ethylene production in isolated rib segments. Kinetin-treated flowers also showed a delay in endogenous ethylene production as well as reduced sensitivity to applied ethylene (Jeffcoat, 1977). In carnation, Mor *et al*. (1983) showed that pretreatment of detached petals for 24 hours with 0.1 milimolar BA, kinetin and zeatin blocked their senescence by 8 days. The normally enhanced wilting and increase in the endogenous levels of ACC and ethylene production following exposure of petals to ethylene (16 ml/l for 10 hours) were not observed in BA pretreated flowers (Mor *et al.*, 1983). Similar findings were reported in carnations by Paulin and Muloway (1985) who attributed this to a slow production of methionine by the flower. Benzyladenine application in carnations resulted in 90% reduction in the capacity of carnation flowers to convert exogenously applied ACC to ethylene due to the reduction of the ethylene synthase activity (Eisinger, 1982). Similarly, Cook *et al.*, (1985)

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reported that BA prolonged the vase-life of flowers during the pre-senescent stage (during which they are unresponsive to ethylene), maintained low levels of ACC and limited the capacity of the tissue to convert ACC to ethylene. Benzyladenine was found to be ineffective in lowering the ethylene production and endogenous ACC levels if added after senescence has begun in isolated petals (Mor *et al.*, 1983).

3.0 MATERIALS AND METHODS

The experiments were conducted with tuberose cut-flower inflorescence purchased from a commercial farm, 'Cianda flowers', in Kiambu, a few kilometers from the University of Nairobi Cianda flower Co. is located in an upper midland (UM3) agroecological zone at altitude of 1580-1760 m above sea level (Jaetzold and Schmidt, 1982).

Tuberose flowers were raised from bulbs. The bulbs were planted in raised beds 1m. wide at spacing of 20 cm x 30 cm and depth of 5-10 cm. The bulbs were pretreated with benlate before planting to prevent botrytis and stem rot diseases which are prevalent diseases under cool moist conditions During land preparation, DAP fertilisers at 125 kg/ha was incorporated with farmyard manure

Split application of nitrogenous fertilisers at 100 kgN/ha was done 30 and 60 days after planting Hand weeding was carried out regularly to prevent weed infestation. Irrigation was done twice weekly when necessary to maintain a 8 cm water depth. Weekly spray with ethylene bisthidiocarbamate (Dithane M45) and 0,0 Dimethyl phosphorothidiodide (Rogor E) was done to prevent attack by stem rot disease and red spider mites, respectively.

The flowers were harvested at the commercial stage of harvest (with one floret open) (Watako, 1992) and brought to the laboratory within 2 hours. In the laboratory the flowers were re-cut under water to 60 cm length and the lower leaves discarded.

The total laboratory area was 178 m^2 while the total experimental area was 60 m^2. The treatments were evaluated under a cool white fluorescent (4160 J/sec) at temperature of $23 \pm 1°C$ and RH $70 \pm 10\%$.

3.1 Treatment combinations

Flower stems were held in a flower vase containing 250 mls of the test solutions. A total of 15 treatment combinations were used for the determinations. All the treatments except deionised water, STS and Florissant 500^{TM} were held in solutions containing 0.5% NaOCl and 150 ppm citric acid Both NaOCl and citric acid were used as germicides (Marousky, 1969) and citric acid also eliminated air embolism (Durkin, 1981).

The treatment combinations were:

1 Deionised water

2 Pulsed (10% sucrose), then transferred to deionised water

3   „   „   „   to 25 ppm Accell[TM]

4   „   „   „   to 50 ppm Accell[TM]

5   „   „   „   to 75 ppm Accell[TM]

6   „   „   „   to 100 ppm Accell[TM]

7 Unpulsed, held in 2.5 ppm GA_{4-7}

8   „   held in 7.5 ppm GA_{4-7}

9   „   held in 10.0 ppm GA_{4-7}

10   „   held in 25 ppm Accell[TM]

11   „   held in 50 ppm Accell[TM]

12   „   held in 75 ppm Accell[TM]

13   „   held in 100 ppm Accell[TM]

14 Florissant 500

15 2.0 mM STS

Deionised water was used as the control. Five treatments were pulsed in 10% sucrose solution for 24 hours and one treatment later transferred to 150 ppm citric acid and 0.5% NaOCl while the other four treatments transferred to Accell[TM] solutions at concentrations of 25, 50, 75 and 100 ppm Accell[TM] is prepared by Abbott labs, North Chicago, USA and contains BA and GA_{4-7} at ratio 10:1, thus containing 2.5, 5, 7.5 and 10 ppm GA_{4-7} in addition to 25, 50, 75 and 100 ppm BA Preliminary studies revealed that there was no significant difference between Accell[TM] and the equivalent BA concentrations.

As a control for the pulsed treatments, flowers were directly held in solutions containing Accell[TM] at concentrations 25, 50, 75 and 100 ppm. Similarly, as a control for the GA_{4-7} found in Accell[TM], cut stems were directly held in solutions prepared from provide[TM] (100% GA_{4-7}) (Abbott labs, North Chicago, USA) at concentration 2.5, 7.5 and 10.0 ppm GA_{4-7}.

Tuberose flower stems were also held in Florissant 500[TM] (Roelofarendsreen, Holland) Florissant 500[TM] has T-chloramine at 50 mg per tablet as the active ingredient (Florissant sales B V) T-Chloramine is a chlorine-based compound which acts as a biocide (Florissant sales B.V.). One tablet was dissolved in three litres of deionised water to act as the holding solution as per the

manufacturer's instructions.

Tuberose cut stems were also held for 1 hour in 2.0 mM silver thiosulphate (STS) anionic complex and later transferred to deionised water. The STS was made according to methods developed by Gorin *et al.* (1985) by first dissolving silver nitrate (0.395 grams) and sodium thiosulphate (2.31 grams) separately in 500 mls. of deionised water, then mixing the two solutions to form the complex. Silver nitrate solution was added to the sodium thiosulphate solution to give a 1.4 molar concentration as silver thiosulphate is only stable in excess sodium thiosulphate. The preparation of solutions was done in flasks covered with a black cloth to maintain the stability of the anionic STS complex.

The vase life, floret opening, water uptake and transpiration water loss experiments were replicated 4 times while the dry weight, sucrose and reducing sugar experiments were replicated three times. All the experiments were repeated at least twice. The experiments were carried out using the completely randomised design (CRD) method (Steel and Torrie, 1981). Data was analysed using variance (General linear Model Procedure of PC; SAS Institute Inc., 1995) and the means were compared by the Tukey's procedure using 5% level of probability.

3.2 Experiments

3.2.1 Effect of plant growth regulators on the vase-life and percentage of open florets of tuberose cut-flower

The vase-life was evaluated from the time the flowers were placed in the vase solutions after pretreatment with 10% sucrose. The vase-life was considered terminated when the last open floret wilted and lost decorative value (Watako, 1992; Woodson, 1987). At that point, the unopened flowers were recorded, and the percentage of open florets determined.

3.2.2 Effect of plant growth regulators on water uptake and transpiration water loss of tuberose cut-flowers

The effect of the plant growth regulators on water uptake and transpiration water loss were carried out following procedures outlined in *Gerbera* (Van Meeteren, 1978) and roses (Mayak and

Halevy, 1974) During the experiments, the flowers were placed in boiling tubes holding 60 mls. of the test solutions. The top of the boiling tube was sealed with a piece of polythene to prevent any evaporation thus water loss was only via the flower. At the start of the experiment and after every 48 hours, the weight of the boiling tube with and without the flower were recorded. To eliminate water deficit caused by air embolism upon removal of the inflorescence from the solution, 1 cm portion of the lowest stem part was cut off. This procedure was followed until the flowers wilted. From the change in weight between 2 successive measurements divided by the number of hours during the interval (48 hours) the rate of water uptake in grams/hr/flower (weighing without the flower) and the rate of transpiration (weighings with the flower) were calculated. The solutions were refilled after every 48 hours using equivalent solutions.

3.2.3 Effect of the plant growth regulators on the dry weight of tuberose cut-flowers

Five grams of the petals from the lowest and the middle florets and 2.5 grams of the petals from the topmost florets/buds were weighed. After weighing, the petals were oven-dried at 60 °C for 72 hours and the final weight taken. The dry weight was expressed as grams per unit weight of flower petals.

3.2.4 Effect of plant growth regulators on the sugar levels of tuberose cut-flower

Five grams of the petals from the lowest and middle florets and 2.5 grams of the topmost florets/buds were used in the determination of reducing sugars and sucrose. A smaller amount of petals of the topmost floret was used because of the difficulty in obtaining 5 grams due to the small size of the top florets. Determinations were carried at days 0, 3, 6, 9 and 12.

The samples were kept on ice during all stages of extraction. After weighing, the samples were homogenised twice with 2.5 mls of distilled water to obtain maximum recovery of soluble sugars. The samples were then centrifuged at 10,000 rpm for 15 minutes and filtered through a filter paper ready for sugar determination.

3.2.4.1 Sucrose

Sucrose was determined calorimetrically using the anthrone test (Harper *et al.*, 1979 cited by Amuyunzu, 1994). Anthrone test solution was prepared by dissolving 1 gram of anthrone in 1 litre of 78% sulphuric acid. Mineral acids not only cleave glycosidic bonds but also cause the dehydration of sugars to yield furfurals and levullinic acids (Pomeranz and Meloan, 1973). The furfurals formed by acid dehydrations of sugars react with any variety of phenolic compounds (phenols, anthrones etc) or aromatic amines to give highly conjugated coloured products (Pomeranz and Meloan, 1973). The amount of colour formation is a function of the amount and nature of the sugar being analysed (Pomeranz and Meloan, 1973).

In this method, each sample extract was diluted in water in the ratio 1:300, and from this dilution, 0.5 ml was used as test samples. The standard consisted of 0.5 ml of sucrose solutions having different concentrations: 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml. To both the test samples and standards, 0.5 mls of 30% potassium hydroxide was added and then boiled for 10 minutes in a water bath after which 5 ml of the anthrone test solution was immediately added and the resulting solution mixed well using a vortex stirrer. At this point, the solutions produced a bright green colour and after they had cooled to room temperature their optical density was measured in a spectrophotometer at 620 nm wavelength. The amount of sucrose in the test samples was estimated by use of a standard curve and expressed as mg sucrose per unit weight of flower petal tissue.

3.2.4.2 Reducing sugars

The amounts of reducing sugar were determined following procedures developed by Sumner (1921), cited by Amuyunzu (1994). This is a calorimetric measurement that involves a colour reagent, a standard solution and the test sample. The colour reagent was prepared by dissolving 20 grams of potassium sodium tartrate-4-hydrate and 0.5 grams of 3, 5-dinitrosalicyclic acid in 10 ml of 2N sodium hydroxide and 110 ml of distilled water to make a total volume of 120 mls. The resulting solution was kept in the dark at all times.

The standard consisted of glucose solutions of different concentrations (0, 0.33, 0.67, 1.00, 1.33, 1.67 and 2.00 mg/ml) while the test sample involved diluting the sample extract (filtrate) in

distilled water in the ratio 1 : 25. This was followed by adding 2.4 ml of the coloured reagent to test tubes containing 0.6 ml each of the standard and test samples and then boiling for 5 minutes in a water bath. At this point the solutions produced an orange colour. After the solutions had cooled to room temperature, 3 ml distilled water was added. Their optical density was then measured in a spectrophotometer at 550 nm and by use of a standard curve the amounts of reducing sugars was determined and expressed as mg of reducing sugar per unit weight of petal tissue.

4.0 RESULTS

4.1 Effect of plant growth regulators on the vase-life of tuberose cut-flowers

The vase-life of tuberose cut-flowers held in deionised water was about 13 days (Table 1.0). Gibberellic acid (GA_{4-7}) treatments had no significant effect on the vase-life of tuberose cut-flowers (Table 1.0) while BA (BA values were obtained by subtracting GA_{4-7} values from Accell^{TM} values and adding deionised water values) at 25 ppm increased the vase-life by 3 days (Table 1.0). Similarly, the lower concentrations of 25 and 50 ppm Accell^{TM} were effective, increasing the vase-life by 3 and 2 days respectively (Table 1.0). Preliminary studies revealed that there was no significant difference between Accell^{TM} and the equivalent BA concentrations. The higher Accell^{TM} concentrations of 75 and 100 ppm however no significant effect on the vase-life of tuberose cut-flower, compared to untreated cut-flowers (Table 1.0). 100 ppm Accell^{TM} infact proved toxic by causing dark necrotic spots on the leaves and lower parts of the stems. Accell^{TM} at 25 ppm improved the vase-life compared to the 2.5 ppm GA_{4-7} treatment (Table 1.0) while 75 and 100 ppm Accell^{TM} had no effect compared to GA_{4-7} at 7.5 and 10 ppm (Table 1.0).

The vase-life of tuberose cut-flowers pretreated with 10% sucrose before holding in deionised water was increased by 4 days (Table 1.0). There was no significant difference between the sucrose pulse before holding in deionised water and 25, 50 and 75 ppm Accell^{TM} in the absence of sucrose (Table 1.0). However, the vase-life of tuberose cut-flowers pre-treated with 10% sucrose before holding in deionised water was significantly longer than that of cut-flowers held in 100 ppm Accell^{TM} in the absence of sucrose (Table 1.0).

Interestingly, pulsing of the cut-flowers with 10% sucrose for 24 hours improved the effectiveness of 25 ppm Accell^{TM} treatments as the vase-life of cut-flowers pulsed in 10% sucrose before holding in 25 ppm Accell^{TM} was significantly longer than that of cut-flowers transferred to deionised water after pulsing (Table 1.0). However, there was no beneficial effect in pulsing flowers in 10% sucrose and holding in 50, 75 and 100 ppm Accell^{TM}, in relation to pulsing in 10% sucrose before transferring to deionised water (Table 1.0).

Florissant 500^{TM} caused no improvement on the vase-life of the cut-flowers (Table 1.0). However, STS proved effective in improving the vase-life by a significant 7 days (Table 1.0).

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Table 1.0 Effect of plant growth regulators on the vase-life and percentage of open florets of tuberose cut-flower.

Preservatives[x]	Vase life (Days)	% floret opening
Deionised water	[y]13.2[g]	62.8[g]
2.5 ppm GA$_{4-7}$	13.2[g]	63.3[g]
7.5 ppm GA$_{4-7}$	13.5[fg]	63.7[g]
10.0 ppm GA$_{4-7}$	13.4[fg]	64.3[fg]
25 ppm BA	15.8[def]	73.0[cde]
75 ppm BA	14.4[efg]	68.2[defg]
100 ppm BA	14.0[fg]	65.1[fg]
25 ppm Accell[z]	15.8[def]	73.9[c]
50 ppm Accell	15.2[def]	71.9[cdef]
75 ppm Accell	14.7[efg]	68.7[defg]
100 ppm Accell	14.2[fg]	66.6[efg]
10 % sucrose + Deionised water	16.8[cde]	75.5[c]
10 % sucrose + 25 ppm Accell	19.8[ab]	84.6[ab]
10 % sucrose + 50 ppm Accell	18.8[abc]	82.4[ab]
10 % sucrose + 75 ppm Accell	17.5[bcd]	78.5[bc]
10 % sucrose + 100 ppm Accell	15.6[def]	73.2[cde]
STS	20.3[a]	88.0[a]
Florissant 500	14.0[fg]	65.8[fg]

x - To all preservatives except deionised water, Florissant 500[TM] and STS, 0.5% NaOCl and 150 ppm citric acid were added.

y - mean separation within column by Tukey's procedure. Means in each column assigned different letters are significantly different at 5% level of significance.

Z - Accell[TM] test solution contains BA: GA$_{4-7}$ at 10:1. Thus, 25 ppm Accell[TM] contains BA equivalent of 25 ppm and GA$_{4-7}$ equivalent of 2.5 ppm while 75 ppm Accell[TM] contains 75 ppm BA and 7.5 ppm GA$_{4-7}$ and 100 ppm Accell[TM] has 100 ppm BA and 10.0 ppm GA$_{4-7}$

4.2 Effect of plant growth regulators on the opening of tuberose florets

On average, 63% of untreated tuberose inflorescence opened (Table 1.0). Gibberellic acid treatments on their own had no significant effect on the opening of tuberose florets while 25 ppm BA increased the percentage of open florets (Table 1.0). Similarly, Accell[TM] at 25 and 50 ppm significantly increased the percentage of open florets, the lower concentration of 25 ppm resulting in the highest percent increase of 11% over the control (Table 1.0). The higher concentrations of 75 and 100 ppm however, caused no significant increase in the percentage of open florets (Table 1.0) There was no significant difference between Accell[TM] and the equivalent BA concentrations (Table 1.0). Accell[TM] at 25 ppm significantly increased the percentage of open florets in relation to 2.5 ppm $GA_{4,7}$ treatment (Table 1.0).

Pretreatment of tuberose cut-flowers with 10% sucrose before holding in deionised water resulted in about 13% more open florets (Table 1.0). There was no difference between cut-flowers pulsed in sucrose before transferring to deionised water and cut-flowers held in 25 or 50 ppm Accell[TM] in the absence of sucrose (Table 1.0). However, cut-flowers held in 75 and 100 ppm Accell[TM] in the absence of sucrose had lower floret opening than flowers pulsed in 10% sucrose and held in deionised water (Table 1.0).

Pulsing of tuberose cut-flowers in 10% sucrose improved the effectiveness of the 25 and 50 ppm Accell[TM] treatments (Table 1.0). The percentage of open florets of cut-flowers held in 25 and 50 ppm Accell[TM] after sucrose pretreatment was significantly higher than in either cut-flowers pulsed in 10% sucrose and held in deionised water or in cut-flowers held in 25 and 50 ppm Accell[TM] in the absence of sucrose (Table 1.0). However, pretreatment of flowers in 10% sucrose before holding in 75 and 100 ppm Accell[TM] was not beneficial, in comparison to holding in deionised water after pulsing in 10% sucrose (Table 1.0).

Holding tuberose inflorescence in Florissant 500[TM] resulted in no significant increase in floret opening (Table 1.0). However, a one-hour pretreatment with STS proved to be the most effective treatment in improving the percentage opening of the florets by a significant 26% (Table 1.0).

4.3 Effect of plant growth regulators on the water uptake (g/hr/flower) of tuberose cut-flower stems

The rate of water uptake declined as the cut-flowers senesced (Fig 1.0). The rate of decline differed with the preservative solutions used (Fig. 1.0).

At the start of the experiment (day 2), cut-flowers which were initially pulsed in 10% sucrose had a significantly higher rate of water uptake than unpulsed flowers (Fig. 1 0, Table 2 0) Among the unpulsed flowers, there was no significant difference in the rate of water uptake at the start of the experiment (Fig. 1.0, Table 2.0). From the 4th day until the end of the experiment period however, cut-flowers held in deionised water, $GA_{4,7}$ and Florissant 500^{TM} had the lowest rates of water uptake (Fig. 1.0, Table 2.0).

From day 4, cut-flowers held in Accell^{TM} had a significantly increased rate of water uptake in relation to the control, with the lowest concentrations of 25 and 50 ppm having the highest effect (Table 2.0). However, from day 10, 100 ppm Accell^{TM} had no significant effect in water uptake (Table 2.0). The cut-flowers held in Accell^{TM} also had a significantly higher rate of water uptake in relation to $GA_{4,7}$ treatments (Table 2.0). However, from day 10, there was no significant difference in the rate of water uptake between cut-flowers held in 100 ppm Accell^{TM} and 10.0 ppm $GA_{4,7}$ (Table 2.0). Therefore, since $GA_{4,7}$ had no effect on water uptake, most of the observations from the use of Accell^{TM} are attributed to the BA effect (Table 2.0).

There was no significant difference in the rate of water uptake between pulsed flowers at the start of the experiment (Fig. 1.0, Table 2.0). However, from the 4th to the 6th day, cut-flowers transferred to deionised water after pulsing had a significantly lower rate of water uptake in relation to cut-flowers transferred to Accell^{TM} (Table 2.0). From the 8th day however, there was no significant difference in the rate of water uptake between cut-flowers pulsed and held in deionised water and cut-flowers transferred to 75 and 100 ppm Accell^{TM} (Table 2.0). Cut-flowers pulsed and held in Accell^{TM} had a significantly higher rate of water uptake than cut-flowers held in the corresponding Accell^{TM} concentrations, in the absence of sucrose (Table 2.0).

A one-hour STS pre-treatment improved the rate of water uptake (Fig. 1.0, Table 2.0). From the 4th day, the rate of water uptake was significantly higher in STS pretreated cut-flowers in relation to the control (Fig 1.0, Table 2.0).

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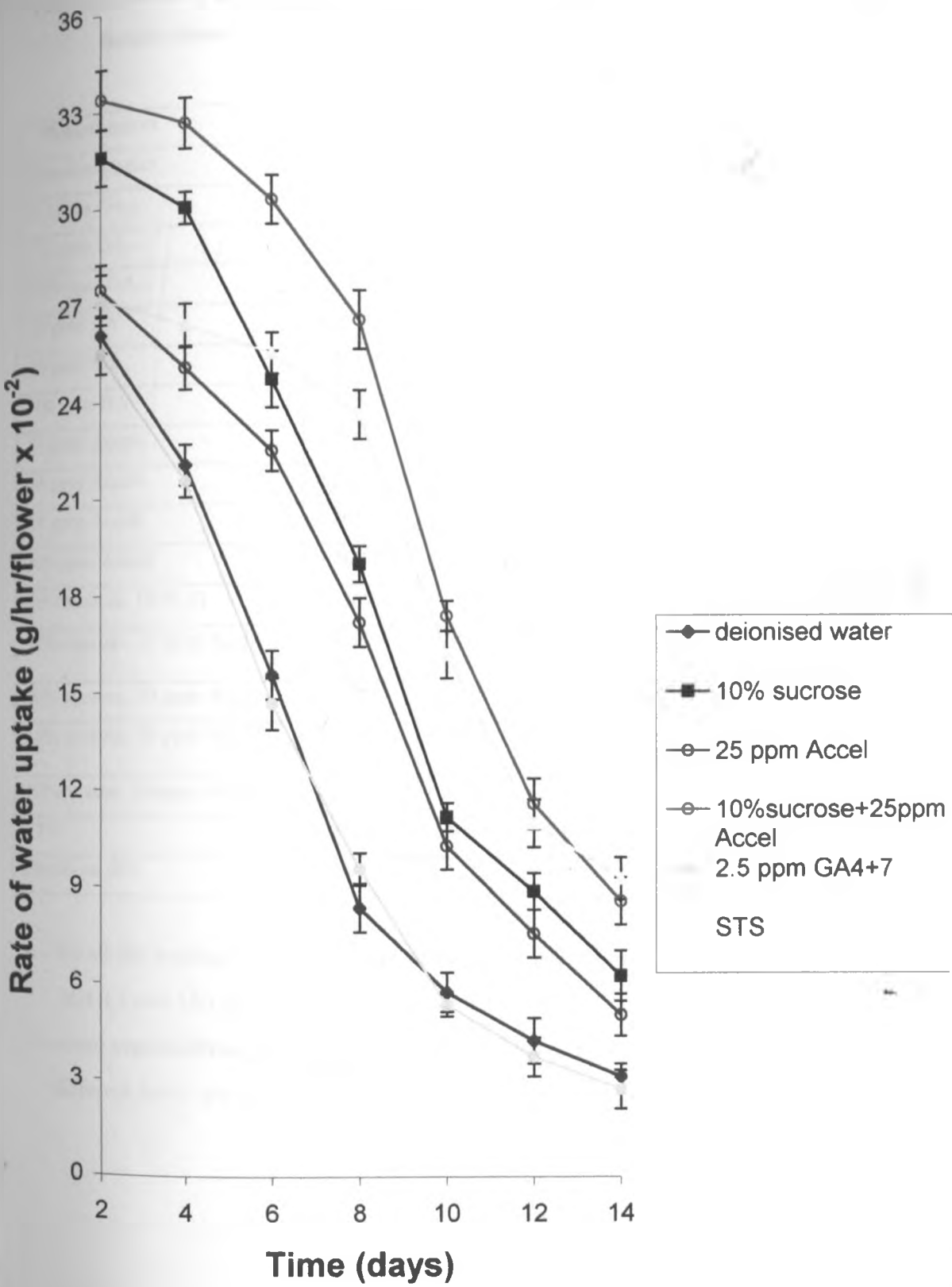
Fig. 1.0 Effect of plant growth regulators on the water uptake (g/hr/flower x 10^{-2}) of Tuberose cut-flowers

Table 2.0 Effect of plant growth regulators on water uptake (g/hr/flower) of tuberose cut-flower stems

Preservatives	Time (Days)						
	2	4	6	8	10	12	14
Deionised water	0.261^b	0.220^g	0.155^m	0.083^i	0.057^{hi}	0.042^{hi}	0.032^{efg}
2.5 ppm GA	0.255^b	0.215^g	0.146^m	0.095^{ij}	0.053^i	0.037^i	0.027^g
7.5 ppm GA	0.262^b	0.218^g	0.161^{lm}	0.099^{ij}	0.061^{hi}	$.044^{ghi}$	0.027^g
10.0 ppm GA	0.265^b	0.224^g	0.153^m	0.085^i	0.065^{ghi}	0.039^i	0.029^{fg}
25 ppm BA	0.285^b	0.255^{cd}	0.233^{efg}	0.159^{fg}	0.106^{de}	0.08^{cde}	0.055^{bc}
75 ppm BA	0.264^b	0.241^{cdefg}	0.192^{ijk}	0.119^{hi}	0.079^{fgh}	0.06^{fgh}	0.035^{efg}
100 ppm BA	0.259^b	0.232^{defg}	0.188^{ijkl}	0.113^{hij}	0.065^{ghi}	0.05^{ghi}	0.038^{def}
25 ppm Accell	0.275^b	0.254^{cde}	0.224^{fgh}	0.171^{ef}	0.102^{def}	0.075^{cdef}	0.05^{cde}
50 ppm Accell	0.273^b	0.250^{cdef}	0.215^{ghi}	0.178^{ef}	0.099^{def}	0.07^{def}	0.047^{cde}
75 ppm Accell	0.265^b	0.239^{cdefg}	0.198^{hij}	0.135^{gh}	0.087^{efg}	0.062^{efg}	0.04^{defg}
100 ppm Accell	0.263^b	0.236^{cdefg}	0.186^{jkl}	0.115^{hi}	0.069^{ghi}	0.047^{ghi}	0.035^{efg}
10% sucrose. DI H$_2$O	0.316^a	0.291^b	0.246^{def}	0.189^{de}	0.111^{de}	0.088^{cd}	0.062^{bc}
10% sucrose. 25 ppm Accell	0.334^a	0.325^a	0.305^a	0.264^a	0.173^a	0.115^a	0.085^a
10% sucrose. 50 ppm Accell	0.339^a	0.317^{ab}	0.298^{ab}	0.248^{ab}	0.169^{ab}	0.111^a	0.073^{ab}
10% sucrose. 75 ppm Accell	0.327^a	0.310^{ab}	0.273^{bcd}	0.225^{abc}	0.147^{bc}	0.091^{bc}	0.061^{bc}
10%sucrose. 100ppm Accell	0.323^a	0.305^{ab}	0.276^{bc}	0.215^{cd}	0.123^{cd}	0.079^{cdef}	0.062^{bc}
STS	0.272^b	0.263^c	0.254^{cde}	0.235^{abc}	0.169^{ab}	0.109^{ab}	0.092^a
Florissant 500	0.266^b	0.227^{fg}	0.165^{klm}	0.086^i	0.055^{hi}	0.035^i	0.031^{efg}

x - To all the holding solutions except deionised water, Florissant 500^{TM} and STS, 0.5% NaOCl and 150 ppm citric acid were added.

y - mean separation within columns by Tukey's procedure. Means in each column assigned different letter are not significantly different at 5% level of probability

4.4 Effect of plant growth regulators on the transpiration water loss (g/hr/flower) of tuberose cut-flowers.

A decline in transpiration water loss was observed as senescence progressed (Fig 2.0). The rate of decline differed with the preservatives used (Fig 2.0).

Gibberellic acid. Florissant 500[TM] and STS had no significant effect on the transpiration rate of tuberose cut-flowers (Table 3.0). Cut-flowers held in Accell[TM] however, had a significantly higher rate of transpiration than the control and GA_{4-7} treatments (Table 3.0). There was no significant difference in transpiration rate between cut-flowers held in Accell[TM] and the BA equivalents (Table 3.0).

The transpiration rate of cut-flowers initially pulsed in sucrose and held in deionised water was lower than the control (upto day 6) but thereafter, there was no significant difference in transpiration between the two treatments (Fig. 2.0, Table 3.0). Similarly, the transpiration rate of cut-flowers pulsed in sucrose before holding in deionised water was significantly lower than that of cut-flowers held in Accell[TM] in the absence of sucrose (Table 3.0). Pulsing of the flowers with 10% sucrose before holding in Accell[TM] similarly lowered the transpiration rate (until day 6) compared to cut-flowers held in Accell[TM] in the absence of sucrose (Table 3.0). From day 8 however, there was no significant difference in the transpiration rate between the Accell[TM] treatments, whether pulsed or not (Table 3.0).
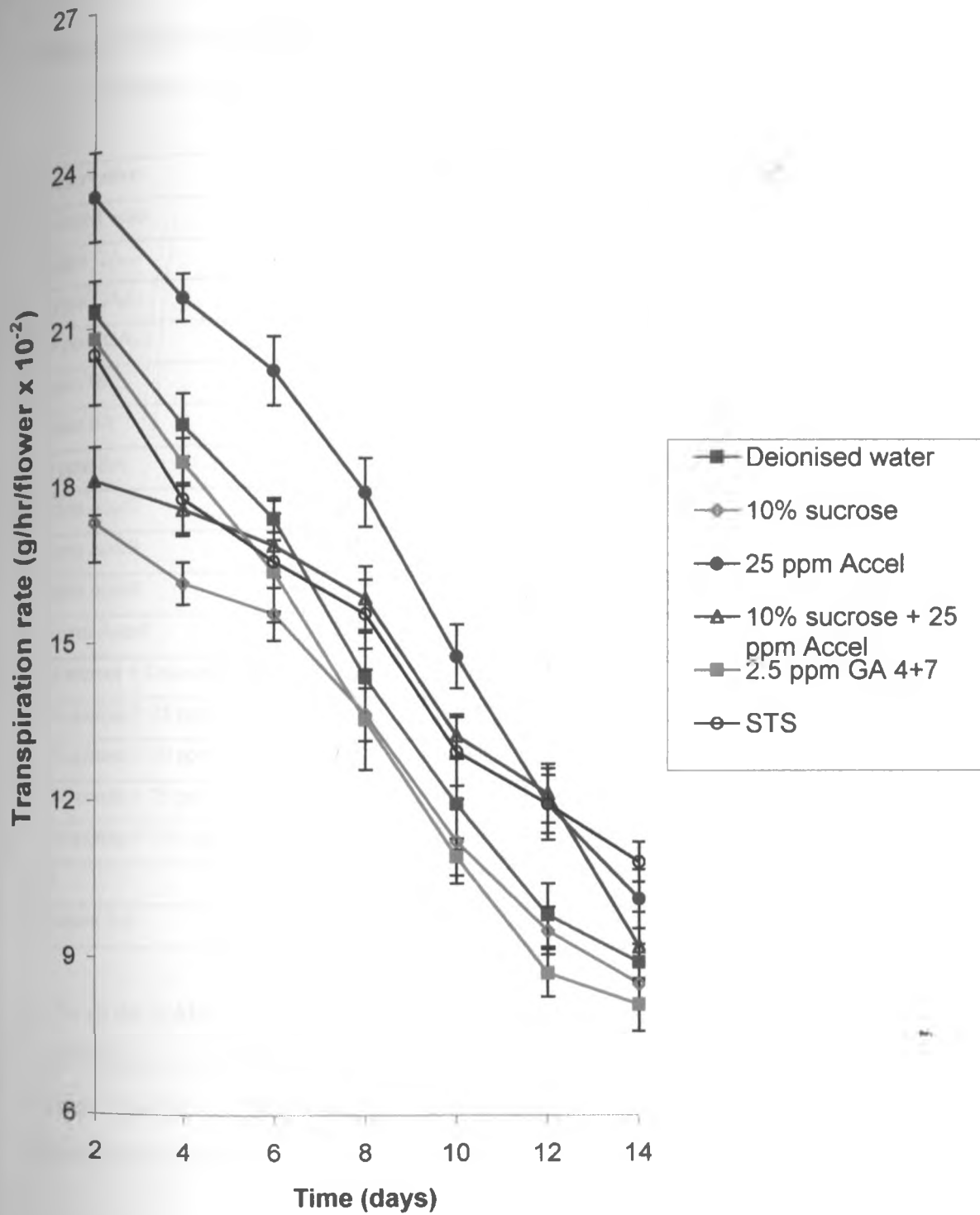
Fig. 2.0 Effect of plant growth regulators on the transpiration rate (g/hr/flower x 10^{-2}) of Tuberose cut-flowers.

Table 3.0 Effect of the plant growth regulators on the transpiration (g/hr/flower) of tuberose cut-flowers.

Time (Days)

Preservative	2	4	6	8	10	12	14
Deionised water	[y]0.213[abcd]	0.191[bcde]	0.173[bcdefg]	0.143[def]	0.119[cdef]	0.098[bcd]	0.089[def]
2.5 ppm GA$_3$	0.208[bcde]	0.184[def]	0.155[g]	0.135[ef]	0.109[ef]	0.087[d]	0.081[def]
7.5 ppm GA$_3$	0.201[cdef]	0.188[de]	0.169[bcdefg]	0.131[f]	0.114[def]	0.083[d]	0.069[f]
10.0 ppm GA$_3$	0.206[bcde]	0.183[def]	0.158[fg]	0.142[def]	0.105[f]	0.091[cd]	0.074[ef]
25 ppm BA	0.24[a]	0.222[a]	0.219[a]	0.186[ab]	0.157[a]	0.127[a]	0.109[ab]
75 ppm BA	0.239[a]	0.222[a]	0.2[abc]	0.193[a]	0.144[abcd]	0.12[ab]	0.115[a]
100 ppm BA	0.232[ab]	0.213[abc]	0.197[abcd]	0.167[abcde]	0.156[a]	0.115[abc]	0.108[ab]
25 ppm Accell	0.235[ab]	0.215[ab]	0.201[ab]	0.178[abc]	0.147[abc]	0.119[ab]	0.101[abcd]
50 ppm Accell	0.241[a]	0.205[abcd]	0.189[abcdef]	0.175[abcd]	0.153[ab]	0.123[ab]	0.095[abcde]
75 ppm Accell	0.227[abc]	0.219[ab]	0.193[abcde]	0.181[abc]	0.139[abcde]	0.105[abcd]	0.095[abcde]
100 ppm Accell	0.225[abc]	0.205[abcd]	0.185[bcdefg]	0.166[a]	0.143[abcd]	0.108[abcd]	0.091[bcdef]
10% sucrose + Deionised water	0.173[f]	0.161[f]	0.155[g]	0.136[ef]	0.112[ef]	0.094[bcd]	0.085[cdef]
10 % sucrose + 25 ppm Accell	0.181[ef]	0.168[ef]	0.175[bcdefg]	0.166[abcde]	0.132[abcdef]	0.119[abc]	0.092[bcde]
10 % sucrose + 50 ppm Accell	0.195[def]	0.169[ef]	0.171[bcdefg]	0.168[abcde]	0.137[abcde]	0.115[abc]	0.093[abcde]
10 % sucrose + 75 ppm Accell	0.189[def]	0.177[ef]	0.168[defg]	0.161[abcdef]	0.127[abcdef]	0.098[bcd]	0.087[bcdef]
10 % sucrose + 100 ppm Accell	0.193[def]	0.175[ef]	0.166[efg]	0.159[abcdef]	0.124[bcdef]	0.098[bcd]	0.085[cdef]
STS	0.205[bcde]	0.177[ef]	0.175[bcdefg]	0.155[bcdef]	0.129[abcdef]	0.119[ab]	0.108[ab]
Florissant 500	0.212[abcd]	0.19[cde]	0.173[bcdefg]	0.147[cdef]	0.102[f]	0.095[bcd]	0.069[f]

x - To all the holding solutions except deionised water, Florissant 500[TM] and STS, 0.5% NaOCl and 150 ppm citric acid were added.

y - mean separation within columns by Tukey's procedure. Means in each column assigned different letters are significantly different at 5% level of significance.

4.5 Effect of plant growth regulators on the water balance (water uptake - transpiration loss (g/hr/flower) of tuberose cut-flowers.

The rate of water uptake was higher than the transpiration rate at the initial stages of the experiment (Table 4.0). From the 6th day however, the rate of transpiration was higher, starting with cut-flowers held in deionised water, Florissant 500[TM] and GA_{4-7} (Table 4.0). Cut-flowers held in Accell[TM] had higher rates of transpiration than water uptake from the 8th day (Table 4.0).

Cut-flowers pulsed in 10% sucrose before holding in either deionised water or 75 ppm or 100 ppm Accell[TM] had higher rates of transpiration than water uptake From the 10th day (Table 4 0). Cut-flowers pulsed in 10% sucrose and held in 25 and 50 ppm Accell[TM] and flowers pretreated with STS had higher rates of transpiration than water uptake from the 12th day (Table 4.0).

Table 4.0 Effect of plant growth regulators on the water balance (water uptake – transpiration) (g/hr/flower) of tuberose cut-flowers.

Preservatives	2	4	6	8	10	12	14
Deionised water	0.03	0.029	-0.018	-0.06	-0.062	-0.056	-0.057
2.5 ppm GA_{4+7}	0.047	0.031	-0.017	-0.04	-0.056	-0.05	-0.054
7.5 ppm GA_{4+7}	0.061	0.03	-0.008	-0.032	-0.053	-0.039	-0.042
10.0 ppm GA_{4+7}	0.059	0.041	-0.005	-0.057	-0.04	-0.052	-0.045
25 ppm BA	0.045	0.033	0.024	-0.027	-0.051	-0.047	-0.054
75 ppm BA	0.025	0.019	-0.008	-0.074	-0.065	-0.06	-0.07
100 ppm BA	0.027	0.019	-0.009	-0.054	-0.091	-0.065	-0.07
25 ppm Accell	0.037	0.039	0.023	-0.007	-0.045	-0.044	-0.051
50 ppm Accell	0.032	0.049	0.026	-0.003	-0.054	-0.053	-0.048
75 ppm Accell	0.038	0.02	0.005	-0.046	-0.052	-0.043	-0.055
100 ppm Accell	0.038	0.031	0.001	-0.051	-0.074	-0.061	-0.066
10% sucrose + Deionised water	0.133	0.12	0.091	0.053	-0.001	-0.004	-0.023
10 % sucrose + 25 ppm Accell	0.163	0.157	0.13	0.098	0.041	-0.006	-0.007
10 % sucrose + 50 ppm Accell	0.144	0.148	0.127	0.08	0.032	-0.004	-0.02
10 % Sucrose + 75 ppm Accell	0.145	0.133	0.105	0.064	-0.005	-0.006	-0.026
10 % sucrose + 100 ppm Accell	0.134	0.13	0.11	0.056	-0.001	-0.023	-0.023
STS	0.067	0.086	0.081	0.08	0.032	-0.01	-0.016
Florissant 500	0.054	0.037	-0.008	-0.061	-0.047	-0.06	-0.038

4.6 Effect of the plant growth regulators on the dry weight (g) of tuberose flower petals.

The dry weight of the petals of tuberose cut-flowers differed with the floret positions (Fig. 3.0). In the petals of the lowest florets, pulsed cut-flowers had significantly higher dry weights than unpulsed flowers throughout the entire period of determination (Fig. 3.0a). There was no significant difference in the dry weights of the lowest petals of the pulsed cut-flowers in days 0, 3 and 9 (Fig. 3.0a). However, cut-flowers pulsed and held in 25 and 50 ppm Accell^{TM} had significantly higher dry weights than cut-flowers pulsed and transferred to either deionised water or 75 and 100 ppm Accell^{TM} in day 6 (Fig. 3.0a, Table 5.0). Among unpulsed flowers, there was no significant difference in dry weights at days 0 and 3 (Fig. 3.0a, Table 5.0). However, in day 6, cut-flowers held in 25 and 50 ppm Accell^{TM} or the BA equivalents or flowers pretreated with STS had significantly higher dry weights than flowers held in deionised water or GA$_{4-7}$ treatments (Fig. 3.0a, Table 5.0).

In the petals of the middle florets, an increase in the dry weight was observed in day 3 before declining (Fig. 3.0b). There was no significant difference in the dry weights of the petals between the plant growth regulators in day 0 (Fig. 3.0b). In subsequent days however, cut-flowers pulsed in 10% sucrose had significantly higher dry weights than unpulsed flowers (Fig. 3.0b), with cut-flowers pulsed and held in 25 ppm Accell^{TM} having the highest increase (Fig. 3.0b, Table 5.0). Among unpulsed flowers no significant difference in dry weight was observed in day 0 and day 3 (Fig. 3.0b, Table 5.0). However, in day 6 and 9, cut-flowers held in 25 and 50 ppm Accell^{TM} and cut-flowers pretreated with STS had significantly higher dry weights than cut-flowers held in 75 and 100 ppm Accell^{TM}, GA$_{4-7}$ and deionised water (Table 5.0).

In the petals of the topmost florets/buds, an increase in the dry weights was observed from day 0 to day 6 before declining in cut-flowers pulsed and held in deionised water or 25, 50 and 75 ppm Accell^{TM} and in cut-flowers pretreated with STS (Fig. 3.0c, Table 5.0). In cut-flowers pulsed and held in 100 ppm Accell^{TM} and all unpulsed cut-flowers (except STS), an increase in the dry weights was observed from day 0 to day 3 before declining (Fig. 3.0c, Table 5.0). There was no significant difference in dry weights in day 0 and 3 (Fig. 3.0c, Table 5.0). In day 6 however, cut-flowers pulsed in 10% sucrose (except pulsed and held in 100 ppm Accell^{TM}) and cut-flowers pretreated with STS had significantly higher dry weights than the other treatments (Fig. 3.0c, Table 5.0).

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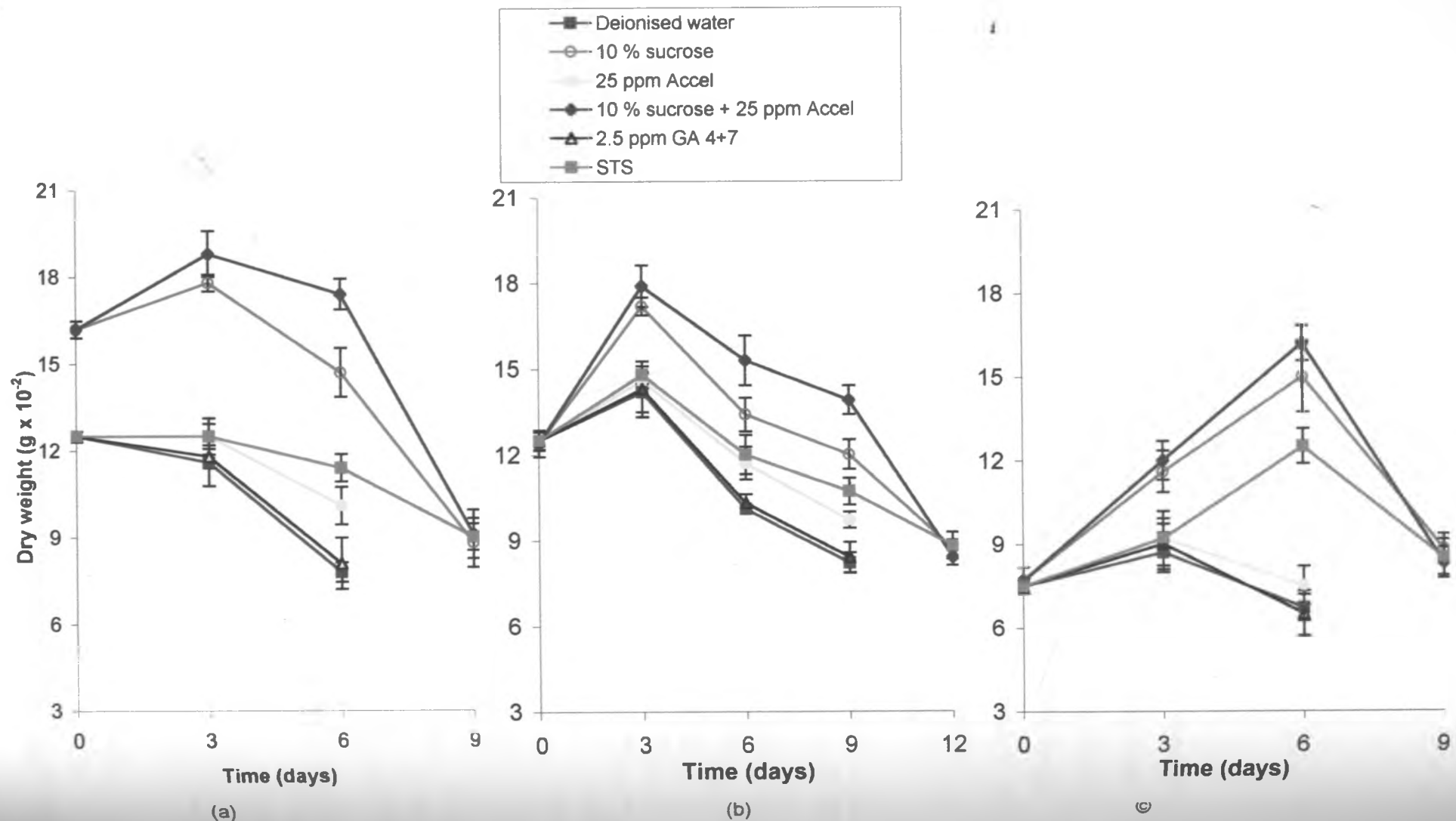
Fig. 3.0 Effect of the plant growth regulators on the dry weight (g) of the petals of the (a) *Lowest* (b) *Middle* (c) Topmost florets of Tuberose cut flowers.

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Table 5.0 **Effect of plant growth regulators on the dry weight of tuberose cut-flowers**

Preservatives	Lowest florets			Middle florets				Topmost florets/ buds		Average
	day 3	Day 6	Day 9	Day 3	Day 6	Day 9	Day12	Day 3	Day 6	
Deionised water	^{y}0.116^b	0.078^{ef}	-	0.142^b	0.101^f	0.082^{ef}		0.092^a	0.067^c	^{z}0.101^{no}
2.5 ppm GA$_{4+7}$	0.118^b	0.081^{ef}	-	0.143^b	0.103^f	0.084^{def}		0.099^a	0.065^c	0.101^{mn}
7.5 ppm GA$_{4+7}$	0.119^b	0.077^{ef}	-	0.141^b	0.102^f	0.081^f		0.095^a	0.066^c	0.102^l
10.0 ppm GA$_{4+7}$	0.119^b	0.080^{ef}	-	0.144^b	0.100^f	0.082^{ef}		0.09^a	0.064^c	0.100^o
22.5 ppm BA	0.123^b	0.098^{cd}	-	0.144^b	0.115^{cd}	0.097^{cd}		0.09^a	0.077^c	0.108^{ghi}
67.5 ppm BA	0.118^b	0.093^{de}	-	0.146^b	0.106^{def}	.095^{cde}		0.086^a	0.068^c	0.103^{kl}
90 ppm BA	0.119^b	0.085^{def}	-	0.144^b	0.106^{def}	0.085^{def}		0.095^a	0.073^c	0.104^{jk}
25 ppm Ac	0.125^b	0.101^{cd}	-	0.145^b	0.117^c	0.097^{cd}		0.097^a	0.075^c	0.108^{fgh}
50 ppm Ac	0.124^b	0.098^{cd}	-	0.143^b	.113^{cde}	0.093^{def}		0.095^a	0.071^c	0.107^{hi}
75 ppm Ac	0.121^b	0.092^{def}	-	0.146^b	0.107^{def}	0.086^{def}		0.089^a	0.067^c	0.104^{ij}
100 ppm Ac	0.122^b	0.087^{def}	-	0.146^b	0.105^{ef}	0.085^{def}		0.093^a	0.07^c	0.104^{hi}
10 % suc + DI water	0.178^a	0.147^b	0.088^a	0.172^b	0.134^b	0.12^b	0.089^a	0.114^a	0.15^{ab}	0.138^{de}
10 % suc + 25 ppm Ac	0.187^a	0.174^a	0.094^a	0.179^a	0.153^a	0.139^a	0.095^a	0.112^a	0.162^a	0.148^a
10 % suc + 50 ppm Ac	0.184^a	0.169^a	0.093^a	0.175^a	0.148^a	0.132^{ab}	0.092^a	0.106^a	0.156^{ab}	0.145^{bc}
10 % suc + 75 ppm Ac	0.182^a	0.159^{ab}	0.09^a	0.176^a	0.141^{ab}	0.126^{ab}	0.09^a	0.116^a	0.146^{ab}	0.141^{cd}
10 % suc + 100 ppm Ac	0.185^a	0.155^{ab}	0.09^a	0.176^a	0.137^b	0.122^b	0.085^a	0.095^a	0.072^c	0.13^d
STS	0.130^b	0.114^c	0.09^a	0.148^b	0.120^c	0.107^c	0.09^a	0.095^a	0.125^b	0.117^{efg}
Florissant 500	0.119^b	0.076^f	-	0.142^b	0.103^f	0.081^f		0.097^a	0.092^c	0.101^l

Suc=sucrose   Ac.=Accell

x- To all holding solutions except deionised water, STS and Florissant 500^{IM}, 0.5% NaOCl and 150 ppm citric acid were added.

y- Mean separation within columns (except the last column) by Tukey's procedure Means assigned the same are not significantly different at p = 0.05.

z- Mean separation in the last column by mean _+ SD. Means assigned different letters are significantly different at P = 0.05.

Analysis of the average amounts of dry weights in all floret positions in all days of determination revealed that cut-flowers held in deionised water, GA_{4-7} at 2.5, 7.5 and 10 ppm and Florissant 500^{TM} had reduced dry weights while Accell^{TM} or it's BA equivalents, STS pretreatment and sucrose had increased dry weights (Table 5.0). However, the sucrose pretreated cut -flowers had the highest dry weights (Table 5.0).

4.7 Effect of plant growth regulators on the sucrose levels (mg/g) of tuberose cut-flower petals

The sucrose levels in the petals of tuberose cut-flowers differed depending on whether the flowers were pulsed or not (Fig. 4.0). The sucrose levels in the petals of the lowest florets of the pulsed flowers was significantly high in day 0, in relation to the levels in unpulsed flowers (Fig. 4.0 a) However, in subsequent days, the sucrose levels in the petals of the lowest florets dropped such that no significant difference in the sucrose levels was observed between the treatments (Fig. 4.0a)

In the petals of the middle florets, an increase in the sucrose levels was observed from day 0 to day 3 before declining (Fig. 4.0b). There was no difference in the sucrose levels between the treatments at day 0 (Fig. 4.0b). At day 3 however, pulsed flowers had significantly higher sucrose than unpulsed flowers (Fig.4.0b). After day 3, the sucrose levels in the petals declined such that at day 6, no difference in the sucrose levels was observed among all the treatments (Fig. 4.0b). In the petals of the topmost florets/buds, a slight increase in the sucrose levels was observed from day 0 to day 3 before declining in day 6 (Fig. 4.0c). No difference in the sucrose levels of the petals of the topmost florets/buds was observed between all the treatments in all days of determination (Fig. 4.0c).

The sucrose levels in the petals of tuberose cut-flowers were influenced by the floret position (Fig 4.0) The petals from the middle florets had the highest level of sucrose followed by petals from the topmost florets/buds while florets from the lowest positions had the least amount (Fig 4.0).
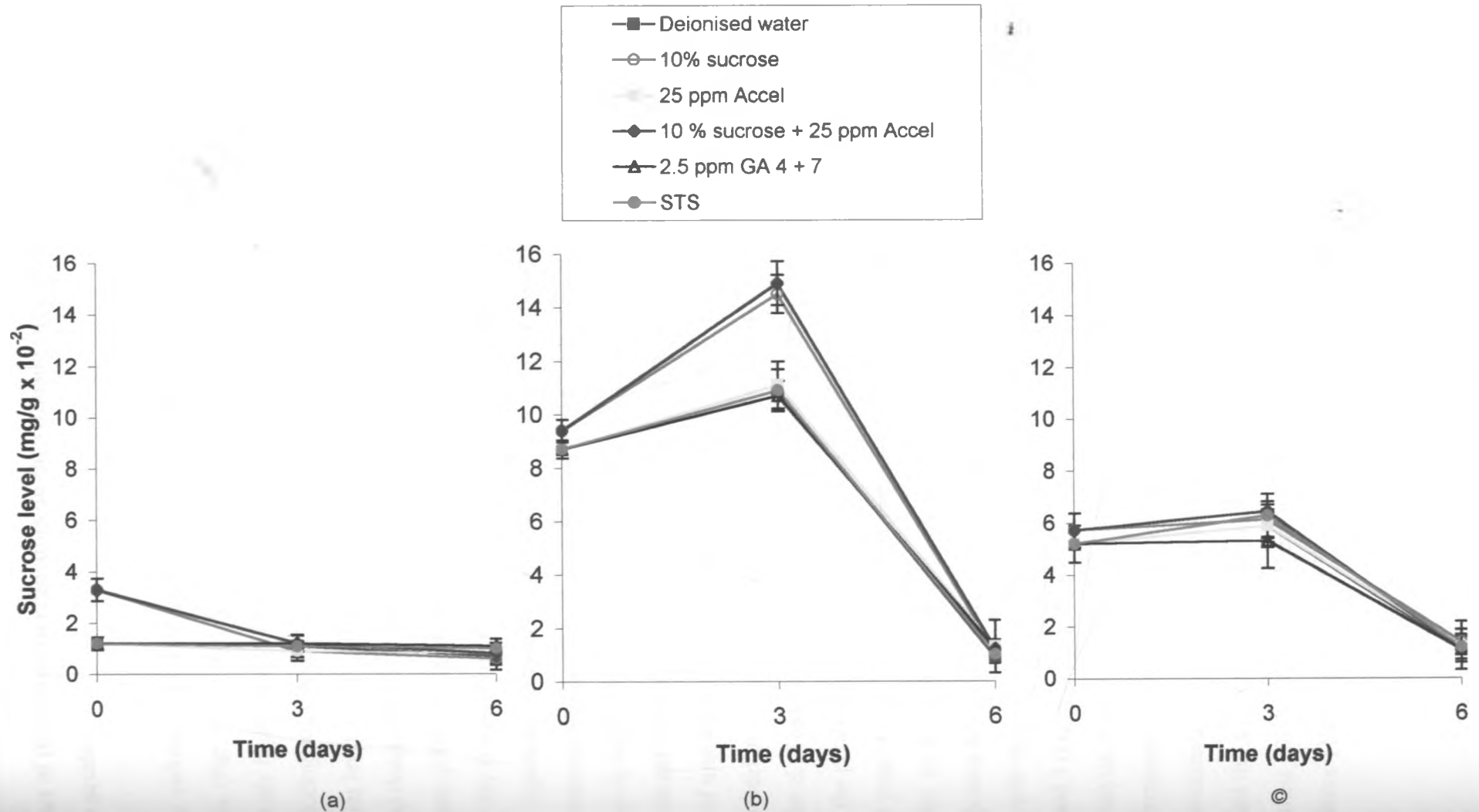
Fig 4.0 Effect of plant growth regulators on the sucrose levels in the petals of the lowest florets (a) middle florets (b) *(b)* and topmost florets of tuberose cut flowers

4.8 Effect of plant growth regulators on the reducing sugar levels (mg/g) of tuberose cut-flower petals.

The reducing sugar levels of the petals of tuberose cut-flowers differed with the holding solutions (Fig 5.0, Table 6.0). In the petals of the lowest florets, pulsed cut-flowers had a significantly higher level of reducing sugar than unpulsed flowers at day 0 (Fig. 5.0a, Table 6.0). The reducing sugar levels in the petals of the lowest florets of pulsed flowers then increased to a maximum level in day 3 before declining, while a gradual decline was observed from day 0 in unpulsed florets (Fig 5.0a, Table 6.0).

In the petals of the middle florets, an increase in the levels of reducing sugar to a maximum level in day 6 was observed, whether the flowers were pulsed or not (Fig 5.0b, Table 6.0). There was no significant difference in the reducing sugar levels of the petals of the middle florets between all the treatments in day 0 and day 3 (Fig. 5.0b, Table 6.0). However, in day 6, pulsed flowers had a significantly higher level of reducing sugar than unpulsed flowers (Fig. 5.0b, Table 6.0). There was no significant difference in the levels of reducing sugar accumulating in the petals of the middle florets of unpulsed flowers in days 0 and 3 (Fig. 5.0b, Table 6.0). In day 6 and 9 however, cut-flowers held in 25 and 50 ppm Accell[TM] and cut-flowers pretreated with STS had a significantly higher level of reducing sugar than the other unpulsed flowers (Fig. 5.0b, Table 6.0).

In the petals of the topmost florets/buds of pulsed cut-flowers (except cut-flowers transferred to 100 ppm Accell[TM] after pulsing) and flowers pretreated with STS, the reducing sugar level increased to a maximum level in day 6 (Fig. 5.0c, Table 6.0). In unpulsed inflorescence and inflorescence held in 100 ppm Accell[TM] after pulsing, the reducing sugar was highest in day 3 (Fig 5.0c, Table 6.0). However, there was no significant difference between the preservatives at both day 0 and 3 (Fig. 6.0c, Table 6.0).

Analysis of the average amount of reducing sugar accumulating in all floret positions in all days of determination revealed that GA$_{4,7}$ treatments had no significant effect on the levels of reducing sugar accumulating in the petals while Accell[TM] at 25-100 ppm or it's BA equivalents significantly increased the amount of reducing sugars in the petals, the lowest concentration of 25 ppm having the highest effect (Table 6.0). There was no significant difference between Accell[TM] and it's BA equivalents (Table 6.0). Accell[TM] treated flowers had significantly higher reducing sugars than

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GA_{4-7} treatments (Table 6.0).

Pulsing of tuberose cut-flowers in 10% sucrose increased the amount of reducing sugars accumulating in the petals (Table 6.0). Pulsing of tuberose cut-flowers with 10% sucrose also increased the effectiveness of the Accell^{TM} treatments in accumulating reducing sugars (Table 6.0). Whereas the amount of reducing sugars in Accell^{TM} treatments ranged from 0.050 to 0.055 mg/g in the absence of sucrose, pulsing increased the reducing sugar levels to 0.087 to 0.101mg/g (Table 6.0). Similarly, the reducing sugar levels in cut-flowers held in 25 and 50 ppm Accell^{TM} after pulsing were significantly higher than in cut-flowers transferred to deionised water after pulsing (Table 6.0). There was no significant difference in the reducing sugar levels in cut-flowers pulsed and held in 75 ppm Accell^{TM} in relation to cut-flowers pulsed and held in deionised water while cut-flowers pulsed and held in 100 ppm Accell^{TM} had a lower amount of reducing sugar (Table 6.0).

A one-hour pretreatment with STS led to a significant increase in the level of reducing sugars in the petals of cut tuberose (Table 6.0) while Florissant 500^{TM} had no effect (Table 6.0).

The amount of reducing sugars accumulating in the petals also differed with the floret positions (Fig 6.0). The lowest florets accumulated the highest amount of reducing sugars followed by the middle florets while the topmost florets accumulated the least amounts (Fig. 6.0).

4.9 Comparison between the sucrose and the reducing sugar levels in the petals of tuberose cut-flowers.

The amount of sucrose in the petals of the lowest florets was consistently lower than the amount of reducing sugars throughout the entire period of determination (Fig. 4.0a, Fig. 5.0a). In the petals of the middle florets, the sucrose levels were higher than the reducing sugar levels in both day 0 and 3 but in day 6, the sucrose levels decreased while the reducing sugar remained high (Fig. 4.0b and 5.0b). In the petals of the topmost florets/buds, the sucrose levels were higher in day 0 and day 3 (Fig. 4.0c and 5.0c). In day 6, cut-flowers pulsed in 10% sucrose and held in deionised water, 25, 50 and 75 ppm Accell^{TM} and cut-flowers pretreated with STS had higher amounts of reducing sugar than sucrose while in the other treatments, both the reducing sugar and sucrose were low (Fig 4.0c and 5.0c).

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(a)

(b)

(c)
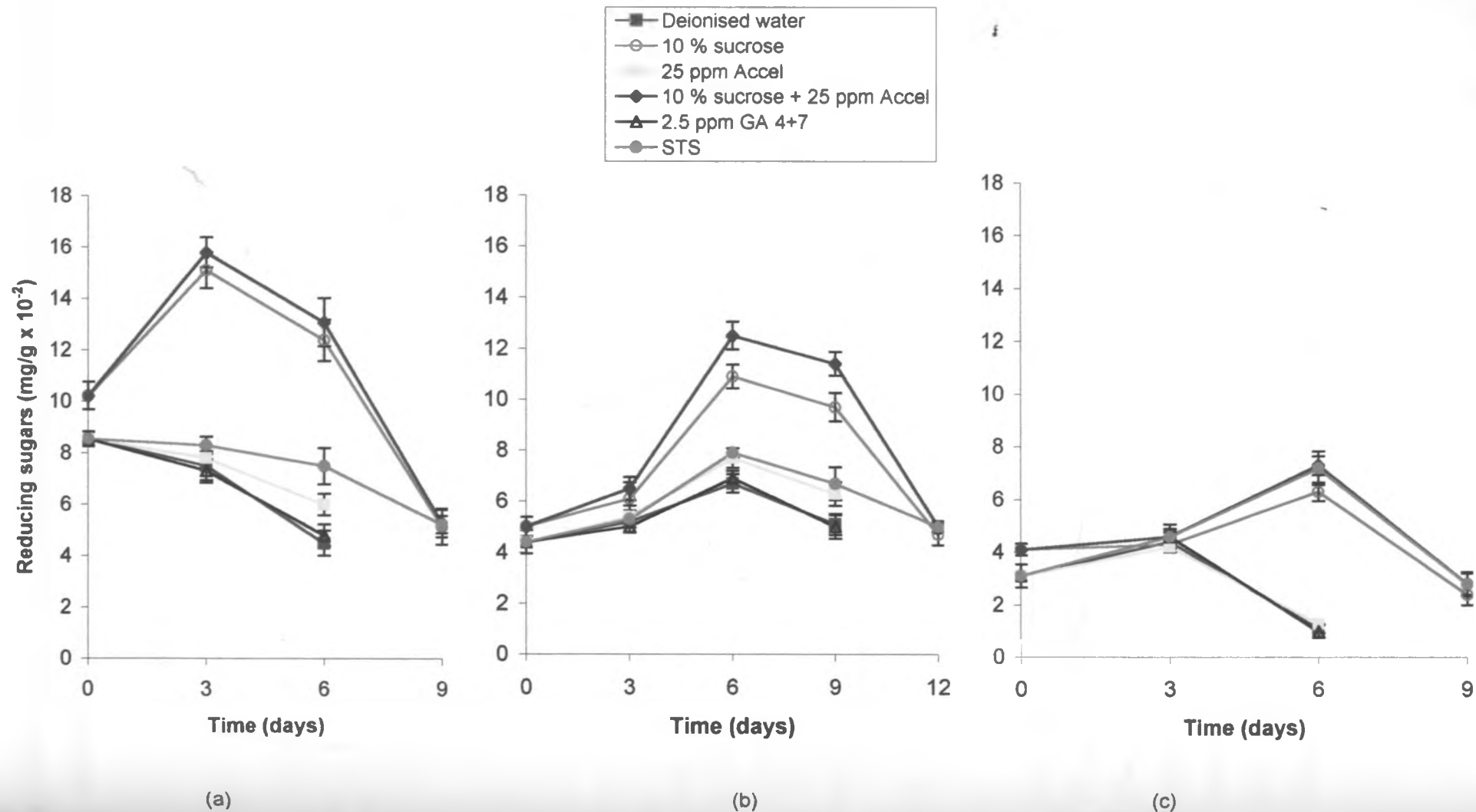
Fig. 5.0 Effect of plant growth regulators on the reducing sugar levels (mg/g) of the petals of the lowest florets(a) middle flore (c) of tuberose cut flowers

Table 6.0 Effect of plant growth regulators on the reducing sugar levels (mg/g) of tuberose cut-flowers petals.

Preservatives	Lowest florets			Middle florets				Topmost florets/ buds		Average
	3	6	9	3	6	9	12	3	6	
Deionised water	0.075^b	0.048^{cd}	-	0.052^{abc}	0.067^e	0.05^g	-	0.039^a	0.011^c	0.049^{fgh}
2 5 ppm GA	0.073^b	0.048^{cd}	-	0.05^e	0.069^{cde}	0.05^g	-	0.044^a	0.01^c	0.051^f
7 5 ppm GA	0.071^b	0.047^{cd}	-	0.05^e	0.066^e	0.049^g	-	0.045^a	0.012^c	0.049^{fghi}
10 0 ppm GA	0.076^b	0.044^d	-	0.051^{bc}	0.067^e	0.051^{fg}	-	0.049^a	0.01^b	0.048^{fghi}
25 ppm BA	0.08^b	0.058^{cd}	-	0.055^{abc}	0.075^{cde}	0.064^d	-	0.037^a	0.015^b	0.055^{efgh}
75 ppm BA	0.081^b	0.049^{cd}	-	0.055^{abc}	0.073^{cde}	059^{defg}	-	0.04^a	0.014^b	0.052^f
100 ppm BA	0.074^b	0.049^{cd}	-	0.054^{abc}	0.07^{cde}	0.053^{efg}	-	0.035^a	0.007^b	0.05^{fg}
25 ppm Ac.	0.078^b	0.06^{bc}	-	0.054^{abc}	0.077^{cd}	0.063^{de}	-	0.049^a	0.007^b	0.055^{efg}
50 ppm Ac	0.079^b	0.058^{cd}	-	0.051^{bc}	0.076^{cde}	0.061^{def}	-	0.042^a	0.013^b	0.055^{efgh}
75 ppm Ac.	0.078^b	0.051^{cd}	-	0.052^{abc}	0.072^{cde}	057^{defg}	-	0.045^a	0.013^b	0.051^g
100 ppm Ac.	0.079^b	0.048^{cd}	-	0.053^{abc}	0.07^{cde}	0.053^{efg}	-	0.037^a	0.008^b	0.05^{fg}
10% Suc + DI H2O	0.151^a	0.124^a	0.051^a	0.061^{abc}	0.109^b	0.097^c	047^a	0.042^a	0.063^a	0.093^d
10% Suc+25 ppm Ac.	0.158^a	0.131^a	0.053^a	0.065^a	0.122^a	0.114^a	0.05^a	0.043^a	0.073^a	0.101^a
10% Suc+50 ppm Ac	0.158^a	0.128^a	0.057^a	0.06^{abc}	0119^{ab}	0.109^{ab}	054^a	0.046^a	0.069^a	0.099^b
10% Suc+ 75 ppm Ac	0.155^a	0.123^a	0.049^a	0.056^{abc}	0.112^{ab}	0.104^{abc}	045^a	0.049^a	0.063^a	0.094^c
10% Suc+100ppm Ac.	0.153^a	0.121^a	0.051^a	0.064^{ab}	0.114^{ab}	0.101^{bc}	051^a	0.044^a	0.014^b	0.087^d
STS	0.082^b	0.075^b	-	0.053^{abc}	0.079^c	0.067^d	0.05^a	0.04^a	0.072^a	0.07^e
Florissant 500	0.075^b	0.045^{cd}	-	0.052^{abc}	0.068^{de}	0.051^{fg}	-	0.046^a	0.014^b	0.05^{fgh}

Suc.= sucrose   Ac.= Accell

x- To all holding solutions except deionised water, STS and Florissant 500, 0.5% NaOCl and 150 ppm citric acid were added.

y- Mean separation within columns (except the last column) by Tukey's procedure. Means assigned the same letter are not significantly different at P=0.05.

z- Mean separation within the last column by mean -+ SD Means assigned the same letters are not significantly different at P=0.05.

Values in the last column are averaged for the entire period of determination.
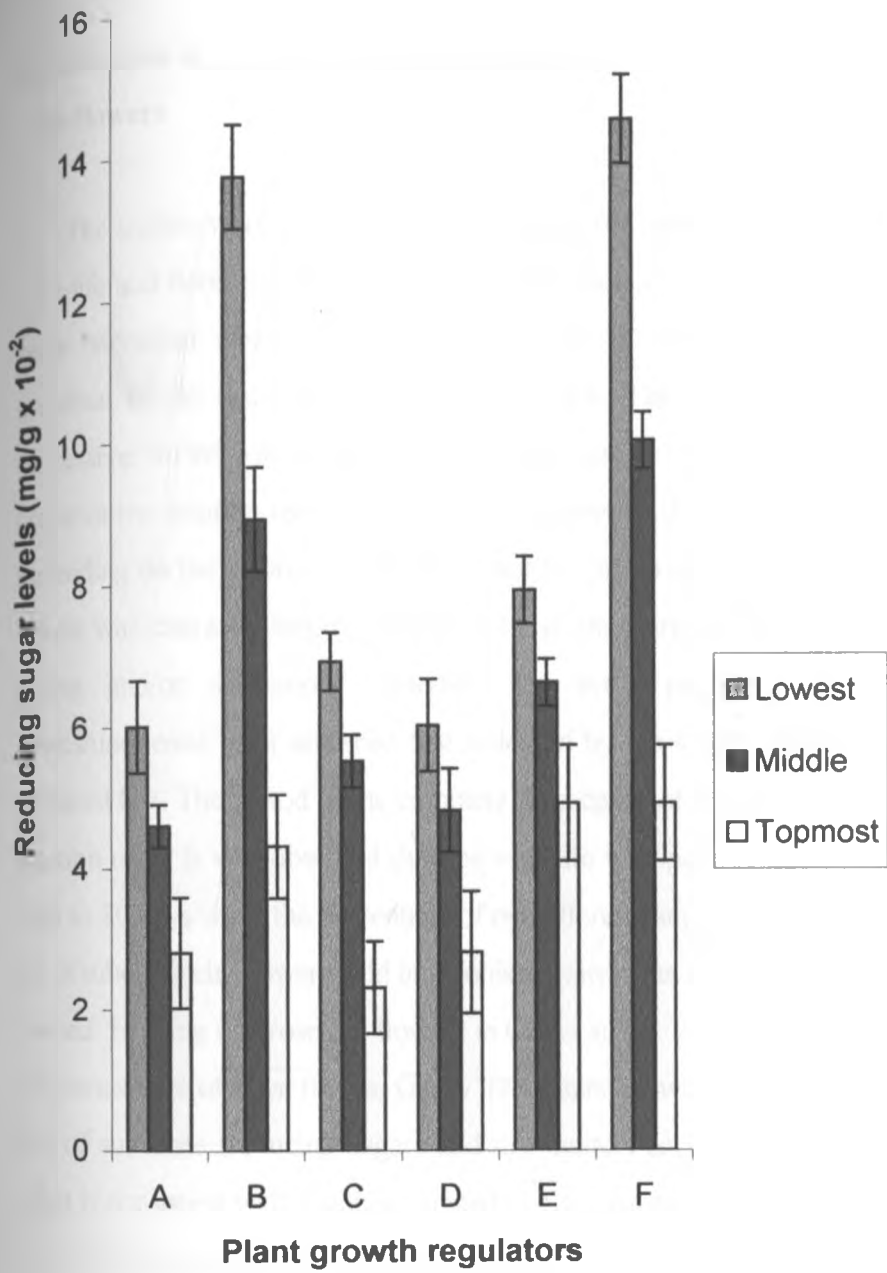
Values are averaged for time period:
    Lowest- 0,3,6 days
    Middle- 0,3,6,9 days
    Topmost- 0,3,6 days

A= Deionised water   E=STS
B= 10% sucrose   F=10% sucrose + 25 ppm Accel
C= 25 ppm Accel   D= 2.5 ppm GA 4+7

Fig 6.0 Effect of floret position on the reducing sugar levels (mg/g) of the peta etals of tuberose cut flowers

5.0 DISCUSSION

5.1 Influence of the plant growth regulators on the vase-life and floret opening of tuberose cut-flowers

The studies were undertaken to investigate the influence of the plant growth regulators on the vase-life and floret opening of tuberose cut-flowers. Tuberose cut-flowers used in the experiments were harvested when the lowest florets opened. Opening of the florets proceeded from the proximal to the distal end of the inflorescence. The time interval between the opening of the successive florets varied greatly depending on the maturity of the individual floret and the preservative solution used. Likewise, the number of days to the senescence of each floret varied depending on the position of the floret and the preservative solution used. Senescence of tuberose florets was characterised by the yellowing of the florets, then browning and ultimately complete drying and/or abscission. Generally, for every preservative solution used, the topmost florets/unopened buds senesced first followed by the lowest florets and finally the middle florets senesced last. The period to the complete senescence of all the florets differed with the preservative solution used. It was observed that the vase-life was increased by a period ranging from about 13 days to 20 days while the percentage of open florets ranged from 63% to 88%.   The vase-life of tuberose cut-flowers held in deionised water was about 13 days and about 63% of the florets opened Holding tuberose cut-flowers in $GA_{4.7}$ at 2.5, 7.5 and 10.0 ppm did not affect the vase-life and percentage of open florets. $GA_{4.7}$ treatments likewise did not affect the water balance and the rate of substrate (reducing sugars and dry matter) accumulation and rate of depletion. This GA effect is consistent with results presented in other cut-flowers by other researchers.

In chrysanthemums, 1-9 ppm $GA_{4.7}$ and silver nitrate with or without sucrose and $\tilde{GA}_{4+7}$ alone was not efficient in extending the vase-life (Garibaldi and Deamborgia, 1988). Holding iris cut-flowers in 10^{-6} g/l $GA_{4.7}$ was as effective as holding in water (Swart, 1985). Treatment of *Lilium longiflorum* bulbs with 100 ppm $GA_{4.7}$ or GA_3 reduced the number of florets initiated, completely inhibited the initiation of secondary florets and increased the number of primary florets which aborted shortly after initiation (De Hertogh and blakely, 1972). Gibberellic acid treatment also had no beneficial effect in *Liatris spicata* (Perez *et al.*, 1986), gladiolus (Nunes, 1989) and hybrid *Limonium* (Doi and Reid, 1995). The use of GA_{4+7} as a post harvest preservative in flowers is a

relatively new procedure (Hutchinson, personal communication). However, other GAs have been widely used.

Application of GA_3 has also been reported to be ineffective in prolonging the vase-life of cut-flowers. Application of GA_3 at 200 ppm to isolated carnation petals grown in agar or liquid medium delayed their senescence (Garrod and Harris, 1978) but treatment of the whole plant with 0.1 to 200 ppm had little or no effect on longevity (Nichols, 1968; Wong *et al.* 1989). Similarly, Cywinska-smother *et al.*, (1978) reported that GA_3 at 100-400 mg/l with either 200 mg/l hydroxyquinoline sulphate (8-HQS) and 5% sucrose or 200 mg/l silver nitrate and 10% sucrose stimulated bud opening but decreased the longevity of open florets and caused discolouration of petals, leaves and calyxes. In orchids, Arditti *et al.*, (1971) reported that GA_3 at higher concentrations induced the post-pollination phenomenon, which is characterised by stigmatic closure, swelling and loss of curvature of the column, wilting of perianth, deformation of the calli and anthocyanin production.

Unlike $GA_{4.7}$ treatments, cut-flowers treated with Accell^{TM} solutions (25 and 50 ppm) had a longer vase-life and higher percentage of open florets, the lower concentration being the most effective, increasing the vase-life by 3 days and floret opening by about 11%. The Accell^{TM} solution used in the experiments contained BA and gibberellic acid ($GA_{4.7}$) at ratio 10 to 1. Preliminary studies on the vase life and floret opening of tuberose cut-flowers between Accell^{TM} and BA revealed that there was no significant difference between these treatments. Therefore, as $GA_{4.7}$ had no effect, it is suggested that BA is the active component of the Accell^{TM} solution, and is responsible for the enhanced vase-life and floret opening observed in tuberose cut-flowers held in 25, 50 and 75 ppm Accell^{TM} solutions.

The higher Accell^{TM} concentrations however had no effect on the vase-life and floret opening of tuberose cut-flowers. The high concentrations probably became toxic, as evidenced by the appearance of the leaves and the stems of the cut-flower. Whereas the leaves and stems of cut-flowers held in 25, and 50 ppm Accell^{TM} remained green throughout the period of experiment, dark necrotic spots appeared on the leaves and lower parts of the stems of cut-flowers held in 100 ppm Accell^{TM}. This toxicity effect of high Accell^{TM} concentration is consistent with findings that supraoptimal levels of kinetin induced ethylene production in excess of that produced by control flowers thereby reducing the vase-life of carnation flowers (Eisinger, 1977).

The increase in the percentage of open florets and vase-life of tuberose cut-flowers by BA

treatment is consistent with findings presented in other cut-flowers. A 30% increase in vase-life was reported when carnations were treated with 225 ppm BA for 2 minutes or 22.5 ppm for 12 hours (Heide and Oydvin, 1969). N^6-benzyladenine also inhibited respiration and retarded senescence in chrysanthemum and carnation flowers (Heide and Oydvin, 1969). Application of BA increased the petal area and number of primary petals produced in carnations (Jeffcoat, 1977). Likewise, kinetin significantly delayed senescence in flowers with stem and leaf tissue removed and also in cut carnation flowers with intact stems (Eisinger, 1977). Further evidence of the cytokinin enhancement of vase-life of carnation cut-flowers was reported by Cook *et al.*, (1985); Cywinska-Smother *et al.*, (1978); Goszczynska and Nowak (1979), Mayak and Dilley (1976a), Paulin and Muloway, (1979), Staden and Bosse (1989), Upfold and Staden (1990) and Wong *et al.*, (1989).

Cytokinins have also been reported to delay senescence in *Alstroemeria* (Dai and Paull, 1991), *Anthurium* (Paull and Goo, 1985; Shirakawa *et al.*, 1964), chrysanthemums (Maclean and Dedolph, 1962), daffodils (Ballantyne, 1966), *Gerbera* (Van Meeteren and Van Gelder, 1980), in excised florets of hyacinth (Wang and Baker, 1979), iris (De Munk and Gijzenburg, 1977; Vonk *et al.*, 1986; Wang and Baker, 1979), *Leucospermum* (Napier *et al.*, 1986) and tulips (Systema, 1981).

The vase-life and floret opening of tuberose cut-flowers was also improved by a 24 hour, 10% sucrose pulse treatment. Pulsing of the cut-flowers also improved the effectiveness of the lower Accell[TM] concentrations (25 and 50 ppm). Whereas the vase-life was improved by Accell[TM] (25 and 75 ppm) by 3 and 2 days respectively and floret opening by 9%-11% in the absence of sucrose, pulsing increased the longevity of Accell[TM]-held flowers by between 6 and 7 days and floret opening by between 20% and 22%. The cut-flowers also had a longer vase-life and higher percentage of open florets, compared to sucrose and Accell[TM] treatments alone. It is therefore suggested that pulsing in sucrose before holding in the Accell[TM] solutions conferred an additive effect on the cut-flower, enhancing its effect in relation to holding in Accell[TM] and sucrose separately. This additive effect is attributed to the BA component of the Accell[TM] solution. Indeed, other researchers have reported additive effects of BA and sucrose on the vase-life of cut-flowers.

A short-term (2 min. at 225 ppm or 12 hours at 22.5 ppm) immersion of carnation stems in BA solutions increased the vase-life by about 30%, compared to those held in water (Heide and Oydvin, 1969). Addition of 5% sucrose and acetic acid to lower the pH to 3.5 resulted in even greater increase in vase-life than BA treatment alone (Heide and Oydvin, 1969). Similarly, Jeffcoat

(1977) reported an increase in the vase-life of carnation flowers treated with BA and 4% sucrose, compared to sucrose and untreated flowers. A 8-day increase in vase-life was observed in carnation cut-flower treated with 5 mg/l BA, 5% sucrose and 300 mg/l 8-HQS, in relation to cut-flowers treated with 5% sucrose and 300 mg/l 8-HQS (Cook et al., 1985). The longevity of carnations was also enhanced by solutions containing 50 mg/litre kinetin and 5-10% sucrose, compared to kinetin and sucrose alone (Mayak and Dilley, 1976a).

However, pulsing in 10% sucrose before holding in 75 and 100 ppm Accell[TM] had no effect as the vase-life and floret opening was not different from that of flowers treated with sucrose alone. This could be attributed to increased toxicity of the higher Accell[TM] concentrations.

A 4-day increase in vase-life and 13% increase in floret opening were observed when tuberose cut-flowers were pulsed for 24 hours, with 10% sucrose. Pulsing in 10% sucrose also resulted in a 4-day increase in vase-life and 17% increase in floret opening in tuberose (Watako, 1992). A sugar-containing vase preservative (1.5%) and/or pretreatment with sugars (20% sucrose for 15-20 hours) also improved the display life of tuberose cut-flowers before and after storage (Naidu and Reid, 1989). Increase in longevity and floret opening by a sucrose pulse has also been reported in *Anthurium* (Paull and Goo, 1982), brodiae (Han et al., 1990), carnation (Aarts, 1957; Nichols, 1968, 1973), chrysanthemums (Borochov et al., 1975; Kofranek and Halevy, 1972), dendrobium (Ketsa and Boonrote, 1990), freesia (Woodson, 1987), gladiolus (Bravdo et al., 1974), gypsophila (Downs et al., 1988; Farnharm, 1975); Lily-of-The-Nile (Mor et al., 1984), *Limonium* (Shillo and Halevy, 1980), roses (Sacalis, 1973) and *Strelitzia* (Halevy et al., 1978).

In this study, the vase-life and floret opening of tuberose cut-flowers was improved by one hour STS pretreatment. The STS pre-treatment in fact proved to be the best method of handling tuberose cut-flowers, as it resulted in a 7- day increase in vase-life and 26% increase in floret opening. This finding is consistent with that of Watako (1992) who reported an increase in the vase-life from 10 days to 23 days and 38% increase in the floret opening by STS pretreatment of tuberose cut-flowers. Similarly, a 60% to 120% increase in vase-life and a substantial reduction in bud abscission (upto 20% of the control) was reported when Lily-of-the-Nile cut-flowers were pretreated with STS as a basal pulse (Mor et al., 1984). More florets of Lilium 'Enchantment' also reached full bloom and the vase-life was improved by a STS pretreatment (Swart, 1980). A short-term (20 minutes) treatment of miniature carnations or overnight treatment with 1 mM STS extended the carnation vase-life as much as continuous use of preservative 'Everbloom' which

contains sucrose, weak acid and certain other components (Reid *et al.*, 1980). Five minutes to 24 hour base treatment of STS (1-4 mM) was also effective in *Alstroemeria* (Chepkairor, 1986; Staby, 1984), *Anthurium* (Paull and Goo, 1982), carnations (Veen and van de Geijn, 1978), gladiolus (Mor *et al.*, 1981), gypsophila (Downs *et al.*, 1988), lilies (Nowak and Mynett, 1985), roses (De Stigter, 1980; Goszczynska and Reid, 1985) and sweet pea (Mor *et al.*, 1984).

Florissant 500^{TM} had no effect on vase-life and floret opening of tuberose cut-flowers. Florissant 500^{TM} has T-Chloramine (a chlorine-based compound) at 50 mg per tablet as the active ingredient (Florissant sales B.V). Although chlorine is a bactericide, in the present study, Florissant 500^{TM} did not increase the rate of water uptake, relative to the control. This could be attributed to the lack of effect of Florissant 500^{TM} on physiological plugging which could have occurred leading to a reduction in water uptake. Florissant 500^{TM} also did not have an effect on the rate of assimilate uptake and subsequent depletion. This could also be attributed to Florissant 500^{TM} inability to counteract ethylene effect, which could have accelerated the rate of depletion of assimilates.

In summary, STS was the most effective preservative in prolonging the vase-life and improving the opening of the florets of tuberose cut-flowers. Pulsing with sucrose also enhanced the postharvest longevity and increased the effectiveness of the Accell^{TM} treatments while GA$_{4-7}$ and Florissant 500^{TM} were ineffective. It is suggested that the effectiveness of these preservatives (sucrose, Accell^{TM} and STS) is due to an improvement of water balance of the tissues, improvement in the accumulation and/or mobilisation of assimilates and delay in the rate of depletion of assimilates or delay in ethylene production and/or action.

5.2 The influence of plant growth regulators on the water balance of tuberose cut-flowers

The vase-life and percentage of open florets of tuberose cut-flowers was influenced by changes in the rate of water uptake and transpiration. Experiments were conducted to determine the rate of water uptake and transpiration water loss of tuberose cut-flowers. It was observed that a decline in water uptake and transpiration occurred with time. This is consistent with the observations of Carpenter and Rasmussen (1973), De Stigter (1980) and Mayak *et al.*, (1974) that water uptake and water loss fluctuates cyclically with an overall-declining trend.

The rate of decline in water uptake differed with the holding solutions. At the start of the experiment (day 2), cut-flowers which were pulsed in 10% sucrose had a significantly higher rate of

water uptake than unpulsed flowers. Among the unpulsed flowers, there was no significant difference in water uptake at the start of the experiments. From the 4th day to the end of the experiment period however, cut-flowers held in $GA_{4,7}$, Florissant 500[TM] and deionised water had significantly lowest rates of water uptake. Cut-flowers held in Accell[TM] had significantly higher rates of water uptake than cut-flowers held in either deionised water or $GA_{4,7}$ (except 100 ppm Accell[TM] from the 10th day), though the rates were significantly lower than in cut-flowers pretreated with STS or pulsed in 10% sucrose.

The decline in water uptake observed in tuberose cut-flowers could be attributed to a decrease in the potential gradient or to an increase in flow resistance, as suggested in *Gerbera* (Van Meeteren, 1978)

The increase in flow resistance could be directly caused by stem plugs by micro-organisms or indirectly through the release of metabolites into the water by the microbes, which block the xylem vessels Microbial growth has been found to parallel the increase in stem resistance to water flow (Larsen and Frolich, 1969). Reduction in water uptake has also been attributed to a build-up of bacterial growth in the vascular tissues of floral stalk (Halevy and Mayak, 1981; Kofranek and Paull, 1972). Similarly, it has been demonstrated that metabolites produced by certain bacteria reduce water conductivity and longevity in carnations (Aarts, 1957; Accati *et al.*, 1981; Mayak and Accati-Garibaldi, 1979).

Sterile water and germicides have been used to control microbial growth and partially decrease the resistance to water flow (Aarts, 1957; Burdett, 1970; Marousky, 1969; Van Meeteren, 1978). In the present study, 0.5% NaOCl was used as a germicide in all treatments except in deionised water, STS and Florissant 500[TM] Sodium hypochloride has also been used as a germicide in tuberose (Ngamau 1992; Watako 1992), gladiolus (Marousky, 1969) and roses (Van Doorn and Perik, 1990). In addition to NaOCl, 150 ppm citric acid was used to lower the pH of the solution to 3-4, thus inhibiting microbial growth (Halevy and Mayak, 1981). Citric acid has been found to be effective in tuberose (Ngamau, 1992; Watako, 1992), roses (Durkin, 1979), chrysanthemums (Kofranek and Halevy, 1972) and *Strelitzia* (Halevy *et al.*, 1978). Thus in the present study, owing to the use of NaOCl, citric acid, STS and Florissant 500[TM] in all the treatments except deionized water, it is doubtful that the increase in the stem blockage was due to microbial occlusions.

In the absence of microbial blockage, the increase in resistance of the tuberose cut-flower stem to water flow could be due to air embolism. Air entering the base of cut stems has been recognised

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as a major factor hindering the rehydration of flowers and Durkin (1979) reported that removing air from water by vacuum increased water flow rates through rose stem sections. Similarly, air embolism in rose stems was effectively overcome by a number of techniques, including acidification of the vase solution and recutting of the stem under water (Durkin, 1981). In the determination of water uptake in the present experiment, the cut ends of the inflorescence were exposed to air when the cut-flower stem was femoved from the solution thus causing air embolism. However, recutting of the stem was carried out and this, together with acidification of the vase solutions (except in STS, Florissant 500^{TM} and deionised water) may have eliminated the air embolism

In the absence of microbial blockage and air embolism, the increase in flow resistance of tuberose cut-flowers could be due to physiological plugs in the stems. Vascular blockages were suggested to be a result of oxidative processes induced from harvesting injury (Durkin and Kuc, 1969). The vascular occlusions could be gummy substances (Aarts, 1957), pectinaceous or carbohydrate (Burdett, 1970; Parups and Molnar, 1972) or could be composed of breakdown products of cell walls (Rasmussen and Carpenter, 1974).

Vascular occlusions could also be due to ethylene-stimulated production of gums at the cut surface. The cut surface of rose stems was reported to produce considerable amount of ethylene (Van Doorn *et al.*, 1980). Cutting of *Anthurium* stems at harvest or recutting later was found to induce ethylene production (Paull and Goo, 1985). Ethephon treatment of *Prunus cesarus* resulted in the blockage of xylem vessels (Olien and Bukovac, 1982). Ethylene also induced vascular plugs in maidenhair ferns (Fujino and Reid, 1983) and *Ricinus commumis* (Vandermolen *et al*, 1983). In tuberose cut-flowers, it is suggested that the reduction in water uptake observed may be due to ethylene stimulated vascular occlusions. This is because cut- flowers treated with preservatives which inhibit ethylene production and/or action (BA, STS and sucrose) had an increased rate of water uptake and longer vase-life while cut-flowers which have no effect on ethylene (deionised water, Florissant 500^{TM} and GA$_{4-7}$) had reduced rates of water uptake and senesced faster.

Like the decline in water uptake, the rate of decline in transpiration water loss differed with the holding solutions. Generally, cut-flowers treated with Accell^{TM} and the BA equivalents had the highest transpiration rate. Cut-flowers pulsed in sucrose initially had the lowest transpiration rate until the 6th day from when the transpiration rate was not significantly different from the control. From the start of the experiment, there was no significant difference in the transpiration rate between cut-flowers pretreated in STS or cut-flowers held in GA$_{4-7}$ and deionised water. Since

there was no significant difference between $GA_{4,7}$ and the control, the increase in the transpiration rate of Accell[TM] is attributed to the BA effect. Indeed, increase in the transpiration rate by cytokinin treatment has been reported in roses (Mayak and Halevy, 1974) who attributed this to an increase in the opening of the stomata by the cytokinin treatment.

Comparison between water uptake and transpiration of tuberose cut-flowers revealed that from the start of the experiments to the 6th day, water uptake was higher than transpiration. This created a favourable water balance allowing retention of water in the tissues leading to the maintenance of fresh weight and cell turgidity. A high level of turgidity is necessary for the growth and expansion of the petals leading to the opening of the florets and delay of flower senescence (Rogers, 1973).

From the sixth day however, cut-flowers held in deionised water, Florissant 500[TM] and GA $_{4,7}$ had a higher transpiration rate than water uptake. The rate of transpiration exceeded water uptake in cut-flowers held in Accell[TM] in the 8th day while flower pulsed in sucrose and transferred to deionised water, 75 and 100 ppm Accell[TM] had a higher transpiration rate from the 10th day. Flowers pulsed in 10% sucrose and held in 25 and 50 ppm Accell[TM] and flowers pretreated with STS had a higher transpiration rate than water uptake from the 12th day. The significance of a higher transpiration rate than water uptake is that it induces water deficit, which reduces the turgidity of the petals. Water deficit also accelerates processes connected with senescence of intact plants as well as detached plant parts, including flowers (Gates, 1968; Livne and Vaadia, 1972). This probably occurred in tuberose flowers since the flowers which developed water deficit earliest had the shortest vase-life while those which developed water deficit later had increased longevity. The enhanced water balance in cut-flowers held in Accell[TM] therefore nullifies the significance of the increased transpiration rate observed, since the net water balance was higher. Similarly, as the water balance of $GA_{4,7}$ treatments was similar to that of the controls, the enhanced water balance observed in Accell[TM] treatments is attributed to the BA component. Indeed, other researchers have also reported an enhancement of the vase-life of cut-flowers by cytokinin treatment through the maintenance of a favourable water balance.

In roses, Mayak and Halevy (1974) reported that kinetin enhanced the fresh weight increase in all flower parts and delayed the subsequent reduction in fresh weight by 1 day. Kinetin also promoted growth and expansion of the petals and maintained the petal turgidity for an extended period (Mayak and Halevy, 1974). Kinetin also enhanced the opening of stomata in the leaves of

cut roses thereby increasing transpiration (Mayak and Halevy, 1974). The higher rate of water uptake however more than compensated for the increased transpiration water loss thereby delaying senescence (Mayak and Halevy, 1974).

In carnation, Paulin and Muloway (1979) found that kinetin increased fresh weight and transpiration and suggested that since water uptake follows transpiration uptake, increase in transpiration increase the flowers' fresh weight. Carnation flowers treated with BA also did not show a change in fresh weight over a 10-day period while control flowers lost over 60% of their initial weight (Cook *et al.*, 1985). Application of BA to the flower buds of chrysanthemum at an early stage of development also increased the fresh weight (Jeffcoat, 1977). Addition of 4% sucrose to the BA solution further improved the effectiveness of BA in prolonging the turgidity of the petals (Jeffcoat, 1977). Similar effects of cytokinins on water balance have been reported in *Anthurium* (Paull and Goo 1985); carnation (Cook *et al.*, 1985) and *Gerbera* (Van Meeteren, 1979).

The BA effect on the water balance and consequently vase-life and percentage of open florets of tuberose cut-flowers could be linked to the protective effect of cytokinins on the cell integrity. In cut-flowers, the cell integrity is disrupted by an increase in the microviscosity and permeability of membranes (Borochov *et al.*, 1978) leading to ion leakage and water loss from the cells (Nichols, 1968; Van Meeteren, 1979). Treatment of *Gerbera* cut-flowers and probably tuberose with BA retarded the decrease in the petal water content and the increase in ion leakage from the cells (Van Meeteren, 1979). These effects could however be linked to BA effects on ethylene action since subtle changes in the membrane structure and function are induced by ethylene (Thompson *et al*, 1982) and both the site of conversion of ACC to ethylene as well as ethylene receptor sites are thought to be associated with cellular membranes (Abeles, 1973).

Apart from cytokinins, sucrose treatment has also been reported to increase the vase-life of flowers by maintaining a favourable water balance. The higher rate of water uptake in tuberose cut-flowers pretreated with sucrose in relation to untreated flowers was also reported by Naidu and Reid, (1989) and Watako (1992). Likewise, gladiolus shoots absorbed sucrose in increasing sucrose concentration of upto 40% in the holding solution (Bravdo *et al.*, 1974). Transfer of the shoots from the sugar solutions to water resulted in increased water uptake, which was dependent on the amount of sucrose previously absorbed (Bravdo *et al.*, 1974).

The transpiration rate of sucrose-pretreated tuberose cut-flowers was lower than that of the control. This could be attributed to a partial closure of stomata by sucrose-treated flowers resulting

in a reduction of water loss as was reported in carnations (Aarts 1957) and roses (Marousky, 1969, 1971). The partial closure of stomata reduced transpiration and ensured that water uptake was higher than water loss, thus maintaining the fresh weight and petal turgidity of the florets and delaying senescence of the cut-flower.

The effect of sucrose on the water balance of tuberose cut-flowers could be related to the sucrose effect on the osmotic concentration of the petal tissues. It is suggested that the supplied sucrose accumulated in the cells and increased the osmotic potential of the petals (Halevy, 1976) enabling the treated flowers to absorb and retain much more water than untreated flowers thus maintaining the turgidity of the expanding petals and consequently increasing the vase-life. The effect of sucrose on the osmotic concentration has also been reported in roses (Halevy, 1976; Halevy and Mayak, 1979).

The effect on sucrose on the water balance of tuberose cut-flowers could also be linked to the maintenance of metabolism resulting in a healthy flower with intact membranes. As in carnation flowers, the beneficial effect of sugars on the water balance could be attributed to the sugars' ability to maintain the mitochondria structure (Kaltaler and Steponkus, 1974) and membrane integrity (Aarts, 1957; Acock and Nichols, 1979; Coorts, 1975; Sacalis, 1973), and thus maintaining the turgidity of the petal tissues.

Besides BA and sucrose treatments, an improvement of the vase-life of cut-flowers through the maintenance of a favourable water balance has also been reported with STS pretreatment. Treatment of maidenhair fern with 0.25 mM STS increased the inflorescence fresh weight and improved water relations (Fujino *et al.*, 1983). Similarly, STS, in combination with sucrose or 8-HQS, maintained water uptake resulting in a 30-50% increase in carnation vase-life (Piskornik, 1981, 1985). In *Anthurium*, silver nitrate pulse reduced the rate of decline in water uptake in relation to untreated flowers (Paull and Goo, 1985).

The effect of STS on the water balance of tuberose cut-flowers could be attributed to Ag biocidal activity. Silver ions from the STS complex could be acting as a biocide in the floral stem base thus reducing or eliminating bacterial build up in the vascular tissue of cut stems as reported in carnations (Halevy and Mayak 1981; Kofranek and Paull, 1974) and *Anthurium* (Paull and Goo, 1985). The Ag could also be interfering with wound ethylene binding sites at the stem base (Sisler, 1982). Pulse treatment of cut *Anthurium* stems and probably tuberose with Ag inhibited ethylene-induced vascular occlusions (Paull and Goo, 1985). This resulted in increased water uptake

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enabling more water to reach the distal buds thus promoting cell enlargement and floret opening. The increased water uptake also helped in the redistribution of absorbed substrates thus strengthening the capability of the young floret sinks resulting in better opening.

5.3 Influence of the plant growth regulators on the accumulation and utilisation of dry matter

As the water uptake and transpiration water loss of tuberose cut-flowers reduced, an increase in the dry weight was observed. The highest dry weights were however observed in the 3rd day in the petals of the lowest and middle florets. In the petals of the topmost florets/unopen buds, the highest dry weights were observed in the 6th day. Cut-flowers pulsed in 10% sucrose had the highest dry weights while flowers held in deionised water, Florissant 500^{TM} and GA_{4-7} had the lowest. The dry weights of cut-flowers held in Accell^{TM} or the BA equivalents and flowers pretreated with STS were higher than the dry weights of flowers held in deionised water and GA_{4-7} but lower than in flowers pulsed in 10% sucrose. The trend of change in dry weight was observed to be almost similar to the trend of change in reducing sugars.

5.4 Influence of the plant growth regulators on the accumulation and utilisation of sugars in tuberose cut-flowers

In addition to water balance, the rate of accumulation and utilisation of substrates may have influenced the vase-life and percentage of open florets of tuberose cut-flowers. In the present study, changes in the amounts and rate of depletion of sucrose and reducing sugars were determined. The changes in the rate of depletion of these assimilates could be used as an indication of the change in respiration because in cut-flowers, assimilates are utilised to provide the energy and carbon skeletons required for bud opening (Doi and Reid, 1995) and also to maintain respiration in the open florets thus prolonging their longevity (Coorts, 1975, Rogers, 1973).

Tuberose cut-flowers used in the experiments were either pulsed in 10% sucrose for 24 hours before being transferred to the holding solutions or were directly held in the holding solutions without a sucrose pulse. Analysis of the sucrose levels revealed there was no significant difference in the sucrose levels in the petals of the florets of all the treatments in all days of determination,

except in day 0 in the lowest florets and day 3 in the middle florets. The sucrose levels in the petals of the lowest florets of pulsed cut-flowers were highest in day 0 but declined in subsequent days while in unpulsed cut-flowers, the sucrose levels were consistently low. In the petals of the middle and topmost florets, the sucrose levels increased to a maximum at day 3 and then declined to negligible levels. Comparison between sucrose and reducing sugar in the petals of the lowest florets revealed that the sucrose levels were far much lower than reducing sugars throughout the entire period of determination. In the petals of the middle and topmost florets/buds, the sucrose levels were initially higher but declined as the reducing sugars increased. Thus, at petal wilting, a substantial amount of reducing sugar remained while sucrose almost disappeared. These observations are consistent with findings reported in carnations (Nichols, 1973; Weinstein, 1957), roses (Ho and Nichols, 1977) and narcissus (Nichols, 1973). In narcissus cv 'Actaea' it was observed that the ratio of reducing sugar to sucrose depended on the stage at which the cut-flower was harvested (Nichols, 1973). When the flower was cut at the 'goose neck' stage (when the bud is reflexed at right angles to the stem), reducing sugar in the buds increased to a maximum level which roughly coincided with full opening (Nichols, 1973). After flower opening, sucrose disappeared as the reducing sugars were accumulating in the corolla (Nichols, 1973). The reducing sugars subsequently decreased until half the maximum content remained at wilting of the corolla (Nichols, 1973).

In the present study, since sucrose was used for pulsing and not reducing sugars, it is suggested that a rapid inversion of sucrose to reducing sugars probably occurred in the petal tissues. Indeed, reducing sugars rather than sucrose have been demonstrated to be the main constituents of sugar pools in mature petal (Nichols, 1973; Weinstein, 1957) and flower petal tissues have been demonstrated to contain high activity of invertase (Hawker *et al.*, 1976). This rapid inversion of sucrose to reducing sugars in the petals probably explains the lack of significant difference in the levels of sucrose observed between the treatments in tuberose cut-flower.

Tuberose cut-flowers bear a large number of florets, which develop sequentially from the proximal to the distal end of the inflorescence. Analysis of the amounts of assimilates (reducing sugars and dry matter) accumulating in the petals revealed a sequential pattern since the lowest florets accumulated the highest amount of assimilates followed by the middle florets while the topmost floret/buds accumulated the least. This reveals the competitive nature of the tuberose florets for assimilates. It is suggested that the lowest floret accumulated the highest amount of

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assimilates due to its closest proximity to the assimilate source (leaves and pulsing solutions) and extra assimilates were translocated to successive florets only when the lowest floret had accumulated adequate assimilates to ensure opening. Thus, the amount of assimilates available to the cut-flower determines the proportion of buds which will open. In the present study, pulsed cut-flowers accumulated a higher level of assimilates in all the florets, in contrast to unpulsed cut-flowers. This probably explains the higher percentage of open florets observed in pulsed flowers. Similar observations have been reported in tuberose (Naidu and Reid 1989; Watako 1992), gypsophila (Farnharm, 1975) and in hybrid *Limonium* (Doi and Reid 1995). Likewise, Spikman (1989) demonstrated that the lack of opening and death of the small buds at the apex of freesia inflorescence is mainly due to the deficiency of carbohydrates.

The tuberose cut-flowers used in the experiments were harvested when the lowest floret opened. The opening of the middle floret occurred between the 3rd and 6th day while that of the topmost florets occurred after the 6th day. In the determination of reducing sugar and dry weight in the petals of the middle and topmost florets, it was observed that florets which opened (all the middle florets and sucrose and STS-pretreated top florets), had the highest amount of reducing sugar and dry weight in the 6th day. The period of opening of the florets thus coincided with the period when the reducing sugar and dry matter levels were highest. This support the postulation that assimilates are utilised for bud opening and that a particular amount of assimilates is reguired to facilitate opening.

A decline in the levels of reducing sugar and dry weight of the petals was observed as senescence progressed. The decline could be attributed to the depletion of assimilates with time. In tuberose cut-flowers, the rate of depletion of assimilates differed with the preservatives used. Generally, sucrose and STS-pretreated cut-flowers had a reduced rate of assimilate depletion. This is consistent with the findings of Nichols and Ho (1975) in carnations that a flower ageing in water lost about 20% of its dry weight in 5 days while supplying 4% sucrose in the feeding solution reduced the loss to 8% during the same period. The extended longevity of pulsed cut-flowers is therefore attributed to the maintenance of the pool of dry matter and respirable substances in the petals thus delaying senescence as reported by Coorts, (1975) and Rogers, (1973).

In addition to the depletion of assimilates from the petals through respiration, a reduction in the amount of assimilates could be caused by the translocation of assimilates from the petals to the ovary as was demonstrated in senescing carnations (Nichols, 1976; Nichols and Ho, 1975) and

orchids (Hsiang, 1951). This translocation is promoted by pollination (Hsiang, 1951) and ethylene (Nichols and Ho, 1975). Though tuberose cut-flowers have been reported to be insensitive to ethylene (Naidu and Reid, 1989), senescence in flowers increase with flower age due to the sensitivity of petals to ethylene (Barden and Hanan, 1972; Marousky and Harbough, 1979; Woodson *et al.*, 1985). Thus, as senescence of tuberose cut-flowers progressed, ethylene production may have been stimulated. This in turn promoted the translocation of the assimilates from the petals to the ovary accompanied by petal wilting. Exogenous sugar treatments have been reported to delay the onset of autocatalytic ethylene production (Dilley and Carpenter, 1975) and flowers supplemented with sucrose senesced less rapidly when treated with ethylene than untreated flowers (Mayak and Dilley, 1976a). This sucrose effect on ethylene production probably explains why pulsed tuberose cut-flowers had a lower rate of depletion of substrates in the petals and consequently longer vase-life.

Inspite of assimilate loss through respiration and/or translocation from the petals to the ovary, a substantial amount of reducing sugars remained in the petals at the end of the determination period. This shows that petal wilting in tuberose cut-flower may not be caused entirely by the depletion of respirable substrates or ethylene-induced translocation of assimilates to the ovary. The presence of the pool of reducing sugars at petal wilting may be due to the progressive inability of the mitochondria to utilise substrates as was demonstrated in roses (Kaltaler and Steponkus, 1974) and carnations (Sacalis and Chin, 1977), due to a change in the mitochondria integrity, which affects its functional capacity (Kaltaler and Steponkus, 1974). The main effect of applied sugars in carnations and roses and probably in tuberose was to maintain mitochondria structure and functions (Kaltaler and Steponkus, 1974; Sacalis and Chin, 1977). This may account for the higher amount of substrates and the resultant higher percentage of open florets and longer vase-life observed in pulsed cut-flowers, in relation to unpulsed flowers. However, the effect of sugar on the mitochondria may not be a specific effect and may stem from the general protective effect of sugars on the membrane integrity (Santarius, 1973).

Although unpulsed tuberose cut-flowers generally accumulated a lower amount of assimilates (reducing sugars and dry matter) than pulsed flowers and the rate of depletion was more rapid, differences were observed between the preservatives on the actual amounts and rate of decline. In relation to untreated cut-flowers or $GA_{4,7}$ treatments, Accell[TM] at 25, 50 and 75 ppm or the BA equivalents increased the level of dry matter and reducing sugars accumulating in the petals and

delayed the rate of depletion. This enabled more substrates to be utilised for opening of the flower buds and maintained respiration in the open flower for a longer period, hence accounting for the increased percentage of the open florets and longer vase-life observed in these treatments in relation to the control.

The increase in the amount of assimilates by Accell[TM] and BA equivalent treatments was probably achieved through the strengthening of the sink activity of the petals leading to an increase in the movement of assimilates to the flower, as was reported iris (De Munk and Gijzenberg, 1977), roses (Halevy, 1987) and tulips (Nowak and Rudnicki, 1979). Likewise, the slowing down of the rate of depletion of assimilates is consistent with findings reported in carnations (Paulin and Muloway, 1979), *Leucospermum* (Napier *et al.*, 1986) and roses (Halevy and Mayak, 1975). This could be attributed to a reduction in the respiration rate by BA treatment thus enabling respiration to proceed for a longer period of time resulting in a longer vase-life. Indeed, the respiration rate of carnations was reduced by a BA treatment through a reduction in glycolysis (Paulin and Muloway, 1979). Likewise, BA treatment reduced the respiration rate of *Anthurium* (Shirakawa *et al.*, 1964), carnations and chrysanthemums (Maclean and Dedolph, 1962) and daffodils (Ballantyne, 1966).

The amount of substrates accumulating in the petals and the subsequent rate of depletion in tuberose cut-flowers was also influenced by STS pretreatment. The STS effect could be attributed to its biocidal effects leading to an increased water uptake. The increased water uptake then helped in the redistribution of the absorbed substrates thus strengthening the capability of the young floret sinks resulting in better opening. The STS pretreatment also may have reduced the petals' respiration rate as reported in carnations (Veen, 1979) thus reducing the rate of depletion of assimilates and consequently delaying senescence.

5.5 Interaction of the plant growth regulators with ethylene

In addition to the improvement of water balance and assimilate utilisation by BA, sucrose and STS, the increase in the vase-life and floret opening of tuberose cut-flowers could be attributed to the antagonism of ethylene production and/or action. Although ethylene production was not determined in the present study, ethylene has been reported to induce abortion or lack of full opening in flower buds of brodiae (Han *et al.*, 1990), lilium Enchantment' (Swart, 1980) and freesia (Spikman, 1987). Ethylene also accelerates floret yellowing and/or abscission in snapdragon

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(Farnharm *et al.*, 1980), *Delphinium, Euphorbia* 'scarlet plume' and sweet pea (Nowak and Rudnicki, 1990).

In tuberose cut-flowers, conflicting roles of ethylene on flower opening and vase-life have been presented. Tuberose florets were reported to produce very little ethylene and to be unaffected by exposure to exogenous ethylene (Naidu and Reid, 1989). This is in contrast with findings by Khondakhar and Muzandur (1985) that floret and bud abscission were reduced and a 4-day increase in vase-life observed when tuberose cut-flowers were treated with 3% sucrose, 0.03% HQC and 0.01% silver nitrate. Likewise, Watako (1992) reported an improvement in floret opening and vase-life after pretreatment with STS.

A possible ethylene effect on untreated tuberose cut-flowers senescence could be through the induction of vascular occlusions leading to a reduction in the rate of water uptake. Ethylene has been reported to induce vascular plugs in *Anthurium* (Paull and Goo, 1985), maidenhair ferns (Fujino and Reid, 1983), *Prunus cesarus* (Olien and Bukovac, 1982), *Ricinus communis* (Vandermolen *et al.*, 1983) and roses (Van Doorn *et al.*, 1980). By antagonising ethylene production and/or action, BA, sucrose and STS-treated cut-flowers reduced stem vascular occlusions hence maintaining increased water uptake and retention leading to maintenance of cell turgidity. This probably explains the increased percentage of flower opening and vase-life of cut-flowers treated by these preservatives.

In addition to effects on water balance, ethylene probably accelerates senescence in untreated tuberose cut-flowers through effects on assimilate uptake and utilisation. As in freesia inflorescence (Spikman, 1987), the young buds at the apex of tuberose cut-flowers probably produce considerable amount of ethylene. The ethylene synthesised in the young buds may then block the transport of assimilates to the buds inducing their abortion. Indeed, ethephon treatment in carnations inhibited the transport of ^{14}C-metabolites to the flower buds leading to the abortion of the flower buds (Halevy, 1987). The use of ethylene antagonists such as STS, sucrose and BA may have blocked ethylene production by the small buds at the apex of the inflorescence thus facilitating transport of water and substrates to these buds consequently increasing the percentage of open florets.

Ethylene could also promote senescence in tuberose cut-flowers by enhancing the translocation and accumulation of sugars and other inorganic materials in the ovary, leading to the loss of fresh and dry weight of the petals of untreated cut-flowers as reported in carnations (Nichols, 1973).

Ethylene could also induce an increase in the respiration rate of the untreated cut-flowers (Pratt and Goeschl, 1964) leading to a rapid depletion of carbohydrates. The treatment of tuberose cut-flowers with BA, STS and sucrose may therefore have reduced the ethylene effects on assimilate transport and utilisation and increased the percentage of open florets and vase-life. These effects of BA, sucrose and STS on ethylene action have also been reported by other researchers.

Pretreatment of carnation petals with 0.1 milimolar BA, kinetin and zeatin blocked their senescence by 8 days (Mor *et al.*, 1983). The normally enhanced wilting and increase in endogenous levels of ACC and ethylene production following exposure of the petals to ethylene was not observed in the BA pretreated carnation flower petals (Mor *et al.*, 1983). Cytokinin application also prevented ethylene or CEPA-induced flower blasting, reduced the peak ethylene production by presenescent flowers by 55% or more and resulted in a higher percentage of open florets (Eisinger, 1977). Similarly, BA has been demonstrated to prolong the presenescent stages during which flowers are unresponsive to ethylene (Halevy and Mayak, 1981). Cytokinins also maintain low levels of ACC and limits the conversion of ACC to ethylene (Eisinger, 1982; Paulin and Muloway, 1985). Cytokinins have also been demonstrated to decrease the sensitivity to ethylene and delay senescence in carnations (Eisinger, 1982; Mayak and Dilley, 1976a; Paulin and Muloway, 1979), iris (Vonk *et al.*, 1986), morning glory (Kende and Hanson, 1976) and tulips (De Munk and Gijzenberg, 1977; Hang and Rees, 1977).

The sucrose effect on ethylene action has also been widely reported. A high sucrose level in the holding solution (upto 16%) markedly delayed the onset of autocatalytic ethylene production in carnations (Dilley and Carpenter, 1975). Flowers supplemented with sucrose also senesced less rapidly when treated with ethylene than untreated flowers (Mayak and Dilley, 1976a). The sucrose effect could be linked to a reduction in the activity of Ethylene Forming Enzyme (EFE) (Apelbaum *et al.*, 1981) which catalyses the conversion of ACC to ethylene or through the restriction of free radicals which promote ethylene biosynthesis (Asada and Kiso, 1973). Sucrose effect could also be linked to CO_2 production as sucrose supply increases CO_2 production (Aarts, 1957; Dilley and Carpenter, 1975). Carbon dioxide is a competitive inhibitor of ethylene action (Burg and Burg, 1966, Smith and Parker, 1966). The sucrose effect could also be related to its interaction with other plant hormones. Sucrose enhances the ability of cytokinins in delaying the senescence of flowers (Cook *et al.*, 1985; Cywinska-Smother *et al.*, 1978; Mayak and Dilley, 1976a) and antagonises the effect of ABA in promoting the senescence of carnations (Mayak and Dilley, 1976b) and roses

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(Borochov *et al.*, 1976).

The effect of silver ions on ethylene action has also been reported. Silver ion is a potent and specific inhibitor of ethylene action (Beyer, 1975). Pretreatment of snapdragon with STS sufficient to provide 2.5 mM Ag per spike, prevented floret abscission caused either by ethylene or by the addition of ethephon to the solution (Farnharm *et al.*, 1980). The ethylene sensitivity was also reduced by STS treatment and floret longevity extended beyond that of control in *Pelargonium domesticum* (Deneke *et al.*, 1990). When applied alone, STS had no effect on roses but prevented the damage induced by ethephon (De Stigter, 1980). Normal and ethylene-induced floret shattering was also prevented in *Delphinium* and sweet peas (Shillo *et al.*, 1980).

6.0 CONCLUSIONS

This study was undertaken to investigate the effect of various plant growth regulators on the post harvest physiology of tuberose cut-flowers and to determine their possible modes of action. The specific objectives were to determine the influence of benzyladenine, gibberellic acid, sucrose, silver thiosulphate and Florissant 500[TM] on the vase-life, floret opening, rate of water uptake and transpiration and the rates of accumulation and depletion of dry matter (as determined from dry weight changes), sucrose and reducing sugar of tuberose cut-flower.

The study showed that gibberellic acid (GA_{4-7}) at 2.5, 7.5 and 10 ppm, Accell[TM] at 75 and 100 ppm and Florissant 500[TM] had no effect on vase-life and flower opening of tuberose cut-flower. On the other hand, Accell[TM] or BA equivalents at 25 and 50 ppm, 10% sucrose pulse and pretreatment with STS increased the vase-life and the percentage of open florets. The increase in the vase-life by the lower Accell[TM] concentrations was observed to be due to the BA effect because of the lack of GA effect.

Several possible modes of action of BA, sucrose and STS were observed to be responsible for the increase in vase-life and percentage of floret opening of tuberose cut-flowers.

The increase in the vase-life and floret opening by Accell[TM] and BA equivalents is attributed to the maintenance of a favourable water balance. Whereas in untreated flowers and in flowers held in GA_{4-7} water deficit occurred after 6 days, water deficit occurred in Accell[TM] and BA equivalents after 8 days. This is inspite of the increased transpiration rate. The treatments also reduced the rate of depletion of assimilates (dry matter and reducing sugars) thus allowing respiration to proceed for a long period of time. Another possible mode of action of Accell[TM] and the BA equivalents is through antagonism of ethylene action though this is not conclusive since the ethylene profiles were not determined.

Pulsing tuberose cut-flowers with 10% sucrose for 24 hours increased the vase-life and floret opening due to the maintenance of a favourable water balance. Sucrose also increased the pool of respiratory substrates in the petals thus delaying petal senescence. Sucrose pulse increased the effectiveness of 25 and 50 ppm Accell[TM] in delaying the senescence of tuberose cut-flowers since the vase-life and percentage of open florets of flowers pulsed in 10% sucrose and transferred to Accell[TM] concentrations was significantly higher than that of flowers held in Accell[TM] and sucrose separately.

Silver thiosulphate pretreatment of tuberose cut-flowers increased the vase-life and percentage of open florets by maintaining a favourable water balance. This could be attributed to STS biocidal activity or to a prevention of ethylene-stimulated vascular occlusions at the cut surface. STS also reduced the rate of depletion of assimilates from the petals thus allowing respiration to proceed for a longer period of time and consequently increasing the vase-life.

Overall findings

1. The vase-life and floret opening of tuberose cut-flower were increased by BA, STS and sucrose treatments while GA$_{4-7}$, and Florissant 500[TM] were not effective.

2. The increase in the vase-life and floret opening of tuberose cut-flower by BA, STS and sucrose was correlated to the maintenance of a favourable water balance, increase in the pool of respiratory substrates and/or reduction in the rate of depletion and antagonism of ethylene production and/or action.

3. An additive effect of sucrose and Accell[TM] was observed as the sucrose-Accell[TM] combination was more effective than both Accell[TM] and sucrose applied separately.

4. The lower concentrations of Accell[TM] were more effective than the higher concentrations in floret opening, water uptake, dry weights and reducing sugar levels.

7.0 RECOMMENDATIONS

The lower concentrations of Accell^{TM} (25 and 50 ppm) increased the floret opening in relation to the higher concentrations (75 and 100 ppm). However, before the lower BA levels could be recommended for the post harvest treatment of tuberose flowers by the farmers the cost-benefit ratio should be determined. Similarly, the combination of 10% sucrose and 25 ppm Accell^{TM} should be recommended as a postharvest treatment of tuberose cut-flowers after the cost benefit analysis has been undertaken.

As the best preservative observed from this study, STS use should be recommended in tuberose cut-flowers. However, as STS preparation may be complicated especially to the small-scale farmers who are the main producers of tuberose, Florissant 100^{TM} should be recommended as this contains STS as the active ingredient. This may however be a short-term measure since STS causes environmental heavy metal pollution.

Florissant 500^{TM} had no effect on the longevity and opening of tuberose cut-flowers. It is recommended that the use of Florissant 500^{TM} be abandoned by farmers and other chemicals such as BA, sucrose or Florissant 100^{TM} be adopted instead.

Further research work needs to be done to supplement the present pool of information on the post harvest physiology of tuberose cut-flowers. This include work on the following aspects:

1 Investigation on the ethylene profiles at various floret positions at various stages of ageing. This will deal conclusively with the question of whether ethylene plays a role in tuberose cut-flower senescence or not.

2. The amount of substrates (especially sucrose and reducing sugar) present in other flower parts such as the ovary, leaves and stems. This will determine whether substrates are utilised for respiration or whether they are translocated to other plant parts.

3. The nature of the substances plugging the xylem vessels of tuberose cut-flowers need to be established.

4 The effect of higher concentration of GA$_{4,7}$ on the vase-life and floret opening of tuberose cut-flowers.

5. The role of cytokinins in ethylene evolution. This will determine whether cytokinins inhibit   or promote ethylene production in cut-flowers.

6. Evaluation of the effectiveness of alternatives of STS such as 1-MCP,(1-methyl

cyclopropane) on the vase-life and floret opening of tuberose cut-flowers.

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9.0 APPENDIX

A1: Effect of plant growth regulators on the vase-life of tuberose cut-flower.

Source	df	Sum of Squares	Mean squares	Observed F	Critical F(0.05)
Treat	17	340.745	20.04382	23.6467	1.55
Error	54	45.795	0.848056		
Total	71	386.54			

Q tests (Tukey's) for variable: Vase-life   CV= 5.91%

Alpha= 0.05   df= 54   MSE= 0.848 Critical value of q=   5.16 w value=   2.376

TREATMENTS	N	MEAN	W GROUPINGS
STS	4	20.3	a
10% sucrose + 25 ppm Accell	4	19.8	ab
10% sucrose + 50 ppm Accell	4	18.8	abc
10% sucrose + 75 ppm Accell	4	17.5	bcd
10% sucrose + deionised water	4	16.8	cde
25 ppm Accell	4	15.8	def
25 ppm BA	4	15.8	def
10% sucrose + 100 ppm Accel	4	15.6	Def
50 ppm Accell	4	15.2	def
75 ppm Accell	4	14.7	efg
75 ppm BA	4	14.4	efg
100 ppm Accell	4	14.2	fg
Florissant 500	4	14	fg
100 ppm BA	4	14	fg
7.5 ppm GA$_{4-7}$	4	13.5	fg
10 ppm GA$_{4-7}$	4	13.4	fg
Deionised water	4	13.2	g
2.5 ppm GA$_{4-7}$	4	13.2	g

A2. Effect of the plant growth regulators on the percentage of floret opening

Source	df	Sum of squares	Mean square	Observed F	Critical F(0.05)
Treatment	17	4040.73	237.68847	35.53	1.55
Error	54	361.68	6.697778		
Total	71	4402.41			

Q test (Tukey's) for variable: % floret opening   Alpha= 0.05 df= 54

MSE=6.69   CV=3.61% w value= 6.673 Critical value of q= 5.16

Means with the same letter are not significantly different.

TREATMENTS	N	MEAN	W GROUPINGS
STS	4	88	a
10% sucrose + 25 ppm Accell	4	84.6	ab
10% sucrose + 50 ppm Accell	4	82.4	ab
10% sucrose + 75 ppm Accell	4	78.5	bc
10% sucrose + deionised water	4	75.5	c
25 ppm Accell	4	73.9	cd
10% sucrose + 100 ppm Accell	4	73.2	cde
25 ppm BA	4	73	cde
50 ppm Accell	4	71.9	cdef
75 ppm Accell	4	68.7	defg
75 ppm BA	4	68.2	defg
100 ppm Accell	4	66.6	efg
Florissant 500	4	65.8	fg
100 ppm BA	4	65.1	fg
10 ppm GA_{4-7}	4	64.3	fg
7.5 ppm GA_{4-7}	4	63.7	g
2.5 ppm GA_{4-7}	4	63.3	g
Deionised water	4	62.8	g

A3. Effect of the plant growth regulators on the water uptake: DAY 2

Source	df	Sum of Squares	Mean square	Observed F	Critical f (0.05)
Treatment	17	0.0661	0.0039	22.87	1.55
Error	54	0.0092	0.00017		
Total	71	0.0753			

Q test for variable (Tukey's): water uptake (day 2)

Alpha= 0.05   df= 54   MSE= 0.00017

Critical value of q= 5.16   w value= 0.034   CV= 4.59%

TREATMENTS	N	Mean	w GROUPING
10% sucrose + 50 ppm Accell	4	0.339	a
10% sucrose + 25 ppm Accell	4	0.334	a
10% sucrose + 75 ppm Accell	4	0.334	a
10% sucrose + 100 ppm Accell	4	0.327	a
10% sucrose + deionised water	4	0.316	a
25 ppm BA	4	0.281	b
25 ppm Accell	4	0.275	b
50 ppm Accell	4	0.273	b
STS	4	0.272	b
Florissant 500	4	0.266	b
10 ppm GA_{4+7}	4	0.265	b
75 ppm Accell	4	0.265	b
75 ppm BA	4	0.264	b
100 ppm Accell	4	0.263	b
7.5 ppm GA_{4+7}	4	0.262	b
Deionised water	4	0.261	b
100 ppm BA	4	0.259	b
2.5 ppm GA_{4+7}	4	0.255	b

A4. Effect of plant growth regulators on the water uptake: DAY 4

Source	df	Sum of Squares	Mean Square	Observed F	Critical F(0.05)
Treatment	17	0.0897	0.0053	47.97	1.55
Error	54	0.0061	0.00011		
Total	71	0.0958			

Q Test (Tukey's) for variable: water uptake Day 2.

Alpha= 0.05   df= 54   MSE= 0.00011

Critical value of q= 5.16   w value= 0.027   CV=4.08%

TREATMENTS	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	4	0.325	a
10% sucrose + 50 ppm Accell	4	0.317	ab
10% sucrose + 75 ppm Accell	4	0.31	ab
10% sucrose + 100 ppm Accell	4	0.305	ab
10% sucrose + deionised water	4	0.291	b
STS	4	0.263	c
25 ppm BA	4	0.255	cd
50 ppm Accell	4	0.254	cde
25 ppm Accell	4	0.25	cdef
75 ppm BA	4	0.241	cdefg
75 ppm Accell	4	0.239	cdefg
100 ppm Accell	4	0.236	cdefg
100 ppm BA	4	0.232	defg
Florissant 500	4	0.227	fg
10 ppm $GA_{4,7}$	4	0.224	g
Deionised water	4	0.22	g
7.5 ppm $GA_{4,7}$	4	0.218	g
2.5 ppm $GA_{4,7}$	4	0.215	g

A5. Effect of plant growth regulators on the water uptake: DAY 6

Source	df	Sum of Squares	Mean Square	Observed F	Critical F(0.05)
Treatment	17	0.18	0.0106	96.26	1.55
Error	54	0.006	0.00013333		
Total	71	0.186			

Q Test (Tukey's) for variable: water uptake Day 6

Alpha= 0.05   df= 45   MSE= 0.0001333

Critical value of q= 5.16   w value=0.027   CV=4.88%

TREATMENTS	N	MEAN	W GROUPINGS
10% sucrose + 25 ppm Accell	4	0.305	a
10% sucrose + 50 ppm Accell	4	0.298	ab
10% sucrose + 100 ppm Accell	4	0.276	bc
10% sucrose + 75 ppm Accell	4	0.273	bcd
STS	4	0.254	cde
10% sucrose + deionised water	4	0.246	def
25 ppm BA	4	0.233	efg
25 ppm Accell	4	0.224	fgh
50 ppm Accell	4	0.215	ghi
75 ppm Accell	4	0.198	hij
75 ppm BA	4	0.198	ijk
100 ppm BA	4	0.188	ijkl
100 ppm Accell	4	0.186	jkl
Florissant 500	4	0.165	klm
7.5 ppm GA_{4-7}	4	0.161	lm
Deionised water	4	0.155	m
10 ppm GA_{4-7}	4	0.153	m
2.5 ppm GA_{4-7}	4	0.146	m

A6. Effect of plant growth regulators on the water uptake: DAY 8

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F(0.05)
Treatment	17	0.256	0.015	100.4	1.55
Error	54	0.008	0.00015		
Total	71	0.264			

Q test (Tukey's) for variable : water uptake (Day 8)

Alpha= 0.05   df= 54   MSE= 0.00015

Critical value of q= 5.16   w value= 0.032   CV= 7.85%

TREATMENTS	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	4	0.264	a
10% sucrose + 50 ppm Accell	4	0.248	ab
STS	4	0.235	abc
10% sucrose + 75 ppm Accell	4	0.225	bc
10% sucrose + 100 ppm Accell	4	0.215	cd
10% sucrose + deionised water	4	0.189	de
50 ppm Accell	4	0.178	ef
25 ppm Accell	4	0.171	ef
25 ppm BA	4	0.159	fg
75 ppm Accell	4	0.135	gh
75 ppm BA	4	0.119	hi
100 ppm Accell	4	0.115	hi
100 ppm BA	4	0.113	hij
7.5 ppm $GA_{4,7}$	4	0.099	ij
2.5 ppm $GA_{4,7}$	4	0.095	ij
Florissant 500	4	0.086	j
10 ppm $GA_{4,7}$	4	0.085	j
Deionised water	4	0.083	j

A7. Effect of plant growth regulators on the water uptake DAY 10

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F(0.05)
Treatment	14	0.117	0.0069	76.471	1.55
Error	45	0.005	0.00009		
Total	59	0.122			

Q test (Tukey's) for variable water uptake (day 10)

Alpha= 0.05   df= 54   MSE= 0.00009

Critical value of q= 5.16   w value= 0.024   CV= 9.58%

TREATMENTS	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	4	0.173	a
10% sucrose + 50 ppm Accell	4	0.169	ab
STS	4	0.169	ab
10% sucrose + 75 ppm Accell	4	0.147	bc
10% sucrose + 100 ppm Accell	4	0.123	cd
10% sucrose + deionised water	4	0.111	de
25 PPM BA	4	0.106	de
25 ppm Accell	4	0.102	def
50 ppm Accell	4	0.099	def
75 ppm Accell	4	0.087	efg
75 ppm BA	4	0.079	fgh
100 ppm Accell	4	0.069	ghi
7.5 ppm $GA_{4,7}$	4	0.065	ghi
100 ppm BA	4	0.065	ghi
10 ppm $GA_{4,7}$	4	0.061	hi
Deionised water	4	0.057	hi
Florissant 500	4	0.055	hi
2.5 ppm $GA_{4,7}$	4	0.053	i

A8. Effect of plant growth regulators on the water uptake: DAY 12

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F(0.05)
Treatment	17	0.048	0.0028	51.34143	1.55
Error	54	0.003	0.000055		
Total	71	0.051			

Q test(Tukey's) for variable: water uptake (day 12)

Alpha= 0.05   df= 54   MSE= 0.000055

Critical value of q= 5.16   w value= 0.019   CV=10.91%

TREATMENT	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	4	0.115	a
10% sucrose + 50 ppm Accell	4	0.111	a
STS	4	0.109	ab
10% sucrose + 75 ppm Accell	4	0.091	bc
10% sucrose + deionised water	4	0.088	cd
25 ppm BA	4	0.08	cde
10% sucrose + 100 Accell	4	0.079	cdef
25 ppm Accell	4	0.075	cdef
50 ppm Accell	4	0.07	def
75 ppm Accell	4	0.062	efg
75 ppm BA	4	0.06	fgh
100 ppm BA	4	0.05	ghi
100 ppm Accell	4	0.047	ghi
7.5 ppm GA_{4-7}	4	0.044	ghi
Deionised water	4	0.042	hi
10 ppm GA_{4-7}	4	0.039	I
2.5 ppm GA_{4+7}	4	0.037	I
Florissant 500	4	0.035	I

A9. Effect of plant growth regulators on the water uptake: DAY 14

Source	df	Sum of Squares	Mean squares	Observed F	Critical F (0.05)
Treatment	17	0.0269	0.0016428	27.76	1.55
Error	54	0.0031	0.0000578		
Total	71	0.03			

Q test (Tukey's) for variable: water uptake(day 14)

Alpha= 0.05   df= 54   MSE= 0.0000578

Critical value of q= 5.16   w value= 0.03   CV=5.58%

TREATMENTS	N	MEAN	W GROUPING
STS	4	0.092	a
10% sucrose + 25 ppm Accell	4	0.085	a
10% sucrose + 50 ppm Accell	4	0.073	ab
10%sucrose+ deionised water	4	0.062	bc
10% sucrose + 100 ppm Accell	4	0.061	bc
10% sucrose + 75 ppm Accell	4	0.055	bc
25 ppm BA	4	0.05	bcd
25 ppm Accell	4	0.047	cde
50 ppm Accell	4	0.045	cdef
75 ppm BA	4	0.04	cdefg
75 ppm Accell	4	0.038	defg
100 ppm BA	4	0.035	defg
100 ppm Accell	4	0.032	efg
Deionised water	4	0.031	efg
Florissant 500	4	0.029	efg
10 ppm GA_{4-7}	4	0.027	gh
2.5 ppm GA_{4-7}	4	0.027	g
7.5 ppm GA_{4-7}	4		g

A10. Effect of plant growth regulators on transpiration rate: DAY 2

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F(0.05)
Treatment	17	0.0303	0.00185	12.9311	1.55
Error	54	0.0075	0.00014		
Total	71	0.0378			

Q test (Tukey's) for variable: Transpiration rate (day 2)

Alpha= 0.05   df= 54   MSE= 0.00016667

Critical value of q= 5.16   w value= 0.030   CV= 5.58%

TREATMENT	N	MEAN	W GROUPING
50 ppm Accell	4	0.241	a
25 ppm BA	4	0.24	a
75 ppm BA	4	0.239	a
25 ppm Accell	4	0.235	ab
100 ppm BA	4	0.232	ab
75 ppm Accell	4	0.227	abc
100 ppm Accell	4	0.225	abc
Deionised water	4	0.213	abcd
Florissant 500	4	0.212	abcd
2.5 ppm $GA_{4.7}$	4	0.208	bcde
10 ppm $GA_{4.7}$	4	0.206	bcde
STS	4	0.205	bcde
7.5 ppm $GA_{4.7}$	4	0.201	cdef
10% sucrose + 50 ppm Accell	4	0.195	def
10 % sucrose + 100 ppm Accell	4	0.193	def
10% sucrose + 75 ppm Accell	4	0.189	def
10% sucrose + 25 ppm Accell	4	0.181	def
10% sucrose + deionised water	4	0.173	f

A11. Effect of plant growth regulators on transpiration: DAY 4

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.0287	0.0016	18.301	1.55
Error	54	0.005	0.000091		
Total	71	0.033			

Q test (Tukey's) for Variable: transpiration rate (day 4)

Alpha= 0.05   df= 54   MSE= 0.000091

Critical value of q= 5.16   w value=0.032   CV=6.84%

TREATMENTS	N	MEAN	W GROUPING
25 ppm BA	4	0.222	a
75 ppm BA	4	0.222	a
25 ppm Accell	4	0.219	a
100 ppm BA	4	0.215	ab
75 ppm Accell	4	0.215	abc
100 ppm Accell	4	0.205	abcd
50 ppm Accell	4	0.205	abcd
Deionised water	4	0.191	bcde
Florissant 500	4	0.19	cde
7.5 ppm GA_{4+7}	4	0.188	de
2.5 ppm GA_{4+7}	4	0.184	def
10 ppm GA_{4+7}	4	0.183	def
10% sucrose + 75 ppm Accell	4	0.177	ef
STS	4	0.177	ef
10% sucrose + 100 ppm Accell	4	0.175	ef
10% sucrose + 50 ppm Accell	4	0.169	ef
10 5 sucrose + 25 ppm Accell	4	0.168	ef
10% sucrose + deionised water	4	0.161	f

A12. Effect of plant growth regulators on transpiration: DAY 6

Source	df	Sum of Squares	Mean Square	Observed F	Critical F (0.05)
Treatment	17	0.0224	0.0013	8.789	1.55
Error	54	0.0079	0.000155		
Total	71	0.0303			

Q test (Tukey's) for variable: transpiration rate (DAY 6)

Alpha= 0.05   df= 54   MSE= 0.000155

Critical value of q= 5.16   w value= 0.032   CV= 6.84%

TREATMENTS	N	MEAN	W GROUPING
25 ppm BA	4	0.219	a
25 ppm Accell	4	0.201	ab
100 ppm BA	4	0.2	abc
75 ppm BA	4	0.197	abcd
75 ppm Accell	4	0.193	abcde
50 ppm Accell	4	0.189	abcdef
Florissant 500	4	0.185	abcdef
100 ppm Accell	4	0.175	bcdefg
STS	4	0.173	bcdefg
Deionised water	4	0.171	bcdefg
10% sucrose + 50 ppm Accell	4	0.169	bcdefg
7.5 ppm $GA_{4.7}$	4	0.168	bcdefg
10% sucrose + 25 ppm Accell	4	0.166	bcdefg
10% sucrose + 75 ppm Accell	4	0.158	defg
10% sucrose + 100 ppm Accell	4	0.155	defg
10 ppm $GA_{4.7}$	4	0.155	efg
10% sucrose + deionised water	4	0.155	efg
2.5 ppm GA_{4+7}	4		f

A13. Effect of plant growth regulators on transpiration: DAY 8

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.023	0.00137	7.958	1.55
Error	54	0.009	0.00017		
Total	71	0.032			

Q test (Tukey's) for variable: transpiration rate (day 8)

Alpha= 0.05   df=54   MSE= 0.00017

Critical value of q= 5.16   w value= 0.034   CV= 8.15%

TREATMENT	N	MEANS	W GROUPING
75 ppm BA	4	0.193	a
25 ppm BA	4	0.186	ab
75 ppm Accell	4	0.181	abc
25 ppm Accell	4	0.178	abc
50 ppm Accell	4	0.175	abcd
10% sucrose + 50 ppm Accell	4	0.168	abcde
100 ppm BA	4	0.167	abcde
10% sucrose + 25 ppm Accell	4	0.166	abcde
100 ppm Accell	4	0.161	abcde
10% sucrose + 75 ppm Accell	4	0.166	abcdef
10% sucrose + 100 ppm Accell	4	0.159	abcdef
STS	4	0.155	bcdef
Florissant 500	4	0.147	cdef
Deionised water	4	0.143	def
10 ppm $GA_{4,7}$	4	0.142	def
10% sucrose + deionised water	4	0.136	ef
2.5 ppm $GA_{4,7}$	4	0.135	ef
7.5 ppm $GA_{4,7}$	4	0.131	f

A14: Effect of plant growth regulators on transpiration DAY 10

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.0215	0.0012714	9.0314	1.55
Error	54	0.0075	0.000147		
Total	71	0.029			

Q test (Tukey's) for variable: Transpiration rate DAY 10

Alpha= 0.05   df= 54   MSE= 0.000147

Critical value for q= 5.16   w value= 0.03   CV= 9.10%

TREATMENT	N	MEAN	W GROUPING
25 ppm BA	4	0.157	a
100 ppm BA	4	0.156	a
50 ppm Accell	4	0.153	abc
25 ppm Accell	4	0.147	abcd
75 ppm BA	4	0.144	abcd
100 ppm Accell	4	0.143	abcde
75 ppm Accell	4	0.139	abcde
10% sucrose + 50 ppm Accell	4	0.137	abcdef
10% sucrose + 25 ppm Accell	4	0.132	abcdef
STS	4	0.129	abcdef
10% sucrose + 75 ppm Accell	4	0.127	abcdef
10% sucrose + 100 ppm Accell	4	0.124	bcdef
Deionised water	4	0.119	cdef
7.5 ppm $GA_{4,7}$	4	0.114	def
10% sucrose + Deionised water	4	0.112	ef
2.5 ppm $GA_{4,7}$	4	0.109	ef
10 ppm $GA_{4,7}$	4	0.105	f
Florissant 500	4	0.102	f

A15: Effect of plant growth regulators on transpiration DAY 12

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.0134	0.000762	9.952429	1.55
Error	54	0.006	0.000113		
Total	71	0.0194			

Q test (Tukey's) for variable: Transpiration rate (Day 12)

Alpha= 0.05   df= 54   MSE= 0.000113   Critical value of q= 5.16

w value= 0.027   CV= 9.89%

TREATMENT	N	MEAN	W GROUPING
25 ppm BA	4	0.127	a
75 ppm Accell	4	0.123	ab
10% sucrose + 25 ppm Accell	4	0.121	ab
75 ppm BA	4	0.12	ab
25 ppm Accell	4	0.119	ab
STS	4	0.119	ab
10% sucrose + 50 ppm Accell	4	0.115	abc
100 ppm BA	4	0.115	abc
100 ppm Accell	4	0.108	abcd
75 ppm Accell	4	0.105	abcd
10% sucrose + 100 ppm Accell	4	0.098	bcd
Deionised water	4	0.098	bcd
10% sucrose + 75 ppm Accell	4	0.098	bcd
10% sucrose + deionised water	4	0.095	bcd
Florissant 500	4	0.094	bcd
10 ppm GA_{4-7}	4	0.091	cd
2.5 ppm GA_{4-7}	4	0.087	d
7.5 ppm GA_{4-7}	4	0.083	d

A16. Effect of plant growth regulators on transpiration: DAY 14

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0 05)
Treatment	17	0.012	0.00857142	9.642857143	1.55
Error	54	0.004	0.00008888		
Total	71	0.016			

Q test (Tukey's) for variable: transpiration rate (Day 14)

Alpha= 0.05   df= 54   MSE= 0.000088888

Critical value for q= 5.16   w value=0.022   CV=9.19%

TREATMENTS	N	MEAN	W GROUPING
75 ppm BA	4	0.115	a
25 ppm BA	4	0.109	ab
STS	4	0.108	ab
100 ppm BA	4	0.106	abc
25 ppm Accell	4	0.101	abcd
50 ppm Accell	4	0.095	abcde
75 ppm Accell	4	0.095	abcde
10% sucrose + 50 ppm Accell	4	0.093	abcde
10% sucrose + 25 ppm Accell	4	0.092	bcde
100 ppm Accell	4	0.091	bcdef
Deionised water	4	0.089	bcdef
10% sucrose + 75 ppm Accell	4	0.087	bcdef
10 % sucrose + deionised water	4	0.085	cdef
10% sucrose + 100 ppm Accell	4	0.085	cdef
2.5 ppm $GA_{4,7}$	4	0.081	def
10 ppm $GA_{4,7}$	4	0.074	ef
7.5 ppm $GA_{4,7}$	4	0.069	f
Florissant 500	4	0.069	f

A17. Effect of plant growth regulators on the dry weight of the petals of the lowest florets: DAY 3

Source	df	Sum of Squares	Mean Square	Observed F	Critical F
Treatment	17	0.0423	0.002528	44.9257	2.66
Error	36	0.002	0.0000556		
Total	53	0.0443			

Q test (Tukey's) for variable Dry weight of petals of the lowest floret(day 3) CV=5.37% Alpha= 0.05 df= 36 MSE= 0.00005566 Critical value of q= 5.38 w value= 0.023

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.187	a
10% sucrose + 100 ppm Accell	3	0.185	a
10% sucrose + 50ppm Accell	3	0.184	a
10% sucrose + 75 ppm Accell	3	0.182	a
10% sucrose + deionised water	3	0.178	a
STS	3	0.13	b
25 ppm Accell	3	0.125	b
50 ppm Accell	3	0.124	b
25 ppm BA	3	0.123	b
100 ppm Accell	3	0.122	b
75 ppm Accell	3	0.121	b
7.5 ppm $GA_{4.7}$	3	0.119	b
10 ppm $GA_{4.7}$	3	0.119	b
Florissant 500	3	0.119	b
100 ppm BA	3	0.119	b
2.5 ppm $GA_{4.7}$	3	0.118	b
75 ppm BA	3	0.118	b
Deionised water	3	0.116	b

A18. Effect of plant growth regulators on the dry weights of the petals of the lowest florets: DAY 6

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F(0.05)
Treatments	17	0.061	0.0036142	128.1557	2.66
Error	36	0.001	0.0000283		
Total	53	0.067			

Q test (Tukey's) for variable: Dry weight of the petals of the lowest florets (day 6)

Alpha= 0.05   df= 36   MSE=0.0000283

Critical value of q= 5.38   w value= 0.016   CV=4.85%

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.174	a
10% sucrose + 50 ppm Accell	3	0.169	a
10% sucrose + 75 ppm Accell	3	0.159	ab
10% sucrose + 100 ppm Accell	3	0.155	ab
10% sucrose + Deionised water	3	0.147	b
STS	3	0.114	c
25 ppm Accell	3	0.101	cd
50 ppm Accell	3	0.098	cd
25 ppm BA	3	0.098	cd
75 ppm BA	3	0.093	de
75 ppm Accell	3	0.092	def
100 ppm Accell	3	0.087	def
100 ppm BA	3	0.085	def
2.5 ppm $GA_{4,7}$	3	0.081	ef
10 ppm $GA_{4,7}$	3	0.08	ef
Deionised water	3	0.078	ef
7.5 ppm $GA_{4,7}$	3	0.077	ef
Florissant 500	3	0.076	f

A19: Effect of plant growth regulators on the dry weight of the petals of the middle florets: DAY 3

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.0109	0.000641	228.99	2.66
Error	36	0.0001	0.00000283		
Total	53	0.011			

Q Test (Tukey's) for Variable: dry weight of the petals of the middle floret (day3)

w value=0.005 Critical value of q=5.38 Alpha=0.05 CV=4.79% df=36

TREATMENT	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.179	a
10% sucrose + 50 ppm Accell	3	0.176	ab
10% sucrose + 100 ppm Accell	3	0.176	ab
10% sucrose + 75 ppm Accell	3	0.175	ab
10% sucrose + deionised water	3	0.172	b
STS	3	0.148	c
100 ppm Accell	3	0.147	cd
25 ppm Accell	3	0.146	cde
50 ppm Accell	3	0.145	cde
25 ppm BA	3	0.145	cde
100 ppm BA	3	0.145	cde
10 ppm $GA_{4.7}$	3	0.144	cde
75 ppm BA	3	0.144	cde
75 ppm Accell	3	0.143	cde
2.5 ppm $GA_{4.7}$	3	0.143	cde
Deionised water	3	0.142	de
Florissant 500	3	0.142	de
7.5 ppm $GA_{4.7}$	3	0.141	e

A20: Effect of plant growth regulators on the dry weight of the petals of the middle florets DAY 6

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F (0.05)
Treatment	17	0.0156	0.001114285	111.4285	2.66
Error	36	0.0003	0.00001		
Total	53	0.0159			

Q test (Tukey's) for variable: dry weights of the petals of the lowest florets: day 6

Alpha=0.05　　　df=36　　　MSE=0.0000083

Critical value of q=5.38　　　w value=0.009　　　CV=2.46%

TREATMENTS	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	3	0.153	a
10% sucrose + 50 ppm Accell	3	0.148	a
10% sucrose + 75 ppm Accell	3	0.141	ab
10% sucrose + 100 ppm Accell	3	0.137	b
10% sucrose + deionised water	3	0.134	b
STS	3	0.12	c
25 ppm Accell	3	0.117	c
25 ppm BA	3	0.115	cd
50 ppm Accell	3	0.113	cde
75 ppm Accell	3	0.107	def
75 ppm BA	3	0.106	def
100 ppm BA	3	0.106	def
100 ppm Accell	3	0.105	ef
2.5 ppm GA_{4-7}	3	0.103	f
Florissant 500	3	0.103	f
7.5 ppm GA_{4-7}	3	0.102	f
Deionised water	3	0.101	f
10 ppm GA_{4-7}	3	0.1	f

A21: Effect of plant growth regulators on the dry weight of the petals of the middle florets: DAY 9

Source	df	Sum of Squares	Mean Square	Observed F	Critical F (0.05)
Treatment	17	0.0200	0.001271	69.20543	2.66
Error	36	0.0006	0.0000172		
Total	53	0.0206			

Q test (Tukey's) for variable: dry weight of the petals of the middle florets (day 9)

Alpha=0.05   df=36   MSE=0.0000172

Critical value of q =5.38   w value= 0.013   CV=4.16%

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.139	a
10% sucrose + 50 ppjm Accell	3	0.132	ab
10% sucrose + 75 ppm Accell	3	0.126	ab
10% sucrose + 100 ppm Accell	3	0.122	b
10 % sucrose + deionised water	3	0.12	b
STS	3	0.107	c
25 ppm Accell	3	0.097	cd
25 ppm BA	3	0.095	cde
50 ppm Accell	3	0.093	def
75 ppm BA	3	0.087	def
75 ppm Accell	3	0.086	def
100 ppm Accell	3	0.085	def
100 ppm BA	3	0.085	def
2.5 ppm GA$_{4,7}$	3	0.084	def
Deionised water	3	0.082	ef
10 ppm GA$_{4,7}$	3	0.082	ef
7.5 ppm GA$_{4,7}$	3	0.081	f
Florissant 500	3	0.081	f

A22: Effect of plant growth regulators on the dry weight of the petals of the topmost florets/buds: DAY 3

Source	df	Sum of Squares	Mean Square	Observed F	Critical F (0.05)
Treatment	17	0.004515	0.0002655	0.8849NS	2.66
Error	36	0.00090044	0.0003		
Total	53	0.0135198			

A23: Effect of plant growth regulators on the dry weight of the petals of the topmost florets/buds: DAY 6

Source	df	Sum of Square	Mean Square	Observed F	Critical F (0.05)
Treatment	17	0.0690455	0.0040615	34.582147	2.66
Error	36	0.004228054	0.000117445		
Total	53	0.07327356			

Q test (Tukey's) for variable:dry weight of the petals of the topmost floret/buds: day 6

Alpha=0.05   df=36   MSE=0.000117445   Critical value of q=5.38

w value= 0.034   CV=11.86%

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.162	a
10% sucrose + 50 ppm Accell	3	0.156	ab
10% sucrose + deionised water	3	0.15	ab
10% sucrose + 75 ppm Accell	3	0.146	ab
STS	3	0.125	b
25 ppm BA	3	0.077	c
25 ppm Accell	3	0.075	c
10% sucrose + 100 ppm Accell	3	0.072	c
100 ppm BA	3	0.072	c
50 ppm Accell	3	0.071	c
100 ppm Accell	3	0.07	c
Florissant 500	3	0.068	c
75 ppm BA	3	0.068	c
Deionised water	3	0.067	c
75 ppm Accell	3	0.067	c
7.5 ppm $GA_{4.7}$	3	0.066	c
2.5 ppm $GA_{4.7}$	3	0.065	c
10 ppm $GA_{4.7}$	3	0.065	c

A24: Effect of the preservatives on the sucrose levels of the petals of the lowest florets: DAY 3

Source	df	Sum of Squares	Mean Square	Observed F	Critical F (0.05)
Treatment	17	0.0016	0.00011	0.786NS	2.66
Error	36	0.0041	0.00014		
Total	53	0.00057			

A25: Effect of the plant growth regulators on the sucrose levels of the petals of the lowest florets DAY 6

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F	
Treatment	17	0.0018	0.00129		0.82NS	2.66
Error	36	0.0047	0.00157			
Total	53	0.065				

A26: Effect of the plant growth regulators on the sucrose levels of the petals of the middle florets: DAY 3

Source	df	Sum of Square	Mean Square	Observed F	Critical F
Treatment	17	0.0158	0.00931	84.49315	2.66
Error	36	0.0004	0.0000113		
Total	53	0.0162			

Q test (Tukey's) for variable: sucrose levels in the petals of the middle florets (day 3) df=36
CV=2.81%   Alpha=0.05   MSE=0.0001133   Critical value of q =5.38   w value=0.010

TREATMENTS	N	MEAN	W GROUPINGS
10% sucrose + 25 ppm Accell	3	0.149	a
10% sucrose + 75 ppm Accell	3	0.148	a
10% sucrose + 100 ppm Accell	3	0.146	a
10 % sucrose + 50 ppm Accell	3	0.144	a
10% sucrose + deionised water	3	0.142	a
25 ppm Accell	3	0.111	b
75 ppm Accell	3	0.109	b
100 ppm Accell	3	0.109	b
STS	3	0.109	b
25 ppm BA	3	0.109	b
10 ppm $GA_{4,7}$	3	0.109	b
50 ppm Accell	3	0.108	b
7.5 ppm $GA_{4,7}$	3	0.108	b
2.5 ppm $GA_{4,7}$	3	0.107	b
Florissant 500	3	0.106	b
75 ppm BA	3	0.106	b
100 ppm BA	3	0.105	b
Deionised water	3	0.105	B

A27: Effect of the plant growth regulators on the sucrose levels of the petals of the middle florets: DAY 6

Source	df	Sum of Squares	Mean Square	Observed F	Critical F
Treatment	17	0.004	0.00029	1.00NS	2.66
Error	36	0.013	0.00029		
Total	53	0.017			

A28: Effect of the plant growth regulators on the sucrose levels of the petals of the topmost florets/buds DAY 3

Source	df	Sum of Square	Mean Square	Observed F	Critical F
Treatment	17	0.0044	0.00031	0.795NS	2.66
Error	36	0.0176	0.00039		
Total	53	0.022			

A29: Effect of the plant growth regulators on the sucrose levels of the petals of the topmost florets/buds DAY 6

Source	df	Sum of Square	Mean Square	Observed F	Critical F
Treatment	17	0.0019	0.00013	0.65NS	2.66
Error	36	0.0061	0.0002		
Total	53	0.08			

A30: Effect of the plant growth regulators on the reducing sugar levels in the petals of the lowest florets DAY 3

Source	df	Sum of Squares	Mean Square	Observed F	Critical F
Treatment	17	0.067	0.003915	231.83143	2.66
Error	36	0.0006	0.0000172		
Total	53	0.0676			

Q test (Tukey's) for variable: Reducing sugar levels in the petals of the lowest florets (day 3)
df=36 Alpha=0.05 MSE=0.0000172 Critical value of q=5.38 w value=0.013 CV=4.19%

TREATMENT	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	3	0.158	a
10 % sucrose + 50 ppm Accell	3	0.158	a
10% sucrose + 75 ppm Accell	3	0.155	a
10% sucrose + 100 ppm Accell	3	0.153	a
10% sucrose + deionised water	3	0.151	a
75 ppm BA	3	0.082	b
STS	3	0.082	b
25 ppm BA	3	0.08	b
50 ppm Accell	3	0.079	b
25 ppm Accell	3	0.078	b
75 ppm Accell	3	0.078	b
10 ppm GA_{4-7}	3	0.076	b
Deionised water	3	0.075	b
Florissant 500	3	0.075	b
100 ppm Accell	3	0.075	b
100 ppm BA	3	0.074	b
2.5 ppm GA_{4-7}	3	0.073	b
7.5 ppm GA_{4-7}	3	0.071	b

A31: Effect of plant growth regulators on the reducing sugar levels of the petals of the lowest florets DAY 6

Source	df	Sum of Squares	Mean Squares	Observed F	Critical F
Treatment	17	0.0615	0.003657	144.7715	2.66
Error	36	0.0009	0.0000253		
Total	53	0.0624			

Q test (Tukey's) for variable: Reducing sugar levels of the petals of the lowest florets (day 6) df=30 Alpha=0.05 MSE=0.00003 Critical value of q=5.38 w value=0.015 CV=6.94%

TREATMENTS	N	MEANS	W GROUPINGS
10% sucrose + 25 ppm Accell	3	0.131	a
10% sucrose + 50 ppm Accell	3	0.128	a
10% sucrose + deionised water	3	0.124	a
10% sucrose + 75 ppm Accell	3	0.123	a
10% sucrose + 100 ppm Accell	3	0.121	a
STS	3	0.075	b
25 ppm Accell	3	0.06	bc
50 ppm Accell	3	0.058	cd
25 ppm BA	3	0.057	cd
75 ppm Accell	3	0.051	cd
75 ppm BA	3	0.049	cd
100 ppm BA	3	0.049	cd
100 ppm Accell	3	0.048	cd
2.5 ppm $GA_{4,7}$	3	0.048	cd
7.5 ppm $GA_{4,7}$	3	0.047	cd
Deionised water	3	0.045	cd
Florissant 500	3	0.045	cd
10 ppm $GA_{4,7}$	3	0.044	d

A32: Effect of the plant growth regulators on the reducing sugar levels in the petals of the middle florets DAY 3

Source	df	Sum of Squares	Mean Square	observed F	Critical F
Treatment	17	0.0011075	0.000065107	3.46708	2.66
Error	36	0.0006765	0.00001875		
Total	53	0.001784			

Q test (Tukey's) for variable: reducing sugar levels in the petals of the middle florets (day 3)
df=36 Alpha=0.05 MSE=0.00001875 Critical values of q=5.38   w value=0.013 CV=7.88%

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.065	a
10% sucrose + 100 ppm Accell	3	0.064	ab
10% sucrose + deionised water	3	0.061	abc
10% sucrose + 50 ppm Accell	3	0.06	abc
10% sucrose + 75 ppm Accell	3	0.056	abc
25 ppm BA	3	0.056	abc
75 ppm BA	3	0.056	abc
25 ppm Accell	3	0.054	abc
100 ppm BA	3	0.054	abc
75 ppm Accell	3	0.053	abc
100 ppm Accell	3	0.053	abc
STS	3	0.053	abc
Deionised water	3	0.052	abc
Florissant 500	3	0.052	abc
50 ppm Accell	3	0.051	bc
10 ppm $GA_{4.7}$	3	0.051	bc
2.5 ppm $GA_{4.7}$	3	0.05	c
7.5 ppm $GA_{4.7}$	3	0.049	c

A33: Effect of the plant growth regulators on the reducing sugar levels in the petals of the middle florets (DAY 6)

Source	df	Sum of Squares	Mean Square	Observed F	Critical F	
Treatment	17	0.0221	0.0013	118.18	2.66	
Error	36	0.0004	0.000011			
Total	53	0.0225				

Q test (Tukey's) for variable: reducing sugar levels in the petals of the middle florets (day 6)
df=36 Alpha=0.05 MSE=0.000011 Critical value of q=5.38   w value=0.010 CV=3.95%

TREATMENTS	N	MEANS	W GROUPING
10% sucrose + 25 ppm Accell	3	0.122	a
10% sucrose + 50 ppm Accell	3	0.119	ab
10% sucrose + 100 ppm Accell	3	0.114	ab
10% sucrose + 75 ppm Accell	3	0.112	ab
10% sucrose + deionised water	3	0.109	b
STS	3	0.079	c
25 ppm Accell	3	0.077	cd
50 ppm Accell	3	0.076	cde
25 ppm BA	3	0.075	cde
75 ppm BA	3	0.073	cde
75 ppm Accell	3	0.072	cde
100 ppm Accell	3	0.07	cde
100 ppm BA	3	0.07	cde
2.5 ppm $GA_{4.7}$	3	0.069	cde
Florissant 500	3	0.068	de
10 ppm $GA_{4.7}$	3	0.067	e
Deionised water	3	0.067	e
7.5 ppm $GA_{4.7}$	3	0.066	e

A34: Effect of plant growth regulators on the reducing sugar levels in the petals of the middle florets DAY 9

Source	df	Sum of Squares	Mean Square	Observed F	Critical F
Treatment	17	0.0279	0.0016	149.2	2.66
Error	36	0.0004	0.000011		
Total	53	0.0283			

Q test (Tukey's) for variable: reducing sugar levels in the petals of the middle florets (day 9)
df=36 Alpha=0.05 MSE=0.000011 Critical value of q=5.38 w value=0.01 CV=4.74%

TREATMENTS	N	MEAN	W GROUPING
10% sucrose + 25 ppm Accell	3	0.114	a
10% sucrose + 50 ppm Accell	3	0.109	ab
10% sucrose + 75 ppm Accell	3	0.104	abc
10% sucrose + 100 ppm Accell	3	0.101	bc
10 % sucrose + deionised water	3	0.097	c
STS	3	0.067	d
25 ppm BA	3	0.064	d
25 ppm Accell	3	0.0630	de
50 ppm Accell	3	0.061	def
75 ppm BA	3	0.059	defg
75 ppm Accell	3	0.057	defg
100 ppm Accell	3	0.053	efg
100 ppm BA	3	0.053	efg
Deionised water	3	0.051	fg
10 ppm GA_{4-7}	3	0.051	fg
Florissant 500	3	0.051	fg
2.5 ppm GA_{4-7}	3	0.05	g
7.5 ppm GA_{4-7}	3	0.049	g

A35: Effect of the plant growth regulators on the reducing sugar levels in the petals of the topmost florets/buds. DAY 3

Source	df	Sum of Square	Mean Square	Observed F	Critical F
Treatment	17	0.0036	0.00026	0.838NS	2.66
Error	36	0.0094	0.00031		
Total	53	0.013			

A36. Effect of plant growth regulators on the reducing sugar levels in the petals of the topmost florets/buds: DAY 6

Source	df	Sum of Squares	Mean Square	Observed F	Critical F
Treatments	17	0.069045506	0.0040615	34.5821	2.66
Error	36	0.004228054	0.000117445		
Total	53	0.07327356			

Q test (Tukey's) for variable: dry weight of the petals of the topmost florets/buds day 6. df=30 CV=11.88% Alpha=0.05 MSE=0.000117445 Critical value of q=5.38 w value=0.0034

TREATMENTS	N	MEANS	W GROUPINGS
10% sucrose + 25 ppm Accell	3	0.162	a
10% sucrose + 50 ppm Accell	3	0.156	ab
10% sucrose + deionised water	3	0.15	ab
10% sucrose + 75 ppm Accell	3	0.146	ab
STS	3	0.125	b
25 ppm BA	3	0.077	c
25 ppm Accell	3	0.075	c
10% sucrose + 100 ppm Accell	3	0.072	c
100 ppm BA	3	0.072	c
50 ppm Accell	3	0.071	c
100 ppm Accell	3	0.07	c
Florissant 500	3	0.068	c
75 ppm BA	3	0.068	c
Deionised water	3	0.067	c
75 ppm Accell	3	0.067	c
7.5 ppm $GA_{4\cdot7}$	3	0.066	c
2.5 ppm $GA_{4\cdot7}$	3	0.065	c
10 ppm $GA_{4\cdot7}$	3	0.065	c