THE EFFECT OF BENZYLADENINE AND GIBBERELLINS ON THE POSTHARVEST PHYSIOLOGY OF ALSTROEMERIA (*Alstroemeria aurantiaca* L.) CUT FLOWERS.

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DECLARATION

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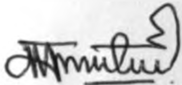

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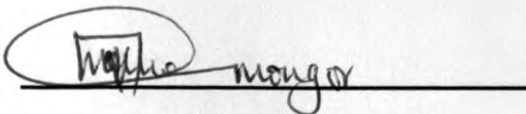

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DEPARTMENT OF CROP SCIENCE

DEDICATION

This thesis is dedicated to my dear parents:

Joseph Mutui Lemba

and

Esther Kanono Mutui

for their tireless encouragement and support during the course of my entire postgraduate program.

and

my younger brothers; James, Peter, Muema and John. I hope this work will be an inspiration to them to achieve even a higher degree.

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TABLE OF CONTENTS

Page

Declaration .... (ii)

Dedication .... (iii)

Acknowledgements .... (iv)

List of Tables .... (vii)

List of Figures .... (ix)

List of Appendices .... (x)

List of Abbreviations .... (xiv)

Abstract .... (xvi)

1.0 INTRODUCTION 1

2.0 LITERATURE REVIEW 10

2.1 Overview of past research 10

2.2 Cut flower senescence 12

2.3 The role of cytokinins in cut flower senescence 15

2.4 The role of gibberellins in cut flower senescence 16

2.5 The role of ethylene in cut flower senescence 18

2.6 Regulation of ethylene biosynthesis by cytokinins 23

2.7 Sucrose as postharvest preservative 25

2.8 Water balance maintenance 28

3.0 MATERIALS AND METHODS 33

3.1 Site 33

3.2 Plant material 33

3.3 Vase solutions 34

3.4 Treatments and Experimental design 36

3.5 Dependent variables determined 37

3.6 Data Analysis 39

4.0 RESULTS 40

4.1 Vase life of *Alstroemeria* cut flowers 40

4.2 Leaf chlorophyll content of *Alstroemeria* 49

4.3 Dry weight of the leaves of *Alstroemeria* 55

4.4 Water content in the leaves of *Alstroemeria* 61

4.5 Total nitrogen content in the leaves of *Alstroemeria* 68

5.0 DISCUSSION **75**

5.1 Vase life of *Alstroemeria* 75

5.2 Leaf chlorophyll content 82

5.3 Dry weight of the leaves 84

5.4 Water content of the leaves 86

5.5 Total nitrogen content in the leaves 88

6.0 CONCLUSIONS AND RECOMMENDATIONS **90**

7.0 REFERENCES **94**

8.0 APPENDICES **113**

LIST OF TABLES

Page

1. The effect of Accel on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers 41
2. The effect of GA_{4+7} (Provide) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers 42
3. The effect of Benzyladenine (Accel - GA_{4+7}) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers 44
4. The effect of plant growth regulators on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers 45
5. The effect of Accel on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers 50
6. The effect of GA_{4+7} (Provide) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers 51
7. The effect of Benzyladenine (Accel - GA_{4+7}) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers. 53
8. The effect of plant growth regulators on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers 54
9. The effect of Accel on the dry weight (g) of the leaves of *Alstroemeria* cut flowers 56
10. The effect of GA_{4+7} (Provide) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers 57
11. The effect of Benzyladenine (Accel - GA_{4+7}) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers 59

12. The effect of plant growth regulators on the dry weight (g) of the leaves of *Alstroemeria* cut flowers 60

13. The effect of Accel on the water content (g) of the leaves of *Alstroemeria* cut flowers 62

14. The effect of GA_{4+7} (Provide) on the water content (g) of the leaves of *Alstroemeria* cut flowers 63

15. The effect of Benzyladenine (Accel - GA_{4+7}) on the water content (g) of the leaves of *Alstroemeria* cut flowers. 65

16. The effect of plant growth regulators on the water content (g) of the leaves of *Alstroemeria* cut flowers 66

17. The effect of Accel on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers 69

18. The effect of GA_{4+7} (Provide) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers 71

19. The effect of Benzyladenine (Accel - GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers. 72

20. The effect of plant growth regulators on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers 73

LIST OF FIGURES

Page

1. Fresh produce exports from Kenya 2
2. Production of cut flowers in Kenya (Ha): 1987-1994 3
3. Cut flowers exports from Kenya (1995) 4

LIST OF APPENDICES

Page

A1. ANOVA table for the effect of Accel on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1) 113

A2. ANOVA table for the effect of Accel on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2) 113

A3. ANOVA table for the effect of GA_{4+7} (Provide) on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1) 114

A4. ANOVA table for the effect of GA_{4+7} (Provide) on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2) 114

A5. ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1). 115

A6. ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2). 115

A7. ANOVA table for the effect of plant growth regulators on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1) 116

A8. ANOVA table for the effect of plant growth regulators on the number of days to the full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2) 116

A9. ANOVA table for the effect of Accel on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 1) 117

A10. ANOVA table for the effect of Accel on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 2) 117

A11. ANOVA table for the effect of GA_{4+7} (Provide) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 1) 118

A12. ANOVA table for the effect of GA_{4+7} (Provide) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 2) 118

A13. ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 1). 119

A14. ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 2). 119

A15. ANOVA table for the effect of plant growth regulators on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 1) 120

A16. ANOVA table for the effect of plant growth regulators on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers (Experiment 2) 120

A17. ANOVA table for the effect of Accel on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 121

A18. ANOVA table for the effect of Accel on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 121

A19. ANOVA table for the effect of GA_{4+7} (Provide) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 122

A20. ANOVA table for the effect of GA_{4+7} (Provide) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 122

A21. ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1). 123

A22 ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2). 123

A23. ANOVA table for the effect of plant growth regulators on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 124

A24. ANOVA table for the effect of plant growth regulators on the dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 124

A25. ANOVA table for the effect of Accel on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 125

A26. ANOVA table for the effect of Accel on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 125

A27. ANOVA table for the effect of GA_{4+7} (Provide) on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 126

A28. ANOVA table for the effect of GA_{4+7} (Provide) on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 126

A29. ANOVA table for the effect of Benzyladenine (Accel -GA_{4+7}) on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 127

A30. ANOVA table for the effect of Benzyladenine (Accel -GA_{4+7}) on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 127

A31. ANOVA table for the effect of plant growth regulators on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 128

A32. ANOVA table for the effect of plant growth regulators on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 128

A33. ANOVA table for the effect of Accel on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 129

A34. ANOVA table for the effect of Accel on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 129

A35. ANOVA table for the effect of GA_{4+7} (Provide) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 130

A36. ANOVA table for the effect of GA_{4+7} (Provide) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 130

A37. ANOVA table for the effect of Benzyladenine (Accel-GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 131

A38. ANOVA table for the effect of Benzyladenine (Accel-GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 131

A39. ANOVA table for the effect of plant growth regulators on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1) 132

A40. ANOVA table for the effect of plant growth regulators on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2) 132

LIST OF ABBREVIATIONS

ABA = Abscisic acid.

ACC = 1 - aminocyclopropane - 1 - carboxylic acid

$AgNO_3$ = Silver nitrate

a.i = active ingredient

ANON = Anonymous

ANOVA = Analysis of Variance

AOA = Aminooxyacetic acid

A.O.A.C = Association of Official Analytical Chemists

AVG = Aminoethoxyvinylglycine

BA = Benzyladenine

BAP = Benzylaminopurine

°C = degree celsius

CO_2 = Carbon dioxide

CRD = Completely Radomised Design

2,4-D = 2,4 - dichlorophenoxyacetic acid

GA = Gibberellic acid

HCDA = Horticultural Crops Development Authority

HQC = Hydroxyquinoline citrate

HQS = Hydroxyquinoline sulphate

IAA = Indole-3-acetic acid

J = Joules

M = Molar

mg = milligram

ml = millitre

mM = Millimolar

NAA = 1- Naphthalene acetic acid

$Na_2S_2O_3$ = Sodium thiosulphate

NPK = Nitrogen, Phosphorous, Potassium

ppb = parts per billion

s = seconds

SAM = S - adenosyl methionine (SAM)

STS = Silver thiosulphate

μm = micromolar

μg = microgram

v = volts

w = watts

ABSTRACT

The objective of this study was to investigate the response to exogenous BA (Accel) and gibberellins (GA_{4+7}) on the vase life and the physiological changes in the leaves of *Alstroemeria* cut flowers. Florissant 200 and 2.0 mM STS (commercial flower preservative solutions) were used as a reference for the best postharvest treatment of *Alstroemeria* cut flowers in order to broaden the applicability of the results to the floriculture industry. The treatments were combined in a factorial manner and laid down in a completely radomised design.

Treatment of *Alstroemeria* cut flowers with 25 or 50 mg/litre BA equivalent (Accel) consistently increased the number of days to full opening of primary florets and delayed the onset of flower senescence as measured by days to 50 % petal fall and 50 % leaf yellowing. Accel (25 mg/litre BA) increased the leaf nitrogen content, chlorophyll and water content of the *Alstroemeria* cut flowers compared to the control. However, Accel (100 mg/litre BA) gave the highest values of dry weight and leaf nitrogen content.

The lower levels of GA_{4+7} (2.5, 5.0 or 7.5 mg/litre) had no effect on the number of days to full opening of primary florets in *Alstroemeria*. However, application of 10.0 mg/litre GA_{4+7} delayed cut flower senescence (increased the days to 50 % petal fall and delayed the onset of 50 % leaf yellowing), increased leaf nitrogen content, chlorophyll and water content but it reduced the dry weight of the leaves.

Accel (25 or 50 mg/litre BA), GA_{4+7} (7.5 or 10 mg/litre) and Florissant 200, increased the number of days to full opening of primary florets, days to 50 % petal fall and delayed the onset of 50 % leaf yellowing comparably in both experiments. However, STS (2.0 mM) had no effect on days to full opening of primary florets, increased the days to 50 % petal fall, dry weight, nitrogen content and decreased both water and chlorophyll content, leading to accelerated onset

of 50 % leaf yellowing in *Alstroemeria*.

Florissant 200, 2.0 mM STS, GA_{4+7} (10 mg/litre) and Accel (25 mg/litre BA) increased the nitrogen content of *Alstroemeria* leaves comparably. However, Florissant 200 had no effect on dry weight and water content of the leaves. These results suggest that Accel (25 mg/litre BA) has the potential to substitute for the use of Florissant 200, as a commercial cut flower preservative to prevent leaf yellowing and prolong cut flower vase life.

CHAPTER 1

INTRODUCTION

1.1 Overview

The horticultural industry is a major contributor to agricultural production in Kenya and has evolved to become an important foreign exchange earner and a leading employer due to its labour intensiveness (Kibanga, 1996). Kenya is endowed with an agro-climatic diversity which allows for a year round production of wide range of horticultural produce (Kibanga, 1996). Exports of fresh horticultural produce (cut flowers, fruits and vegetables) have grown steadily from 3,000 tonnes in 1970 to about 85,000 tonnes in 1996 (Figure 1). Cut flowers are among the most important produce in Kenya's horticultural export industry. In 1996, Kenya exported about 35,000 tonnes (42 % by volume) of cut flowers valued at Ksh 4.4 billion (57 % by value) (Anon, 1996).

In Kenya, commercial cut flower production is done by a few large scale growers and numerous medium and small scale growers. Cut flowers exported from Kenya, in order of importance (acreage and quantity exported) includes roses, carnations, statice, *Alstroemeria*, solidaster. Others includes tuberose, arabicum, delphinium, ornithogalum, chrysanthemums, molucella, lilies, gypsophila, liatris, strelitzia, heliconia, orchids etc (Figures 2, 3). Most of the cut flowers are exported to Europe.

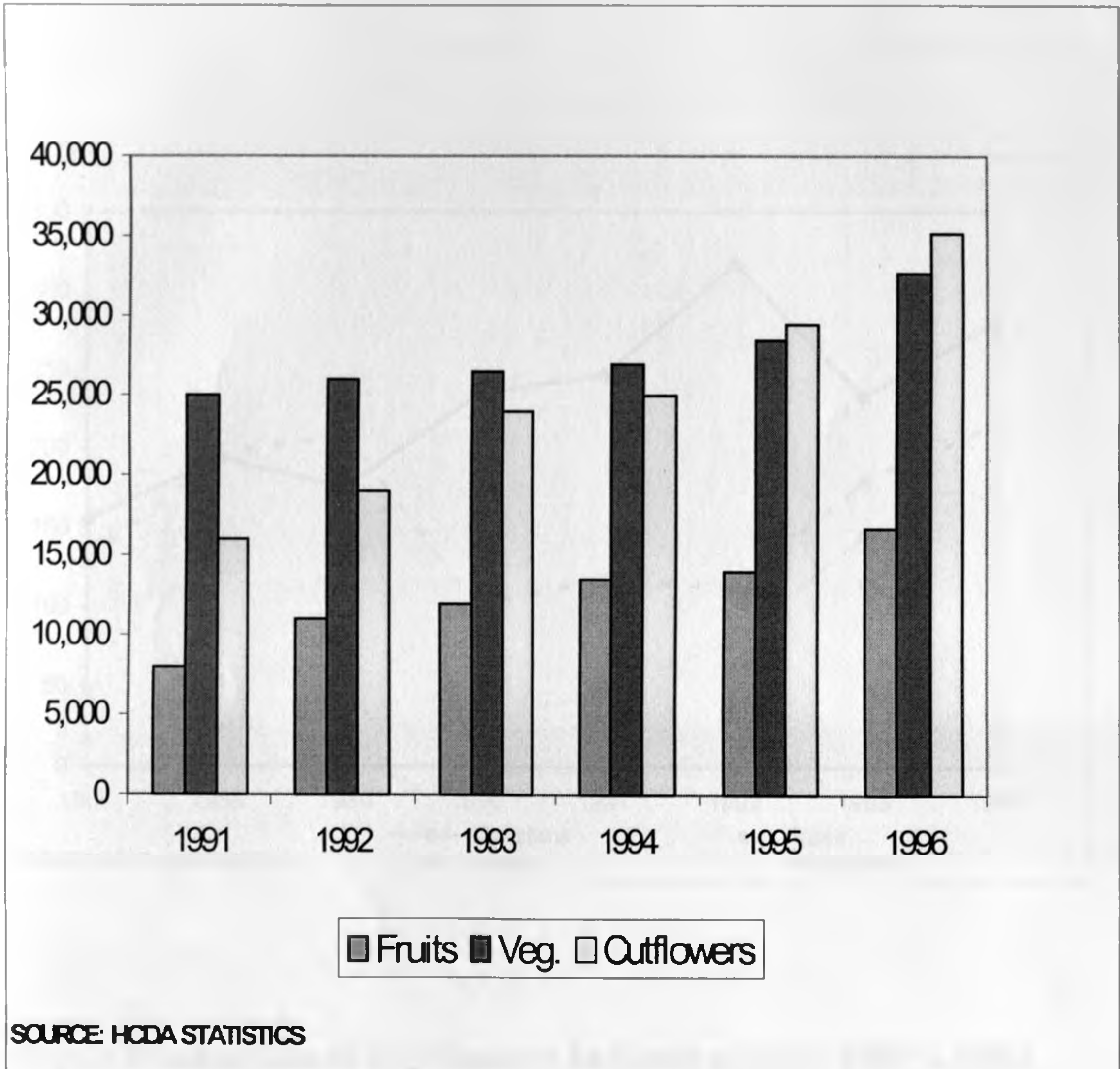

Fig. 1: Fresh produce exports from Kenya (MT).

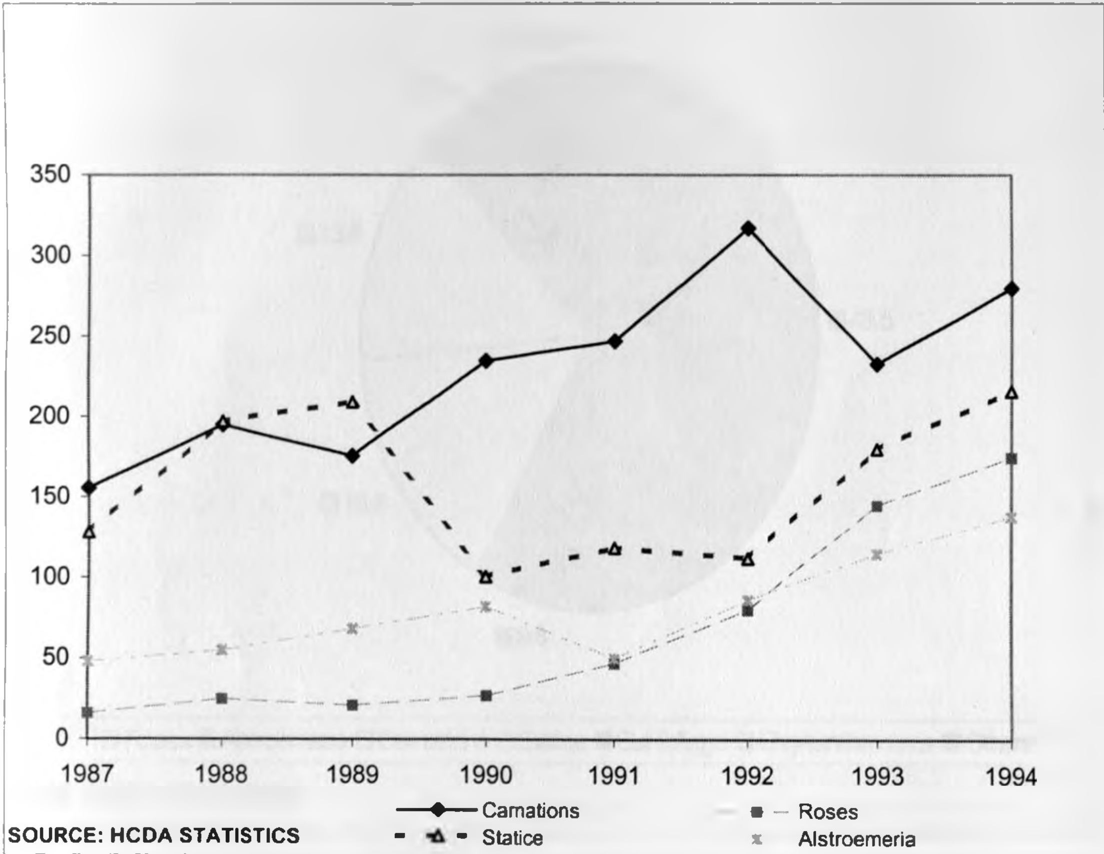

Fig 2: Production of cut flowers in Kenya (Ha): 1987 - 1994

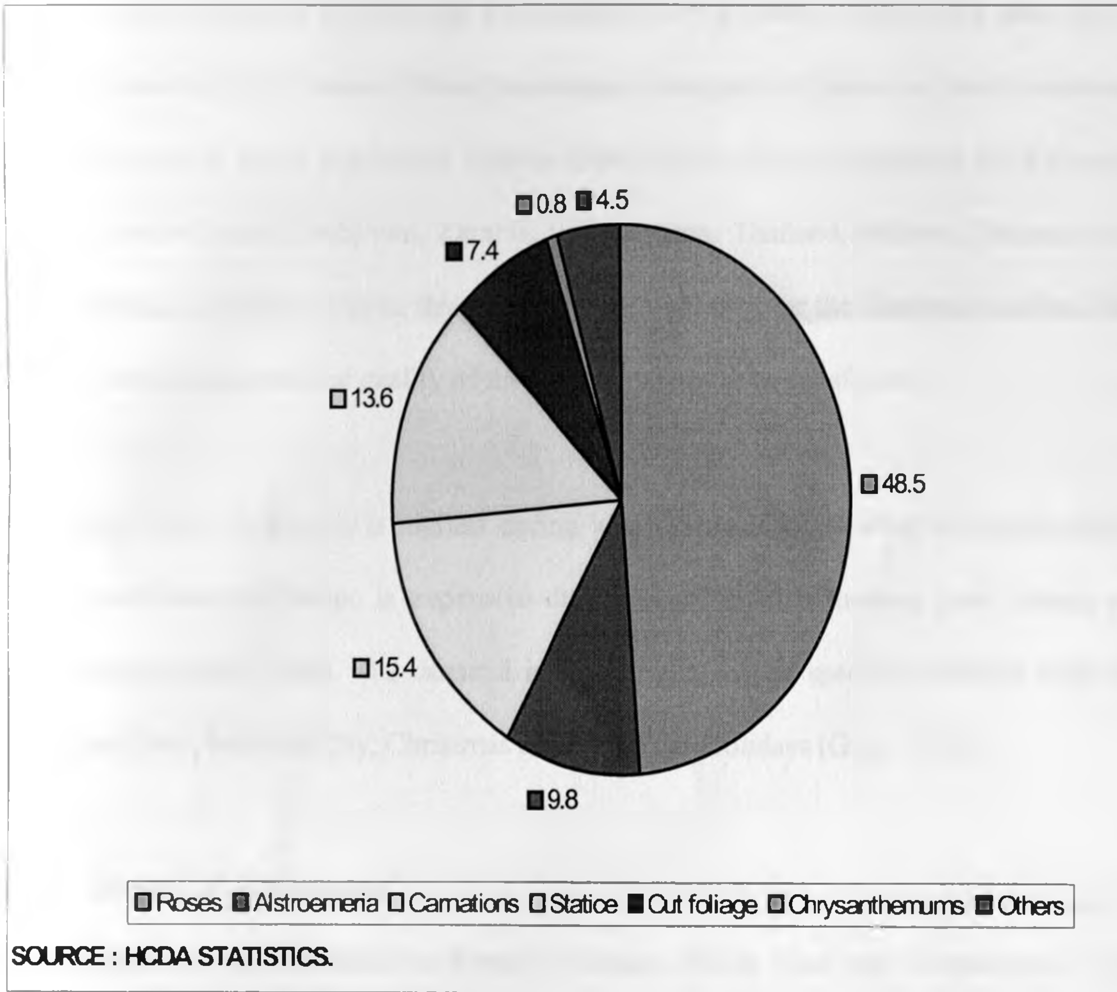

Fig. 3: Cut flowers exports from Kenya (1995).

In 1994, the main markets in order of importance were: the Netherlands (64.4 %), Germany (19 %), United Kingdom (9.9 %) and Switzerland (3.4 %) (Anon,1994). In the same year, Kenya exported a total of about 430 million stems of assorted cut flowers to Dutch Auctions and was second to Israel in terms of volume (Gray, 1996). Other competitors for the same market include Spain, Zimbabwe, Zambia, South Africa, Thailand, Malawi, Tanzania etc (Gray, 1996). In order to win in this cut throat competition for the European market, the quantity to meet demand and quality of the cut flowers has to be satisfactory.

The demand for cut flowers is highest during winter season (September to March) when flower production in Europe is expensive due to supplementary heating and lighting of greenhouses (Anon, 1988). The demand is also influenced by special occasions such as Valentine's Day, Mother's Day, Christmas and New Year Holidays (Gray, 1996).

1.2 History of *Alstroemeria*

Alstroemeria spp are named after Swedish botanist Baron Klas van Alstroemeria who brought the first plants of the genera *Alstroemeria* from South America to Europe (Uphof, 1952). There are up to 60 species, all native to South America with Chile as the centre of distribution (Uphof, 1952). *Alstroemeria species,* along with its improved cultivars and hybrids have recently received a lot of attention as potential cut flowers, since they are popular with consumers and give high yields (Healy and Wilkins, 1979; 1982). Commercial hybrid varieties along with their mutations have undergone extensive breeding over the years and are subject to the plant breeders rights (Heins and Wilkins, 1979). This implies that, the planting materials will be expensive to Kenyan growers, due to the payments of royalties to the breeders. In addition, overseas breeders will not be prepared to give out their proprietary hybrids for multiplication, if they feel they are not sufficiently protected in Kenya, hence we

shall continue propagating old varieties.

Alstroemeria, also known as the Peruvian Lily or Lily-of-the Inca has become a popular cut flower in Kenya especially with small scale growers due to its simple management practices, ease of growth outdoors, under shade netting and presence of a wide range of cultivars (Chepkairor, 1986). It is thought to have been introduced to Kenya in the late 1960's or early 1970's by former British settlers (Chepkairor, 1986). Varieties found in Kenya includes "Marina", "King Cardinal", "Pink Perfection" and "Carmen". Newer varieties currently in the market are; "Cardinal", "Tiara", "Jacqueline", "Panther", "Yellow King", "Zebra", "Sunrise", "Friendship", "Jubilee", and "Golden Delight" (HCDA, 1996). *Alstroemeria* does well in upper highlands where soil temperature is low enough (10-15°C) to induce flowering and some parts of the lower highland zones especially under irrigation. In Kenya, it is grown around Limuru and Naivasha. Other suitable areas includes Kinangop, Kericho, Molo, Kaptagat, Wundanyi, Timboroa and Meru (HCDA, 1996).

1.3 Botany

Alstroemeria belongs to the family *Alstroemeriacea*, formerly *Amaryllidacea* (Uphof, 1952). It is a herbaceous perennial which produces tall slender leafy stems up to 2 metres high. It terminates with red, pink, purple, yellow or white flowers (Uphof, 1952). *Alstroemeria aurantiaca* D. Don ex Sweet, *A. violocea* Phil, *A. haemantha* Ruitz Pav., *A. ligtu* L., *A. pelegrina* L., and *A. pulchra* Sims have been primary contributors to the interspecific hybrids available commercially (Heins and Wilkins, 1979). Most of the species are endemic to very restricted areas (Uphof, 1952).

Alstroemeria cut flowers are propagated by division of the rhizomes with attached roots and shoots or by tissue culture (Ziv *et al.*,1973). Aerial shoots arise from an underground sympodial rhizome (Heins and Wilkins, 1979). The rhizome apex develops from an axillary bud of the first scale leaf of the previous shoot (Buxbaum, 1951). The second axillary bud has the potential to become a lateral rhizome. Aerial shoots can be vegetative or generative depending on the previous environmental conditions (Heins and Wilkins, 1979). The roots are either thin and fibrous or thick and fleshy. The parallel-veined leaf twists at the junction of the sheath and blade causing the leaf to be inverted 180° (Priestly *et al.*, 1935). Stomates which appear on adaxial surface are likewise inverted (Heins and Wilkins, 1979).

The inflorescence is a whorl of simple or compound cymes (Whyte, 1929) which form a terminal branched cluster of flowers. Each cyme can consist of several flowers arising sympodially. The perianth segments are separated. They are sometimes nearly equal and sometimes markedly unequal. There are 6 stamens which are delinate. The inferior ovary is 3-celled and develops into capsule (Healy and Wilkins, 1985a).

Most of *Alstroemeria* varieties are sterile hybrids, hence as explained above, they are propagated by division of underground rhizomes with attached roots (Healy and Wilkins, 1985a) or tissue culture (Ziv *et al.*, 1973). Planting should be done in April or May to produce export flowers in September or December (HCDA, 1996). *Alstroemeria* growth and flowering is influenced by temperature (Heins and Wilkins, 1979; Healy and Wilkins, 1985b). Higher temperatures (25°C) favour production of vegetative shoots while low temperatures (10°C) favour production of higher percentage of flowering shoots (Healy and Wilkins, 1985b). Growth and flowering is also influenced by photoperiodism. Heins and Wilkins (1979) reported that flowering of *Alstroemeria* was favoured by long days (16

hours) or short night interruptions. Short days favour vegetative growth (Heins and Wilkins, 1979).

1.4 Problems Associated With *Alstroemeria*

Leaf chlorosis, loss of leaf turgidity and floret fall are problems associated with postharvest handling of *Alstroemeria* (Halevy and Mayak, 1981). The quality of cut shoots is reported to diminish with premature yellowing of the foliage before senescence of the secondary florets (Hicklenton, 1991). Premature yellowing in *Alstroemeria*, lilies and euphorbia fulgens is thought to be caused by the disturbance of hormone balance of the plant (Hofman, 1988). After cutting of the stems, the supply of hormones from the roots is cut off and in various cases the supply shortage can quickly be seen in the yellowing of the leaves. The premature leaf yellowing was associated with a low level of GA_3 in the leaves of certain cultivars of *Alstroemeria* and the length of darkness experienced during packing (Dai and Paull, 1991).

1.5 Justification

The problem of leaf yellowing in *Alstroemeria* may be eliminated by use of a pretreatment agent containing plant hormones such as gibberellins, auxins and cytokinins (Hofman, 1988). For the ethylene sensitive *Alstroemeria* (orchid types), a combined treatment of silver and plant hormones is preferred in order to prevent premature ageing of buds and dropping of the flowers (Hofman, 1988). This led to the development of pretreatment agents like chrysal-SVB which consist of gibberellins, auxins, cytokinins, tracer for control at Dutch auctions and stabilizers (Hofman, 1988) and Florissant 200, which contains plant growth hormones which prevent leaf yellowing, improves flowering and shelf life (HCDA, 1996). There is some evidence that leaf yellowing can be delayed by a combined pretreatment with two growth regulators, cytokinin and gibberellin, but this treatment is not yet commercially used in the U.S.A (Evans and Reid, 1991).

Excised-leaf experiments demonstrated the potential of GA_3 and BA in delaying leaf senescence in Easter lily (Han, 1995). However, development of leaf chlorosis on intact plants was slower than on excised leaves. Due to this discrepency, Han (1995) recommended further studies, to determine the potential for use of GA_3 and BA on the development and longevity of the flower buds, as well as, on foliar chlorosis to be conducted in a postproduction environment, hence justifying the current study.

1.6 Objectives:

The main objective of this study was to investigate the response of *Alstroemeria* cut flowers to exogenous BA equivalent (Accel), GA_{4+7}, Silver thiosulphate (STS) and Florissant 200, application in terms of the vase life and the physiological changes of the leaves in response to the treatments. The rationale for the treatments being to determine the best concentration of BA equivalent in Accel that has potential for reducing leaf yellowing in *Alstroemeria* and how it compares with recommended commercial preservatives.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Past Research

The short vase life of many cut flowers is a pressing problem in the florist industry (Sacalis, 1973). The length of vase life varies with different cut flowers. Roses might last 5 days (Sacalis, 1973); carnations, 3-7 days (Laurie *et al.*,1980); chrysanthemums, 14 days; *Cymbidium* orchids, 28 days (Laurie *et al.*, 1980) and some parigo hybrids of *Alstroemeria*, 21 days (Healy and Lang, 1989).

Alstroemeria has recently become a popular cut flower in Kenya especially with small scale farmers and in 1995 it was ranked fourth after roses, carnations and statice (Anon, 1995). Verboom (1980) observed that very little research had been conducted to determine optimum postharvest procedures in *Alstroemeria. Alstroemeria* cut flower vase life is influenced by both postharvest handling procedures and pretreatment with plant growth regulators. Vase life of *Alstroemeria* can be prolonged when the stems are picked in the morning, with at least one opened flower (Milde, 1989). Stems left uncut or cut through the blanched area of the stem absorbed less holding solution than stems cut through green stem tissue (Healy and Lang, 1989). Cutting at or above the blanched area did not decrease the vase life of the primary florets, but the vase life of the secondary florets was reduced when the stems were left uncut and the leaves on shoots cut at the blanched area were flaccid (Healy and Lang, 1989).

Furthermore, several researchers have reported physiological maturity and subsequent senescence in *Alstroemeria* (Watada *et al.*, 1984; Milde, 1989; Healy and Lang, 1989; Hicklenton, 1991). Leaf removal when secondary flowers are present can be used to eliminate the possibility of senescing leaves reducing the marketability of the inflorescence without reducing vase life (Milde, 1989; Healy and Lang, 1989; Hicklenton, 1991). Vase life of the primary florets was reduced by the presence of secondary and tertiary flower buds, yet total vase life of a generative shoot was increased by the presence of secondary and tertiary florets that continued to develop after anthesis of the primary floret (Healy and Lang, 1989). Packaging of *Alstroemeria* cut flowers for 2 days is known to shorten flower life and hasten leaf yellowing (Dai and Paull, 1991).

Cytokinins and gibberellins have been reported to improve the postharvest vase life of many cut flowers including *Alstroemeria* (Halevy and Mayak, 1981). The use of 50 mg/litre of either GA_3 or BA independently or in combination, significantly increased the vase life of *Alstroemeria* cut flowers, by allowing full opening of primary through tertiary florets, in the presence of fresh green foliage (Hicklenton, 1991). Premature yellowing of the foliage in *Alstroemeria,* which diminish the quality of cut shoots, can be prevented by the addition of a mixture of auxin (5 mg/litre), cytokinin (2 mg/litre), and gibberellin (7.5 mg/litre) to the vase water (Staden, 1976; 1978). Gibberellin was regarded as the most active component of this solution. The results of Dai and Paull (1991) further confirmed the effectiveness of GA_3 in delaying leaf yellowing in *Alstroemeria* but BA was less effective, when the two chemicals were used seperately. In the same study, addition of oasis floral preservative (OFP) to the GA_3 solution improved its effectiveness.

Ethylene is known to accelerate cut flower senescence through petal shedding, chlorosis and sleepiness among other effects (Halevy and Mayak, 1981). Several compounds have been used to inhibit ethylene synthesis. These ethylene antagonists includes: Aminoethoxyvinylglycine (AVG), Aminooxyacetic acid (AOA) and salts of 8-Hydroxyquinoline (Baker, 1983). Beyer (1976) discovered that Ag+ inhibited the action of ethylene in a number of plant responses and delayed senescence in flowers. Silver thiosulphate anionic complex (STS) pretreatment moved readily through the stems of cut flower and increased the vase life of *Alstroemeria* (Staby and Naegele, 1984; Chepkairor, 1986; Vermeulen, 1986.). Chrysal-SVB had similar effects on the vase life of *Alstroemeria* as freshly prepared silver thiosulphate complex (Chepkairor, 1986). Other reports found STS treatment was ineffective in extending vase life, increasing floret development or increasing stem fresh weight in Buddleia (Behe and Krentz, 1995) and 4 mM STS actually accelerated leaf yellowing in *Alstroemeria* (Dai and Paull, 1991).

2.2 Cut Flower Senescence

Leaf senescence is an integral part of plant development (Thomas and Stoddart, 1980). The obvious character of leaf senescence is yellowing due to breakdown of chlorophyll, which is closely associated with reduction in photosynthetic rate (Thimann, 1980; Giridhar and Thimann, 1987) and this makes other pigments particulary carotenoids and xanthophylls visible. The enzyme chlorophyllase has been implicated in the initial steps of chlorophyll breakdown (Chichester and McFeeter, 1979).

The flower is in most cases the organ with the shortest period of longevity. In polycarpic plants, death is restricted to parts of the flower itself, that is, those which senesce and often abscise soon after flowering (Mayak and Halevy, 1980). Colour fading and discolouration is

a common phenomenon in many flowers during aging and two major pigments that contribute to the colour of the flowers are carotenoids and anthocyanins (Mayak and Halevy, 1980).

Senescence in flowers is characterised by a decline in RNA (Matile and Winkenbach, 1971); protein (Hobson and Nichols, 1977); phospholipids (Beutelmann and Kende, 1977) and other macromolecules, increased activities of RNase and other hydrolytic enzymes (Hobson and Nichols, 1977); ethylene production (Nichols, 1968a); increased microviscosity of membranes (Borochov *et al.*, 1976a) and increased permeability of membranes resulting in ion leakage and water loss (Nichols, 1968a). The process of senescence is genetically programmed and is regulated by plant hormones (Halevy and Mayak, 1979) but can be hastened by water stress (Borochov *et al.*, 1976b).

Cytokinins are apparently involved in the regulation of flower senescence (Mayak and Halevy, 1974), possibly by maintaining RNA and protein levels (Ballantyne, 1966). Although proteins generally decline during senescence of flowers there is some selective protein synthesis (Baker, 1983). MacLean and DeDolph (1962) reported that BA inhibited respiration and retarded senescence in carnations and chrysanthemum flowers. Ballantyne (1965) reported that BA retarded senescence in carnations and daffodils that had been stored for 2 weeks at 0.5°C, but was ineffective on freshly cut daffodils. Combinations of 5×10^{-4} M BA and 10^{-4} M 2,4-D, applied by dipping flowers retarded senescence in freshly cut Daffodils (Ballantyne, 1965). Ballantyne (1966) concluded that retardation of senescence by BA in combination with 2,4-D was not due to an inhibition of respiration, but possibly their effects on protein and nucleic acid metabolism. Heide and Oydvin (1969) also found that short term (2 minutes) at 10^{-3} M or 12 hours at 10^{-4} M immersion of stems in BA solutions

increased display life of carnations by about 30 % compared to those held in water.

Cytokinin activity is higher in young rose petals than in old ones (Mayak and Halevy, 1970). Likewise, the endogenous cytokinin content of a long-lived variety of rose is higher than in short-lived one. Exogenous applications of BA to buds of short-lived variety delayed its senescence. Mayak and Halevy (1974) demonstrated that the main initial effect of Kinetin on leafless rose shoots was to promote water uptake and petal growth. Later effects of Kinetin were a slowing of processes associated with stress and senescence and maintenance of petal turgidity for an extended period. Van Meeteren (1979) found that treatment with 10^{-4} M Kinetin for 2 minutes reduced water loss and ion leakage in *Gerbera* flowers. Eisinger (1977) concluded that stems and leaves of carnation flowers contained an anti-senescence factor since their removal hastened senescence of the flowers. Exogenous application of Kinetin delayed senescence in flowers with stem and leaf tissues removed and also in flowers with intact stems. Cytokinins delays wilting of cut rose flowers by protecting cell integrity (Mayak and Halevy, 1974). In roses, cytokinin rather than auxin was a better inhibitor of flower abscission (Halevy and Kofranek, 1976).

The mode of application has been suggested to affect the potency of cytokinins (MacLean and DeDolph, 1962). Cytokinins were supplied to the stem and leaves of carnation variety "Scania" by immersion, spraying or continously supplied by the stem. A positive effect was only obtained with the BA supplied by immersion for 50 minutes in a 10^{-4} M concentration and with Kinetin supplied continously in 10^{-5} M and 5 X 10^{-6} M concentrations (Paulin and Muloway, 1979). The effects of cytokinins on vase life was associated with a decrease of the water and dry matter loss and a decrease of the glycolysis and of the proteolysis. Goszczynska and Nowak (1979) demonstrated that pulsing of carnation buds before cold

storage with BA caused a slight acceleration of bud opening and increased the vase life of fully opened flowers. The best results were obtained when the mixture of 20 mg/litre NAA and 5 mg/litre BA was in the pulsing solution to prevent senescence. This confirmed earlier observation that BA prevented senescence and improved quality and longevity of carnations (Heide and Oydvin, 1969).

2.3 The Role of Cytokinins in Cut Flower Senescence

Cytokinins have been reported to markedly delay or reverse leaf yellowing of some plant species (Halevy and Mayak, 1981; Leshem, 1986; Thimann, 1987). Mothes and Engelbrecht (1961) demonstrated that if a small basal leaf was treated with kinetin solution, only that area remained green while the rest of the plant turned yellow. Kinetin is however less effective on leaves attached to the mother plant possibly because attached leaves are already receiving adequate levels of endogenous cytokinins from the roots (Thimann, 1980). In water stressed plants, the level of cytokinins decreased while the level of ABA increased and leaves turned yellow fast (Ahorani *et al.*, 1977).

Cytokinins retard the breakdown of chlorophyll and proteins in excised oat leaves and delay the onset of rising respiration associated with leaf senescence (Thimann, 1987). Cytokinin applied to yellowing leaves of Cocklebur (*Xanthium pennsylvanicum* L.) reverses the breakdown of aged chloroplasts, resulting in regreening of leaves (Leshem, 1986). The gradual disorganization of grana in the chloroplasts of senescing Cocklebur leaves actually reverses during the regreening process (Leshem, 1986).

Leaf yellowing of *Alstroemeria hybrida* L. is significantly delayed when the ends of cut stems are immersed in solutions of 50 mg/litre BA, since the foliage colour and colour

intensity did not diminish during 14 days of storage in tap water (Hicklenton, 1991). Leaf yellowing of excised Easter Lily leaves is significantly delayed by application of benzyladenine $\geq$ 50 mg/litre. Rapid development of foliar chlorosis following cold storage is also delayed significantly by application of 500 mg/litre of BA before storage (Han, 1995), implying BA has the potential of delaying leaf senescence. Dai and Paull (1991) showed that 10 mg/litre Zeatin riboside and 100 mg/litre BA increased the time to 50 % petal shedding by 22 % relative to the control with deionised water.

In some plant species (e.g broccoli), pulsing cut stems with BA concentrations as low as 5 mg/litre effectively prevents discolouration, while in others it is ineffective even at higher concentrations (Halevy and Wittwer, 1966). Dai and Paull (1991) were unable to confirm any response to cytokinin in reducing leaf yellowing. Han (1995) found the increased longevity of Easter lily leaves treated with BA to be independent of the concentrations of BA used. Light has been reported to increase the action of cytokinin in oat leaves (Thimann and Satler, 1979). Red light was reported to delay senescence in rice leaves, and far red to antagonise this effect (Mishra and Pradhan, 1973), but so far, such phytochrome like effects have not been reported in other leaves.

2.4 The Role of Gibberellins (GA) in Cut Flower Senescence

Gibberellins were first discovered in Japan when it was noticed that in a field of rice infected by the fungus *Gibberella fujikuroi*, the plants grew excessively tall and often could not support themselves and eventually died from combined weakness and parasite damage (Salisbury and Ross, 1986). Yabuta (1935) isolated an active crystalline compound from the fungus, which was named gibberellin. More than 60 gibberellins have now been discovered in various fungi and plants.

It was confirmed that very small doses of GA (as low as 0.001 μg/litre) may enhance the rate of growth in length of stem internodes or of leaves of selected monocotyledonous and dicotyledonous plants (Salisbury and Ross, 1986). Externally applied GA do not show polar transport, promote root initiation, inhibit root elongation, delay leaf abscission or inhibit lateral buds (Salisbury and Ross, 1986). Some of the general effects of GA on treated plants include: growth promotion in intact plants, flower sex expression, fruit setting, breaking dormancy in seeds and buds, improvement of organ size and hybrid seed production (Wittwer, 1983).

Gibberellins have been extracted from carnation flowers and have been shown to play a role in the control of carnation petal growth (Jeffcoat and Harris, 1972). Aarts (1957) reported that 1 mg/litre GA_3 increased the longevity of *Matthiola incana* spikes. On the other hand, Kelly and Schlamp (1964) observed that GA treatment of potted intact Easter lilies (*Lilium longiflorum*) resulted in 25 to 30 % increase in longevity. In leaves of Taraxacum (dandelion) and Tropaeolum (nasturtium) GA was effective in delaying senescence (Fletcher and Osborne, 1966; Beevers, 1966). As Tropaeolum leaves senesce, their GA content steadily decreases (Chin and Beevers, 1970). A similar decrease occurs in lettuce leaves under water stress (Aharoni *et al.*, 1977). Hence senescence of these leaves is associated with endogenous deficiency of these hormones (Fletcher *et al.*, 1969; Chin and Beevers, 1970). GA effect on delaying leaf senescence is associated with DNA dependent RNA and protein synthesis (Fletcher and Osborne, 1966). GA also prevented rise of endogenous ABA level in detached lettuce leaves (Aharoni and Richmond, 1978).

Application of GA to isolated carnation petals delayed their senescence (Garrod and Harris, 1978). However, treatment to whole flowers had little or no effect on longevity but caused

some petal enlargement (Nichols, 1968b). GA_3 (100 to 400 mg/litre) in opening solution of carnations promoted opening but decreased longevity and caused discoloration of flowers (Cywinska - Smoter *et al.*, 1978). GA (20 to 35 mg/litre) produced a slight increase in longevity of carnations but accelerated bud opening of carnation (Goszczynska and Nowak, 1979) and gladiolus (Ramanuja Rao and Mohan Ram, 1979) after storage. GA had little or no effect on orchids (Arditti, 1979).

Application of GA inhibits chlorophyll loss in the leaves of *Alstroemeria* (Goszczynska and Michalczuk, 1988) and lilies (Nowak and Mynett, 1985). Addition of GA_3 to vase solutions significantly delayed leaf yellowing of cut *Alstroemeria* (Hicklenton, 1991). Leaf yellowing in excised Easter Lily leaves is delayed by application of $\geq$ 250 mg/litre GA_3 (Han, 1995). Han (1995) reported that application of 500 mg/litre GA delayed rapid development of foliar chlorosis following cold storage. However, in most plant species GA_3 does not delay leaf senescence and its content in tissues is not correlated with senescence (Halevy, 1986).

2.5 The Role of Ethylene in Cut Flower Senescence

The role of ethylene in accelerating senescence of cut flowers is widely accepted (Burg, 1973; Halevy and Mayak, 1981; Kader, 1985). The known physiological and biochemical effects of ethylene on cut flowers include increased respiratory activity, increased cell permeability, loss of cell compartmentalization and alteration of auxin transport and metabolism (Pratt and Goeschl, 1969). Morphological effects of ethylene includes in-rolling of petals (sleepiness), fading of petals, wilting and abscission of flowers, chlorosis, epinasty and abscission of leaves (Kader, 1985). Nichols (1976) found that ethylene promotes accumulation of sugars and inorganic materials in the ovary, accompanied by the loss of fresh and dry weight of the petals.

Sources of ethylene includes senescing flowers, climacteric ripening fruits and vegetables, diseased plant tissues e.g *Botrytis spp*, leakage from gas mains, exhaust from internal combustion engines in transport vehicles, improperly vented greenhouse space heaters, manufacturing plants and chopped or mechanically damaged leaves (Rogers, 1973; Hardenburg *et al*., 1986). The threshold level of ethylene in the intercellular spaces of fruit and flowers to induce ripening and senescence respectively, is 0.1 to 1.0 mg/litre (Rogers, 1973). However, carnations show reduced vase life when exposed to 50 parts per billion (ppb) ethylene in the ambient atmospheres (Baker, 1983).

Adams and Yang (1979) elucidated the biosynthetic pathway of ethylene to be as follows: Methionine - -> S-adenosylmethionine (SAM) - -> 1-aminocyclopropane -1- carboxylic acid (ACC) - -> ethylene. Halevy and Mayak (1981) reported that during the process of aging of carnation flowers, three phases can be distinguished. The first phase starts at the beginning of anthesis and is characterized by the enlargement of the inner petals and growth of the styles. In this phase, a low steady rate of ethylene production and a decrease in respiration rate is commonly observed. The second stage usually starts 6 days after anthesis in `White Sim' Carnation. Senescence processes begin, showing typical in-rolling of petals concomitantly with a burst of ethylene production and climacteric respiration. The third stage is the advanced senescence stage; petals wilt, ethylene production and respiration decline.

Exposure of carnation flowers to ethylene or ACC causes autocalytic production of ethylene which accelerates the climacteric phase (Nichols, 1968a; Mor and Reid, 1981). Tissues differ in their response to the presence of ethylene. Young flowers are not responsive, whereas mature flowers respond and transition into the second phase occurs (Mayak and Halevy,

1980). The sensitivity to ethylene can be as a result of an intricate, complex interaction between internal factors such as plant hormones (Sacher, 1973); like abscisic acid (Mayak and Dilley, 1976); carbohydrate reserve (Mayak and Dilley, 1975) and osmotic concentration of the petal tissue (Mayak *et al.*, 1978). The responding system is also affected by the environmental factors like temperature (Maxie *et al.*, 1973), water stress to cut flowers imposed by excessive drying, and cold storage used in the practice of handling flowers (Mayak and Kofranek, 1976), all result in higher sensitivity to ethylene.

Many ornamental crops can be protected from the detrimental effects of ethylene through; storing them at 40°C or below for those not sensitive to low temperatures, good ventilation, filters with an active absorbent of ethylene like potassium permanganate or brominated charcoal, 5 % carbon dioxide in storage, low oxygen concentration (1-3 %), hypobaric storage and use of inhibitors of ethylene biosynthesis and/or action (Rogers, 1973; Hardenburg *et al.*, 1986). Ethylene inhibitors includes: salts of 8- hydroxyquinoline, cobalt ion, rhizobitoxine and its analog like aminoethoxyvinylglycine (AVG), and aminooxyacetic acid (AOA). Inhibitors of ethylene action like silver ion, carbon dioxide, ethylene oxide and substituted benzothiadiazoles (Baker, 1983). Baker *et al.* (1978) found that benzoate, n-propyl gallate, and other free radical scavangers inhibited ethylene production of fruit tissue slices. Cytokinins delay the onset of senescence and reduce ethylene sensitivity and production in carnations (Cook *et al.*, 1985). While AVG is very effective in extending vase life in carnations and other flowers, it is, however too expensive to produce for agricultural applications (Baker, 1983).

Silver salts have long been used as germicides in floral preservatives (Ryan, 1957). Beyer (1976) discovered that Ag^+ inhibited the action of ethylene in a number of plant responses and delayed senescence in flowers. Silver thiosulphate anionic complex (STS) applied to the cut flower stems moved readily than silver nitrate (Veen and Geijn, 1978) and prevents the deleterious effects of ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound. STS is prepared by mixing 2 mM $AgNO_3$ with 16 mM $Na_2S_2O_3$. Pretreatment with STS is essential for flowers sensitive to ethylene, including *Alstroemeria*, sweet pea, carnation, delphinium, lily and snapdragon (Evans and Reid, 1991).

Reid *et al.* (1980a) reported that a short term (20 minute) treatment of miniature carnation with 4 mM STS, or overnight treatment with 1 mM STS extended the vase life as much as continuous use of the commercial preservative such as Everbloom which contains sucrose, weak acid and certain other components. Basal treatment of flowers of *Agapanthus orientalis* with 4 mM STS for 3 hours increased their vase life by 60 % to 120 % and 20 % reduction in bud abscission over control (Mor *et al.*, 1984). Reid *et al.* (1980b) doubled the vase life of carnation cultivar "White Sim" by immersing their stems in 1.0 mM Ag+ for 10 minutes. He further showed that 0.5 μmol Ag^+ was required per stem for maximum vase life while more than 5 μmol Ag^+ was toxic. Mor *et al.* (1989) showed that 10 mM AOA as one hour pulse, applied either before or after cold storage, extended by up to 2.7 days the longevity of roses that had been stored for 3 weeks at 10°C. They further found 0.5 mM STS as 0.5 hour pulse extended the life of fresh and cold-stored roses by 2 and 3 days, respectively.

Pulsing *Alstroemeria* with Chrysal-SVB II, a carrier of STS, GA and a fungicide increased the time to 50 % petal shedding by 22 % relative to the control (deionised water) (Chepkairor, 1986; Dai and Paull, 1991). Pulsing cut Snapdragons with 0.2 mM STS for 16 hours improved flower quality and prolonged vase life (Lee *et al.*,1995). Ethylene caused floret abscission in snapdragons and flowers pretreated with STS, and then held in preservative solution during cold storage, had better quality and longer vase life than those in plain water (Lee *et al.*, 1995). Dai and Paull (1991) found that pulsing *Alstroemeria* for 24 hours with 4 mM STS did not improve petal life and accelerated leaf yellowing. Behe and Krentz (1995) found silver thiosulphate treatment was ineffective in extending vase life, increasing floret development or increasing stem fresh weight in Buddleia. The reason for this could be, while it appears that those flowers which are very sensitive to ethylene benefits from STS treatment, those that are less sensitive will benefit from treatment, only under conditions where they are exposed to high concentrations of ethylene.

The effectiveness of the silver ion in reducing ethylene action declines with increasing ethylene concentration (Yang, 1985). It was assumed that one or more of the co-ordinating ligands (L) in the reception site facilitated the binding of ethylene to the receptor. This results in the formation of a biologically active complex that could be utilized by the plant cells during metabolism. The silver ions when applied interacts with these co-ordinating ligands, resulting in the receptor having little capacity to bind ethylene (Yang, 1985). AOA (2 mM) a known inhibitor of ACC synthase, Cobalt and AVG delays senescence and reduces ethylene responsiveness on carnations (Mor *et al.*, 1983; Cook *et al.*, 1985). The advantage of using STS, over one that inhibits ethylene biosynthesis is that, the former provides protection against exposure to ethylene in the environment (Baker, 1983).

2.6 Regulation of Ethylene Biosynthesis by Cytokinins

Cytokinins are thought to regulate carnation senescence through their interaction with ethylene (Eisinger, 1977). Kende and Hanson (1976) reported that BA delayed ethylene production in isolated rib segments of *Impomoea* flowers. Kinetin treated flowers showed a delay in endogenous ethylene production as well as less sensitivity to applied ethylene (Eisinger, 1977). Eisinger (1977) suggested that cytokinins may control ethylene-induced senescence in carnation.

Cytokinin treatment appears to reduce ethylene synthase activity, since pretreatment with BA resulted in 90 % reduction in the capacity of carnation flowers to convert exogenously applied ACC to ethylene (Eisinger, 1982). BA pretreatment prevented the normal rise in endogenous ACC levels associated with the onset of senescence (Mor *et al.*, 1983; Cook *et al.*, 1985). Cook *et al.* (1985) reported that BA treated flowers inhibited the conversion of exogenously supplied ACC to ethylene and they did not show a decline in flower quality during the course of the experiment. Mor *et al.* (1983) reported that BA treated flowers produced high levels of ethylene in response to exogenous ethylene without affecting the timing of the onset of senescence.

In carnation foliage, BA did not inhibit ethylene production in green leaves but rather slightly increased it (Mor *et al.*, 1983). This indicates that leaves respond to cytokinins in a different manner than petals. Cook *et al.* (1985) concluded that BA appears to prolong the presenescent stage in flowers during which they are unresponsive to ethylene (Halevy and Mayak, 1981), maintain low levels of ACC and limit the capacity to convert ACC to ethylene. BA was ineffective in lowering ethylene production and endogenous ACC levels, if added after senescence had begun in isolated petals (Mor *et al.*, 1983). Mor *et al.* (1983)

concluded that BA does not act as a free radical scavanger.

In the foregoing, it is clear that hormonal control is exerted through a balance between plant hormones interacting with each other and with other internal factors (Mayak and Halevy, 1980). Combined application of benzyladenine and auxin to daffodils was more effective in delaying senescence and climacteric respiration (Ballantyne, 1965) than the additive effects of the two growth regulators. In roses, cytokinin was a better inhibitor of flower abscission than auxin (Halevy and Kofranek, 1976). Treatment of intact plants with GA_3, BA or a combination of the two, before the cold storage, significantly increased longevity of leaves and delayed post-storage development of leaf chlorosis in Easter Lilies (Han, 1995). Neither GA_3 nor BA can be considered to be a universal inhibitor of chlorophyll breakdown or leaf senescence (Hicklenton, 1991). Membrane modification was suggested to be controlled by the cytokinin/ABA balance (Itai and Benzioni, 1976). These researchers did not rule out the possibility of ethylene participation.

Sacalis and Nichols (1979) showed ethylene production to be sharply inhibited by 2,4-D, hence possibility that ethylene and not auxin, is the primary regulator of senescence. The plant hormones, auxin and cytokinin appear to be involved in suppressing flower senescence, and abscisic acid in promoting it. Therefore, conditions or treatments that reduce the level of ABA and that which enhances endogenous levels of auxin and cytokinin may delay senescence of flowers. Indeed BA, a cytokinin appears to block the ethylene receptor site, as well as, the ethylene synthesis pathway, hence delaying senescence in flowers (Cook *et al.*, 1985). However, the mechanism by which GA_3 and BA delays senescence of excised Easter lily leaves is not known. Respiration rates of leaves treated with GA_3 and /or BA declined rapidly not long after the chemical treatments (Han, 1995).

2.7 Sucrose as Postharvest Preservative

Starch and sugar stored in the stem, leaves and petals provide much of the food needed for flower opening and for this reason good growing conditions, proper nutrition and high light intensity are vital in the postharvest flower quality (Evans and Reid, 1991). The supply of sugars to cut flowers promotes bud opening and retards flower senescence. Such effects have been reported for gladiolus (Kofranek and Halevy, 1976), spray carnations (Borochov and Mayak, 1984), gypsophila (Downs *et al.*, 1988) and liatris (Han, 1992). In cut flowers bearing florets that develop sequentially like *Alstroemeria*, tuberose and gladiolus, there is competition among florets for the available carbohydrate. Immature flower buds fail to develop without an additional carbohydrate supply and indeed sucrose treatment increase their quality and longevity of individual florets, perhaps by increasing the pool of respiratory substrate and lowering the osmotic potential of the petals (Halevy and Mayak, 1979; Evans and Reid, 1991).

The concentration of sucrose in the preservative solution depends on the purpose of the solution. Pulse solutions contain up to 20 % sucrose while preservative solutions usually contain 1.5-2 % sucrose (Evans and Reid 1991). The extend to which longevity of cut stems is maintained varies with plant species. Cut roses lasted 8 days in sucrose (Sacalis, 1973); carnations, 14 days (Sacalis, 1973), but Narcissus showed only a small improvement. Heide and Oydvin (1969) found 5 % sucrose and acetic acid to lower pH to 3.5 resulted in even greater vase life of carnations than with BA alone since their effects was additive. Basal treatment of partially opened flowers (2 to 5 florets open) with solutions containing 10-20 % sucrose improved bud opening of *Agapanthus orientalis* (Mor *et al.*, 1984). Halevy *et al.* (1978) found optimum opening of *Strelitzia reginae* Ait. with 20 % sucrose. However, since

the differences between 10 % and 20 % sucrose were not significant, they decided to use 10 % sucrose as a standard control. Doi and Reid (1995) found 20 g/litre of sucrose not only prolonged the longevity of individual florets but also promoted bud opening in hybrid *Limonium*, so that the vase life of cut inflorescences extended to 17 days. Pulse treatment with 100 g/litre of sucrose in combination with Physan at 200 μl/litre for 12 hours partially substituted for a continuous supply of sucrose, in the same study.

The mode of action of sucrose in increasing longevity of cut flowers is controversial. Coorts (1973) and Rogers (1973) considers the main effect of sugar to be a respiratory substrate and a basic metabolite for the growing flower. However, substrate limitations are not entirely responsible for the short vase life of cut flowers since sugar concentrations taken up by flowers and accumulated in petals were probably more than required for metabolism (Kaltaler and Steponkus, 1976; Sacalis., 1973). Acock and Nichols (1979) studied the water relations of cut carnation flowers and confirmed the findings of Halevy (1976) that sucrose in holding solutions decreases the water potential of petals. They concluded however, that the effects of sucrose were metabolic in nature, by maintaining membrane integrity and thus turgor pressure. Halevy (1976) had concluded the effect of sucrose was due to an osmotic adjustment in flowers that resulted in improved water uptake and retention. Earlier, the fact that maltose and mannitol which increases osmotic concentration of cell sap and yet they did not increase longevity of flowers made Aarts (1957) to conclude that beneficial effects of sugars on longevity of flowers was in maintenance of protoplasmic structures and not a reduction in osmotic potential of cell saps in cut flowers.

It was also suggested that the effects of osmotic potential was brought about by maintaining mitochondrial integrity (Kaltaler and Steponkus, 1976), membrane integrity or by enhancing

cuticle synthesis in the petals (Coorts, 1973; Sacalis, 1973). Sugars also reduced water loss through closure of stomates (Marousky, 1973). Other workers have shown that sucrose interacts with the several growth regulators on the senescence of cut flowers by enhancing the effect of cytokinin, delaying endogenous ethylene production and/or reducing the damaging effects of ethylene (Mayak and Dilley, 1976) and antagonising the effects of abscisic acid (Borochov *et al.*, 1976b). However, recent study by Kelly *et al.* (1985), did not show enhancement of cytokinin effects when sugar was present as previously reported.

Flower petal tissue generally contains high activity of invertase (Halaba and Rudnicki, 1989) and indeed, most of the sugar pool of mature petals is composed of inverted sugars (Nichols, 1973). Nichols (1976) reported an increase in the ratio of sucrose to reducing sugars in senescing and in ethylene triggered prematurely wilting carnations. This may be explained by an assumed partial or total activation of the invertase. Halaba and Rudnicki (1989) showed that crude extracts containing an invertase inhibitor prepared from wilting petals of *Alstroemeria* "Zebra" and other flowers reduced invertase activity while incubated together with a crude extract of invertase from fresh petals of the same species. Inhibition levels of 20-30 % were observed, which indicates that invertase activity during petal senescence is controlled by the synthesis of an inhibitor. It was postulated that, this invertase inhibitor is possibly formed in the petals of all flowers at the beginning of wilting and thus controls the translocation of sucrose from wilted petals to other organs of the flower (Halaba and Rudnicki, 1989).

At incipient wilting sucrose disappeared as reducing sugars increased (Nichols, 1973), hence wilting was not caused by depletion of respiratory substrate. Carpenter and Dilley (1975) suggested that, the increased respiratory rate in flowers treated with sucrose may result in an

accumulation of carbon dioxide which could act as an antagonist of ethylene action. In this regard, De Stigter (1980) concluded that, aside from the effect of glucose on energy and hormone levels, its main effect on water balance results from stomatal closure preventing initial water loss and improved water retention and solute uptake capacity by preserving membrane integrity through a metabolism-depended process. Therefore, further research is needed to establish the actual mechanism of sucrose in delaying flower senescence.

2.8 Water Balance Maintenance

Cut flowers have a high surface area to volume ratio and frequently many leaves. They lose water much more readily through stomates in leaves than many other perishable commodities. They should therefore be stored in high humidities (> 95 %) and low temperatures to minimise water loss (Evans and Reid, 1991). Maintenance of improved water status seems to be the most important aspect in extension of longevity in cut flowers (Rogers, 1973). Hence, as discussed above, one of the main effects of applied sugars on flower longevity seems to result from their contribution to the osmotic adjustment of the flowers (Halevy, 1976). A high level of turgidity is necessary for development of flower buds to full-bloom maturity (Rogers, 1973).

Turgidity in plants and flowers is dependent upon a balance between water uptake and transpiration losses (Laurie *et al*., 1980). After cutting the floral stems, the transpiration rate remains nearly constant, while the absorption rate declines. The absorption rate is determined by the water potential gradient along the cut stem and by the resistance to water flow from the vase to the petals (Laurie *et al*., 1980). Since the water in the xylem elements is usually under tension, when a stem is cut, the tension is released and a small air bubble enters each conducting tube (air embolism). These bubbles cannot move far up the stem and may restrict

the flow of water to the flower when the stem is placed in the vase (Evans and Reid, 1991). Embolism is removed by recutting the stems under water (2 cm is removed), conditioning of the flowers by placing them in warm water (38°C to 43°C) in 4°C storage room for 6 to 12 hours and acidifying (pH 3.5) the vase solution (Rogers, 1973; Evans and Reid, 1991). Warm water used in reconditioning is taken up rapidly and in greater quantity and also retention is better than cold water, hence leaves and flowers regain turgidity. It also improves the opening of certain flowers (Iris, Gladiolus, Roses etc) (Hardenburg *et al.*, 1986). Lower pH levels improves water uptake.

Water quality has an important effect on the keeping quality of cut flowers and decorative foliage since alkaline water does not move readily through cut flower stems (Rogers, 1973; Hardenburg *et al.*, 1986; Evans and Reid, 1991). Flouride containing waters can cause a severe necrosis of petal and leaf tissue on gladioli (Marousky and Woltz, 1971). This problem can be overcome by either removing minerals from the water (ion exchange columns or reverse osmosis) or by acidifying the water by addition of 300-500 mg/litre of citric acid (Hardenburg *et al.*, 1986; Evans and Reid, 1991).

Sugar which is normally added to vase solution is an excellent substrate for the growth of fungi and bacteria in water and this growth can be enhanced by materials that leak out of the cut stem end (Evans and Reid, 1991). Stem plugging and reduced water transport capacity is related to substances produced by the bacteria and/or bacteria themselves which clog the fine tubes of the water conducting system (Aarts, 1957; Evans and Reid, 1991). It is important that buckets be cleaned regularly and pretreatment solutions and vase solutions contain material to prevent growth of bacteria (Hardenburg *et al.*, 1986; Evans and Reid, 1991). Treatments that inhibited microbial growth helped flower stems maintain a higher rate of

water transport and increased keeping quality (Laurie, 1936).

Direct or indirect bacterial stem-plugging is not, however the only cause of impeded water movement in cut flowers (Rogers, 1973). It has been shown that flowers kept in solutions containing only bacteriocidal chemicals often keep no longer than those in plain water (Wiggins and Payne, 1963) and this led to the concept of physiological stem-plugging caused by wound gums. This physiological blockage has been reported in *Anthurium* (Paull and Goo, 1982) and in roses is referred to as "bent-neck" (Burdett, 1970). Some authors showed that the xylem of cut rose flowers contained amorphous plugs (Burdett, 1970; Lineberger and Steponkus, 1976), but others have seriously questioned the relevance of these plugs for vascular blockage, as they were found in only a few xylem elements (Rasmussen and Carpenter, 1974; Van Doorn and Perik, 1990). The most direct evidence for the role of physiological processes in the xylem blockage of cut rose flowers "Better times" was given by Marousky (1969, 1971) who found continued reduction in water uptake in cut flowers placed in sterile solutions, since microbial blockage was ruled out.

The cut surface of rose stems was found to produce considerable amounts of ethylene (Van Doorn *et al.*, 1989) and ethylene induced the presence of vascular plugs in *Ricinus communis* (VanderMolen *et al.*, 1983). Olien and Bukovac (1982) reported that ethephon treatment resulted in a blockage in xylem vessels of *Prunus cerasus* (Rosaceae). After the stems of carnations are cut, an impermeable layer of suberin-like material may be deposited on the cut surface (Cline and Neely, 1983). Enzymes involved in polymerization processes leading to deposition of lignin and suberin are inhibited at low pH (Vamos Vigyazo, 1981). If physiological vascular blockage is due to enzyme action, it should be reduced or prevented by enzyme inhibitors or conditions unfavourable for enzyme activity (Rogers, 1973). Indeed,

low pH (3 to 4) improved the keeping life of cut flowers (Aarts, 1957; Marousky, 1971) and it also inhibited enzymes involved in polymerization process, leading to deposition of lignin and suberin (Vamos Vigyazo, 1981). Buys and Cours (1980) reported that, formation of oxidation products by reactivation of polyphenoloxidase or peroxidase enzymes are responsible for the blockage in floral stem. Paull and Goo (1985) and Walker (1969), suggested that this vascular occlusion was due to ethylene-stimulated production of gums.

Researchers have shown that water relations of cut flowers can be influenced by plant growth regulators and other chemicals such as antimicrobial compounds (Baker, 1983). Cytokinins (BA, Kinetin), gibberellins and auxins (2, 4-D) reduced loss of fresh weight in cut flowers (Ballantyne, 1965; Mayak and Halevy, 1974; Han, 1995) by increasing both water uptake and petal expansion. Low levels of ABA is associated with improved water balance (Borochov *et al.*, 1976b). *Alstroemeria* shoots at times fail to absorb water and the leaves, florets and in severe cases, stems become flaccid when the stem base is either not cut or cut through blanched portion of the stem (Healy and Lang, 1989).

Antimicrobial agents like calcium nitrate and silver nitrate prevents the damaging effects of substances present in bacterial filtrates and silver ion acts also as inhibitor of ethylene action (Mayak *et al.*, 1977; Beyer, 1976). Schnabl (1976) showed that spraying with aluminium sulphate (0.1 %) caused partial closing of stomates and reduced transpiration of cut rose flowers. Cobalt ion improves water uptake but it is not known how it exerts its effect on water relations, probably by inhibiting ethylene production or by some other mechanisms (Baker, 1983).

Salts of 8-hydroxyquinoline have been widely used as antimicrobial substances in vase solutions and Larsen and Cromarty (1967) demonstrated that these compounds were wide spectrum microbiocide, inhibiting the growth of yeasts, bacteria and fungi. However, they do not kill them (Evans and Reid, 1991). Marousky (1969) found that 8-hydroxyquinoline citrate (8-HQC) caused reduced stomatal aperture in leaves of cut rose flowers, and decreased the stem resistance to water flow that develops in cut flowers. The effect of 8-HQC on reducing stem resistance to water flow was evident even under aseptic conditions, indicating that the compound inhibited the physiological component of reduced water flow (Marousky, 1969). Many antimicrobial compounds are known but they have not been used as widely as salts of 8-HQC, silver and aluminium.

Van Doorn and Perik (1990) showed that 8-HQC and low pH prevented vascular blockage by reducing the number of bacteria in the stems. However, no evidence was found for hypothesis that 8-HQC and low pH inhibit a stem induced vascular blockage in *Rosa hybrida* L. cvs "Sonia", "Ilona", "Polka" and "Frisco". Marousky (1972) reported that effective flower opening, improved longevity and increased turgidity was achieved in gypsophila by use of 8-HQC and sucrose. A preservative solution containing 150 mg/litre 8-HQC prolonged vase life of cut snapdragons (Lee *et al.*, 1995).

The mechanism of 8-HQC action as a microbiocide is considered to be due to its properties as chelating agent (Rogers, 1973), but it is not known whether its effects on the physiological processes of stomatal closing and vascular resistance to water flow are due to its properties as a chelatig agent (Baker, 1983). Stomatal closing in cut flowers in response to 8-HQC may not be a universal phenomenon, since Gay and Nichols (1977) found no evidence that it caused stomatal closure in chrysanthemum.

CHAPTER 3

MATERIALS AND METHODS

3.1 Site

This study was conducted using *Alstroemeria* flowers cvs `Yellow King' and `Marina', obtained from a commercial farm in South Kinangop whose altitude is between 2000-2500 m above sea level. The two cultivars were obtained from the same farm and were grown under the same agroecological conditions for both experiments. The rain is bimodally distributed. The long rains fall from March to June while short rains fall from October to December. The annual rainfall is between 900-1000 mm. The mean maximum day temperature is about 20.9°C and mean minimum of 10.1°C.

3.2 Plant Material

Two cultivars of *Alstroemeria* `Yellow King' and `Marina' were used for the study. `Yellow King' is a relatively new cultivar compared to 'Marina'. They were propagated through division of underground rhizomes with attached roots. The rhizomes were planted at a depth of 10-15 cm. They were grown on raised beds 1 metre wide with 50 cm paths. Each bed contained 2 rows of plants with 35-40 cm between rows and 40-50 cm within plants. At the time of bed preparation 500 kg/ha Triple Super Phosphate fertilizer was incorporated. After six months of growth, a top dress was applied using 17-17-17 NPK.

During the entire growing season non-flowering stems were pulled out to leave sufficient foliage for the plant to grow. The flowers were grown under rainfed conditions and there was no supplementary irrigation. The field was kept weed free by shallow cultivation to avoid disturbance of the root system.

Flowering shoots of 'Yellow King' and 'Marina' *Alstroemeria,* were harvested on 2nd April and 23rd May 1997, from a 24 month old plants growing in an open field for the first and second experiments, respectively. Marketable inflorescence shoots between 70 cm and 100 cm long were pulled from the rhizome as the primary florets opened. This was done in the morning and taken to the grading shade. They were then sorted and graded to 62 cm, packed and received the same day at our laboratory in Kabete Campus, University of Nairobi for further analysis. The flowers were immediately unpacked and 2 cm of stem was cut off under water to avoid air embolism.

The lower 10 cm of the stems was defoliated to avoid emersion of leaves into vase solutions. These leaves were used for initial (0 day) determination of chlorophyll content, dry weight, moisture content and total nitrogen content. All cut flowers were then pulsed in 2 % sucrose solution for 24 hours (Goszcynska and Nowak, 1979; Cook *et al.*,1985). Results of Kelly *et al.* (1985) did not show enhancement of the cytokinin effect when sugar was present. *Alstroemeria* cut flowers bear florets that develop sequentially leading to competition among florets for available carbohydrate. Immature flower buds fail to develop without an additional carbohydrate supply such as sucrose (Halevy and Mayak, 1979; Evans and Reid, 1991). As the cut flowers were harvested at bud stage, it was necessary to pulse them, to have equal energy level at the beginning of the experiment to avoid this intra floret competition. Each cultivar was held separately in one big bucket with the solution.

3.3 Vase Solutions

Deionised water was used to make preservative solutions. Each preservative solution contained 80 mg/litre 8-hydroxyquinoline sulphate (8HQS) as a biocide (Reid and Kofranek,

1980). The pH of the preservative solutions was in the range 3.0 to 3.5. This was important since lower pH level improves water uptake (Evans and Reid, 1991).

Two percent sucrose solution for pulsing the flowers was prepared by dissolving 20 grams of commercial sucrose in 1 litre of deionised water. Cut stems of the flowers were pulsed for 24 hours.

Silver thiosulphate complex (STS) was prepared according to Gorin *et al.* (1985). Silver nitrate (0.395 g) was first dissolved in 500 ml of deionised water which was added to a solution of 2.31 g sodium thiosulphate dissolved in 500 ml of deionised water while stirring. The concentration of silver was 2.0 mM. The preparation of the stock solutions was done in flasks covered with black cloth to maintain stability of the anionic STS complex which is only stable in excess sodium thiosulphate (Systema, 1980; Gorin *et al.*, 1985).

Accel[R] [a liquid concentrate containing 20 g a.i/litre (w/w) 6-benzyladenine and 2 g a.i/litre (w/w) gibberellins (GA_{4+7}), Abbott Laboratories, Chicago, Illinois] was used to prepare 25, 50, 75 and 100 mg/litre BA equivalent solutions.

To assess if the gibberellins found in Accel affected BA efficacy, Provide [R] [a liquid concentrate containing 21 g a.i/litre (w/w) gibberellins (GA_{4+7}), Abbott laboratories, Chicago, Illinois] at 2.5, 5.0, 7.5, 10.0, 12.5 or 15.0 mg/litre was applied to *Alstroemeria* cut flowers in amounts equivalent to GA_{4+7} found when Accel was applied at 25, 50, 75 or 100 mg/litre BA equivalent.

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Florissant 200, manufactured by Florissant sales b.v., Holland contains plant growth

hormones (probably cytokinins and gibberellins) which prevents leaf yellowing. One tablet of Florissant 200 was added per 3 litres of deionised water. A clean bucket was used and old solution was never mixed with a fresh one, in accordance with manufacturer's advice.

3.4 Treatments and Experimental Design

The experiments were set out as 11 x 2 and 13 x 2 full factorial in the first and second experiments, respectively, and arranged in a completely randomised design (CRD). `Yellow King' and `Marina' *Alstroemeria* cultivars were treated with 11 and 13 preservative solutions giving a total of 22 and 26 treatment combinations, respectively, which were randomised in each of the 3 replicates. Eight stems were used per replicate. The preservative solutions were:

1. Deionised water (control)
2. 25 mg/litre BA equivalent (Accel)
3. 50 mg/litre BA equivalent (Accel)
4. 75 mg/litre BA equivalent (Accel)
5. 100 mg/litre BA equivalent (Accel)
6. Florissant 200
7. 2.0 mM STS (Silver thiosulphate)
8. 2.5 mg/litre GA_{4+7} (Provide)
9. 5.0 mg/litre GA_{4+7} (Provide)
10. 7.5 mg/litre GA_{4+7} (Provide)
11. 10.0 mg/litre GA_{4+7} (Provide)
12. 12.5 mg/litre GA_{4+7} (Provide)
13. 15.0 mg/litre GA_{4+7} (Provide)

All the cut flowers were kept under similar conditions at room temperature 23°C ± 2°C, 74 - 81 % relative humidity, and continuous lighting with 64 cool - white Sylvania fluorescent lamps (65W, 240V) giving a total light intensity of 4160 J/S. The area of the laboratory was 178 m^2 and was maintained free of any contaminant such as old plant material, smoke and dust. The vases were placed on 1.5 m high bench tables.

3.5 Dependent Variables Determined

(a) Vase life determination

(i) Number of days to full opening of the primary florets.

(ii) Number of days from harvest to 50 % petal fall and

(iii) Number of days from harvest to 50 % leaf yellowing

were determined from daily observation (Dai and Paull, 1991).

The fifty percentage point was used since by this time the quality of cut shoots was diminished. Complete petal fall and leaf yellowing would not be of any significance to the customer since aesthetic value of the flowers will have been lost.

(b) Chlorophyll Content Determination

Chlorophyll content was determined from 2 discs per leaf (9 mm diameter) cut using a cork borer from 5 leaves per replicate. The 10 discs were extracted in 4 ml of 0.1N HCl in methanol at 21°C in a dark room for 24 hours. Absorbance of extracts were measured using a WPA S105 Spectrophotometer. The leaf chlorophyll content was measured as absorbance of these extracts at 653 nm (Holden, 1965; Douglas, 1983). The following equation was used to calculate the relative total chlorophyll content (Douglas, 1983).

Chlorophyll (mg/cm² of *Alstroemeria* leaf) = 24.88 x A_{653}

Where: A is absorbance at 653 nm.

24.88 is a molar extinction coefficient.

(c) Dry Weight Determination

Eight grams of lower fresh leaves were weighed immediately after removal from the shoots for all treatments, after 0, 7, 14 and 21 days using Sartorius digital balance ELE. The leaf samples were put in brown paper bags and oven dried at 66°C to constant weight (72 hours) using Memmert, UL80 780218 incubator. Dried leaves were weighed for dry weight determination.

(d) Water Content Determination

Water content of the leaves was determined by subtracting dry weights from their corresponding fresh weights (8 grams), after 0, 7, 14 and 21 days (Ballantyne, 1965).

(e) Total Nitrogen Content Determination

The dried leaves, used in dry weight determination for each treatment were ground using a Coffee Mill (Moulinex, superior `S'). Nitrogen content (%) was analyzed using Microkjeldahl method according to the Association of Official Analytical Chemists (A.O.A.C., 1984).

The nitrogen present in the sample is converted to ammonium sulphate by digestion at 380°C with concentrated sulphuric acid, in the presence of a catalyst, potassium sulphate and mercuric oxide. Ammonia liberated by distilling the digest with 46 % sodium hydroxide solution is absorbed by boric acid and is titrated for quantitative estimation. Total nitrogen

content in the samples was calculated using the equation:

$$\% N = \frac{\text{Titre(ml) x Normality of acid x 14.007 x 100}}{\text{Oven dried weight of sample (O.5 g) x 1000}}$$

Where: 14.007 is the equivalent weight of nitrogen according to the Association of Official Analytical Chemists (A.O.A.C., 1984).

3.6 Data Analysis

Analysis of variance was performed on the data collected using the general linear models (Proc GLM) procedure of the Statistical Analysis System (SAS) program package (SAS, 1990). Linear, quadratic and cubic orthogonal polynomials were tested and appropriate regression models were used to examine the nature of the response to benzyladenine (BA) and gibberellins (GA_{4+7}) concentration (Snedecor and Cochran, 1989). Multiple comparisons among means were done using the Honest Significant Difference (Tukey's) and the protected Least Significant Difference (LSD) at P=0.05. Proc Univariate procedure was carried out on the residuals to support the assumptions of normality made by the researcher.

CHAPTER 4

RESULTS

There were no interactions between the plant growth regulators and *Alstroemeria* cultivars for all dependent variables determined, therefore only the main effects are reported.

4.1 Vase life of *Alstroemeria* Cut Flowers

(a) Number of days to full opening of the primary florets

Treating *Alstroemeria* flowers with 25, 50 or 75 mg/litre BA equivalent of Accel increased the mean number of days to full opening of primary florets. However, the 100 mg/litre BA equivalent (Accel) had no effect on the opening of primary florets (Table 1). As the concentration of Accel increased, there was a decrease in the days to opening of primary florets treated with 100 mg/litre BA equivalent producing no significant effects from control, in both experiments. Accel (25mg/litre BA equivalent) which was the best treatment of the lot, increased the mean number of days by 1.50 and 1.33 days, in the first and second experiments, respectively. The response to Accel concentration was cubic and quadratic in the first and second experiments, respectively. There was cultivar difference, with `Yellow King' taking longer time to open than `Marina', in both experiments (Table 1).

The lower levels of GA_{4+7} (2.5, 5.0, or 7.5 mg/litre) had no effect on the number of days to full opening of primary florets, except 10 mg/litre GA_{4+7} delayed the full opening of primary florets, in experiment one (Table 2). In the second experiment, GA_{4+7} had no effect on the number of days to full opening of the primary florets. The cultivars showed significant differences for most of the parameters under the influence of GA_{4+7} in both experiments.

Table 1: **The effect of Accel on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers**

	First Experiment			Second Experiment		
Accel (BA equivalent mg/litre)	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
0 (control)	4.50c	14.17d	18.50b	4.17c	14.33d	14.50d
25	6.00a	19.50a	20.33a	5.50a	16.17a	20.50a
50	5.50ab	18.50ab	21.00a	5.00ab	15.50ab	18.83b
75	5.33b	17.33b	18.67b	5.00ab	15.33bc	17.50c
100	5.00bc	15.50c	17.00c	4.67bc	14.67cd	16.83c
Significance[z]	L^{***},Q^{**},C^{**}	$L^{****},Q^{****},C^{***}$	L^{***},Q^{**}	L^{**},Q^{*}	L^{***},Q^{**},C^{*}	$L^{****},Q^{****}C^{****}$
LSD	0.63	1.20	0.87	0.77	0.70	0.97
Cultivars						
Yellow King	6.00a	18.13a	18.73b	5.13a	15.33a	17.07b
Marina	4.53b	15.87b	19.47a	4.60b	15.07a	18.20a
Significance	****	****	*	*	ns	***
LSD	0.39	0.76	0.55	0.49	0.45	0.61

[z] The response was linear (L), quadratic(Q) or cubic (c).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 2: The effect of GA_{4+7} (Provide) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers

	First Experiment			Second Experiment		
GA_{4+7} (mg/litre)	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
0 (Control)	4.50b	14.17b	18.50c	4.17ab	14.33c	14.50b
2.5	4.67b	18.50a	20.50b	3.33b	15.33bc	19.33a
5.0	4.67b	18.67a	21.17ab	4.00ab	15.67ab	20.00a
7.5	5.00ab	19.17a	21.67a	4.33ab	16.00ab	21.00a
10.0	5.33a	19.33a	22.00a	4.83a	16.50a	21.83a
12.5	-	-	-	4.33ab	16.00ab	20.83a
15.0	-	-	-	3.83ab	15.50ab	20.33a
Significance[Z]	*	L****,Q***,C**	L**	L*,Q*	****	L***
LSD/HSD[Y]	0.55	1.10	1.10	1.33	1.08	2.59
Cultivars						
Yellow King	5.47a	18.80a	20.53a	4.52a	15.76a	18.67b
Marina	4.20b	17.13b	21.00a	3.71b	15.48a	20.71a
Significance	****	****	ns	***	ns	****
LSD	0.35	0.70	0.70	0.46	0.37	0.90

[Z] The response was linear(L), quadratic(Q) or cubic(C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively.

[Y] Means separated by the protected LSD (P=0.05) in first experiment and Tukey's HSD (P=0.05) in the second experiment respectively; means with the same letter(s) within columns are not significantly different.

The lower BA concentration (25 mg/litre) delayed the full opening of the primary florets while the higher BA concentrations (75 or 100 mg/litre) had no significant effects even though 100 mg/litre BA showed some acceleration in opening of the primary florets (Table 3).

Florissant 200, increased the number of days to opening of primary florets by 1.33 days, in the first experiment and had no effect in the second experiment (Table 4). Florissant 200 was not different from any of the Accel treatments in both experiments. In the first experiment, 2.5 or 5.0 mg/litre GA_{4+7} enhanced the opening of the primary florets of *Alstroemeria* compared to Florissant 200, however 7.5 or 10.0 mg/litre GA_{4+7} were not different from Florissant 200. However, in the second experiment, the response of *Alstroemeria* flowers to GA_{4+7} was not different from Florissant 200 (Table 4). STS (2.0 mM) was not different from all Accel and GA_{4+7} concentrations used in all experiments, in respect to primary floret opening (Table 4).

(b) Number of days from harvest to 50 % petal fall

In the first experiment, holding *Alstroemeria* flowers in Accel increased the number of days to 50 % petal fall (Table 1). Accel (25 mg/litre BA equivalent) was the best treatment and increased the vase life of petals by 5.33 days compared to the control. The response to Accel concentration was cubic. In the second experiment, holding *Alstroemeria* cut flowers in 25, 50, or 75 mg/litre BA equivalent (Accel) increased the number of days to 50 % petal fall (Table 1), and the response was cubic to increasing Accel concentration. However, 100 mg/litre BA equivalent (Accel) had no effect, on the number of days to 50 % petal fall (Table 1). As the concentration of Accel increased, there was a decrease in the mean number of days to 50 % petal fall. The two cultivars responded differently to Accel treatment, in respect to number of days to 50 % petal fall (Table 1). In the first experiment, 'Yellow King' took a longer time for petals to fall than 'Marina' while in the second experiment, the same trend was observed though

Table 3: **The effect of Benzyladenine (Accel-GA_{4+7}) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers.**

	First experiment			Second experiment		
BA (mg/litre)	Days to full opening of primary florets	Days to 50 % petal fall	Days to 50 % leaf yellowing	Days to full opening of primary florets	Days to 50 % petal fall	Days to 50 % leaf yellowing
0(Control)	0.00bc	0.00a	0.00a	0.00c	0.00ab	0.00ab
25	1.33a	1.00a	-0.17a	2.17a	0.83a	1.17a
50	0.83ab	-0.17a	-0.17a	1.00b	-0.17b	-1.00b
75	0.83ab	-1.83b	-3.00b	0.67bc	-0.67b	-3.50c
100	-0.33c	-3.83c	-5.00c	-0.17c	-1.83c	-5.00c
Significance**		****	****	****	****	****
LSD	0.86	1.58	1.47	0.88	0.84	1.89

, **, ns, significant within columns at P=0.01, 0.0001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 4: The effect of plant growth regulators on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers

	First Experiment			Second Experiment		
Concentration (mg/litre)	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Accel						
0 BA (control)	4.50b	14.17c	18.50cd	4.17abc	14.33c	14.50ef
25 BA	6.00a	19.50a	20.33b	5.50a	16.17a	20.50ab
50 BA	5.50ab	18.50a	21.00ab	5.00ab	15.50abc	18.83bcd
75 BA	5.33ab	17.33ab	18.67c	5.00ab	15.33abc	17.50cd
100 BA	5.00ab	15.50bc	17.00d	4.67abc	14.67bc	16.83de
Provide						
2.5 GA_{4+7}	4.67b	18.50a	20.50ab	3.33c	15.33abc	19.33abcd
5.0 GA_{4+7}	4.67b	18.67a	21.17ab	4.00bc	15.67ab	20.00abc
7.5 GA_{4+7}	5.00ab	19.17a	21.67ab	4.33abc	16.00a	21.00ab
10.0 GA_{4+7}	5.33ab	19.33a	22.00a	4.83ab	16.50a	21.83a
12.5 GA_{4+7}	-	-	-	4.33abc	16.00a	20.83ab
15.0 GA_{4+7}	-	-	-	3.83bc	15.50abc	20.33ab
Florissant 200	5.83a	17.67ab	21.33ab	4.33abc	16.00a	19.50abc
2.0 mM STS	5.17ab	19.17a	12.50e	4.33abc	15.83ab	12.50f
Significance	****	****	****	****	****	****
Tukey's HSD	1.02	2.29	1.60	1.36	1.29	2.65

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or non-significant respectively. Means separated by Tukey's HSD (P=0.05); means with the same letter(s) within columns are not significantly different.

the cultivar response was nonsignificant (Table 1).

In experiment one, GA_{4+7} increased the number of days to 50 % petal fall (Table 2), and the response to increasing GA_{4+7} concentration was cubic. However, in the second experiment, 2.5 mg/litre GA_{4+7} had no effect, on the number of days to 50 % petal fall (Table 2). In both experiments, there were no differences on the number of days to 50 % petal fall within the GA_{4+7} concentrations. The cultivars were highly significant and nonsignificant in the first and second experiments, respectively. 'Yellow King' took a longer time for petals to fall than 'Marina', in both experiments (Table 2).

Higher BA concentration (100 mg/litre) accelerated the onset of 50 % petal fall while lower BA concentrations (25 or 50 mg/litre) had no effect, in both experiments (Table 3). BA (75 mg/litre) accelerated the onset of 50 % petal fall, in the first experiment and had no effect, in the second experiment.

The plant growth regulators (Accel, GA_{4+7}, Florissant 200 and 2.0 mM STS) increased the mean number of days to 50 % petal fall (Table 4). Accel (25 or 50 mg/litre BA equivalent) were not significantly different from all the GA_{4+7} concentrations, Florissant 200 and 2.0 mM STS, in the two experiments, in respect to increasing the number of days to 50 % petal fall (Table 4). Increasing BA concetration in Accel above 25 mg/litre led to a decrease in the number of days to 50 % petal fall (Table 4). Holding *Alstroemeria* flowers in Accel (25 mg/litre BA) resulted in the highest number of days to 50 % petal fall.

Florissant 200, increased the vase life of petals by 3.5 and 1.67 days, in the first and second experiments, respectively, in comparison to the control (Table 4). Likewise, 2.0 mM STS

increased the vase life of petals by 5 and 1.5 days, in the first and second experiments, respectively, compared to the control (Table 4).

(c) Number of days from harvest to 50 % leaf yellowing

In experiment one, 25 and 50 mg/litre BA equivalent (Accel), increased the number of days to 50 % leaf yellowing (Table 1). Accel (75 mg/litre BA) had no effect, but 100 mg/litre BA equivalent significantly decreased the number of days to 50 % leaf yellowing (Table 1). In the second experiment, all the Accel levels increased the number of days to 50 % leaf yellowing (Table 1). Higher Accel levels (50, 75 or 100 mg/litre BA equivalent) significantly reduced the number of days to 50 % leaf yellowing when compared to 25 mg/litre BA equivalent (Table 1). The response to Accel (BA equivalent) concentration was quadratic and cubic in first and second experiments, respectively. The cultivars were significant with `Marina' taking a longer time to attain 50 % leaf yellowing than `Yellow King' (Table 1).

All the levels of gibberellins (GA_{4+7}) delayed the onset of 50 % leaf yellowing in *Alstroemeria* cut flowers (Table 2). In both experiments, the levels of GA_{4+7} were not different from each other, except 2.5 mg/litre, in the first experiment. As the concentration of GA_{4+7} increased, there was an increase in the number of days to 50 % leaf yellowing and the response was linear in both experiments. Holding the *Alstroemeria* cut flowers in GA_{4+7} concentration above 10 mg/litre, led to a slight decrease in the vase life (Table 2). The cultivars were significant, in the second experiment alone. `Marina' took a longer time than `Yellow King' before the onset of 50 % leaf yellowing (Table 2).

Holding *Alstroemeria* cut flowers in 75 or 100 mg/litre BA promoted senescence, as measured by the days to 50 % leaf yellowing. However,lower BA concentrations (25 or 50 mg/litre) had

no effect, in both experiments (Table 3).

Accel (25 or 50 mg/litre BA equivalent), all the GA_{4+7} concentrations and Florissant 200, increased the number of days to 50 % leaf yellowing comparably, in both experiments (Table 4). However, there was a slight increase in the mean number of days to 50 % leaf yellowing, as the concentration of GA_{4+7} increased upto 10.0 mg/litre, then a slight decline. Florissant 200 delayed the onset of 50 % leaf yellowing by 2.83 and 5 days, in the first and second experiments, respectively, in comparison to control (Table 4). STS (2.0 mM) significantly accelerated the onset of 50 % leaf yellowing by 6 days, in the first experiment, when compared to the control (Table 3). In the second experiment, 2.0 mM STS accelerated the onset of 50 % leaf yellowing by 2 days, though it was not significant (Table 4).

4.2 Leaf Chlorophyll Content of *Alstroemeria*

The initial chlorophyll content of the cut flowers were: 2.18 and 2.60 mg/cm^2 for 'Yellow King' and 2.83 and 2.20 mg/cm^2 for 'Marina', in the first and second experiments, respectively. However, there was no significant difference on the chlorophyll content of the cultivars, except after 21 days in the first experiment (Table 5). During the experimental period, the chlorophyll content decreased, in all the cut flowers.

In both experiments, Accel consistently retarded the degradation of the leaf chlorophyll content (Table 5). In experiment one, holding *Alstroemeria* cut flowers in bud opening solution of above 50 mg/litre BA equivalent (Accel), resulted in a slight decrease in the leaf chlorophyll content (Table 5). The response to Accel concentration was linear after 7 and 14 days, then quadratic after 21 days, in the first experiment. During the second experiment, the chlorophyll content was highest at 25 mg/litre BA equivalent (Accel), beyond which, there was a slight decrease compared to the control, over the 3 weeks (7, 14 and 21 days). The response to Accel concentration was cubic over the 3 weeks, in the second experiment (Table 5).

In experiment one, during the first 7 postharvest days, 2.5 mg/litre GA_{4+7} had no effect on chlorophyll breakdown, but 5.0, 7.5, or 10 mg/litre GA_{4+7} reduced chlorophyll breakdown (Table 6). After 14 days, only 7.5 or 10 mg/litre GA_{4+7} had significantly higher leaf chlorophyll content. At 21 days after harvest, in the first experiment, all the GA_{4+7} treated cut flowers had significantly higher leaf chlorophyll content than the control (Table 6).

In the second experiment, GA_{4+7} treated cut flowers had significantly higher leaf chlorophyll content (Table 6). However, there were no differences between GA_{4+7} concentrations. The response to GA_{4+7} concentration was linear, 21 days after treatment, in the first experiment.

Table 5: **The effect of Accel on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers**

Accel (BA equivalent mg/litre)	First Experiment: Chlorophyll content of leaves (mg/cm^2)			Second Experiment: Chlorophyll content of leaves (mg/cm^2)		
	7 days	14 days	21 days	7 days	14 days	21 days
0 (control)	1.52c	1.34c	0.81c	0.74c	0.55d	0.38d
25	2.10ab	1.94b	1.75ab	2.28a	2.16a	1.68a
50	2.34a	2.30a	1.93a	1.98ab	1.77b	1.41b
75	2.17ab	2.10ab	1.65ab	1.87ab	1.43bc	1.32bc
100	1.94b	1.84b	1.36b	1.59b	1.34c	1.20c
Significance^Z	L**	L**	L***,Q**	L****,Q***,C**	L****,Q****,C****	L****,Q****,C****
LSD	0.32	0.35	0.41	0.43	0.36	0.17
Cultivars						
Yellow King	1.94a	1.80a	1.22b	1.81a	1.50a	1.21a
Marina	2.08a	2.01a	1.78a	1.57a	1.40a	1.18a
Significance	ns	ns	****	ns	ns	ns
LSD	0.20	0.22	0.26	0.27	0.23	0.11

^{Z} The response was linear(L), quadratic(Q) or cubic (C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); Means with the same letter(s) within columns are not significantly different.

Table 6: The effect of GA_{4+7} (Provide) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers.

	First Experiment			Second Experiment		
GA_{4+7} (mg/litre)	Chlorophyll content of leaves (mg/cm^2)			Chlorophyll content of leaves (mg/cm^2)		
	7 days	14 days	21 days	7 days	14 days	21 days
0 (control)	1.52b	1.34b	0.81c	0.74b	0.55b	0.38b
2.5	1.70ab	1.50ab	1.22b	1.18a	1.06a	0.87a
5.0	1.88a	1.60ab	1.32ab	1.22a	1.14a	0.96a
7.5	1.90a	1.66a	1.38ab	1.27a	1.16a	0.98a
10.0	1.96a	1.68a	1.49a	1.49a	1.25a	1.08a
12.5	-	-	-	1.38a	1.17a	1.02a
15.0	-	-	-	1.32a	1.17a	0.99a
Significance[Z]	ns	ns	L**	L**	L****,Q**,C*	L****,Q**
LSD/HSD[Y]	0.33	0.30	0.22	0.32	0.24	0.25
Cultivars						
Yellow King	1.74a	1.48a	1.12b	1.22a	1.05a	0.95a
Marina	1.84a	1.63a	1.37a	1.24a	1.09a	0.85b
Significance	ns	ns	***	ns	ns	*
LSD	0.21	0.19	0.14	0.11	0.08	0.09

[Z] The response was linear(L), quadratic(Q) or cubic(C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively.

[Y] Means separated by the protected LSD (P=0.05) and Tukey's HSD(P=0.05) for the first and second experiment respectively; means with the same letter(s) within columns are not significantly different.

In the second experiment, the response to GA_{4+7} concentration was linear, cubic and quadratic, after 7, 14 and 21 days, respectively. The cultivar difference was not significant except, after 21 days in the first experiment, in which 'Marina' had higher chlorophyll content than 'Yellow King'. The same trend was observed in the second experiment, except that 'Yellow King' had higher chlorophyll content than 'Marina'(Table 6). During the experimental period, the chlorophyll content in all the cut flowers decreased.

Alstroemeria cut flowers treated with 25 or 50 mg/litre BA, had significantly higher leaf chlorophyll content, however, 100 mg/litre BA had no effect, in both experiments (Table 7). In the first experiment, 75 mg/litre BA had significantly higher chlorophyll content in the leaves, 14 days after treatment. However, it had no effect on the chlorophyll content, 7 and 21 days after treatment. In the second experiment, the reverse was true (Table 7).

In the first and second experiments, there was no difference between Florissant 200 and GA_{4+7} in terms of leaf chlorophyll content (Table 8). In the first experiment, 25 mg/litre BA equivalent (Accel) had significantly higher leaf chlorophyll content compared to GA_{4+7}, except 7 days after harvest, when all the plant growth regulators had the same effect on leaf chlorophyll content (Table 8). In experiment two, 25 mg/litre BA (Accel) reduced chlorophyll breakdown, as evidenced by high retention of leaf chlorophyll content compared to all GA_{4+7} concentrations, Florissant 200, and 2.0 mM STS (Table 8). Florissant 200, was not significantly different from the control, even though it had slightly higher leaf chlorophyll content, during the first experiment, over the 3 weeks. In the second experiment, Florissant 200, had significantly higher leaf chlorophyll content , over the 3 weeks, compared to the control (Table 8). STS (2.0 mM) treatment had no effect, on the leaf chlorophyll content of *Alstroemeria,* in both experiments,except 21 days after treatment, in the second experiment,when it had significantly higher leaf chloropyll content (Table 8).

Table 7: The effect of Benzyladenine (Accel-GA_{4+7}) on the chlorophyll content of the leaves (mg/cm^2) of *Alstroemeria* cut flowers.

BA (mg/litre)	First Experiment			Second Experiment		
	Chlorophyll content of leaves (mg/cm^2)			Chlorophyll content of leaves (mg/cm^2)		
	7 days	14 days	21 days	7 days	14 days	21 days
0 (control)	0.00b	0.00c	0.00b	0.00c	0.00c	0.00d
25	0.40a	0.44ab	0.54a	1.10a	1.10a	0.81a
50	0.45a	0.70a	0.61a	0.76a	0.63b	0.45b
75	0.27ab	0.44ab	0.27ab	0.60ab	0.28c	0.33bc
100	-0.02b	0.17bc	-0.13b	0.09bc	0.09c	0.11cd
Significance	*	**	**	***	****	****
LSD	0.39	0.35	0.47	0.53	0.31	0.23

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 8: The effect of plant growth regulators on the chlorophyll content of the leaves (mg/cm²) of *Alstroemeria* cut flowers.

Concentration (mg/litre)	First Experiment: Chlorophyll content of leaves (mg/cm²)			Second Experiment: Chlorophyll content of leaves (mg/cm²)		
	7 days	14 days	21 days	7 days	14 days	21 days
Accel[R]						
0 BA (control)	1.52cd	1.34de	0.81de	0.74f	0.55e	0.38g
25 BA	2.10ab	1.94abc	1.75ab	2.28a	2.16a	1.68a
50 BA	2.34a	2.30a	1.93a	1.98ab	1.77ab	1.41ab
75 BA	2.17ab	2.10ab	1.65abc	1.87abc	1.43bc	1.32bc
100 BA	1.94abc	1.84abcd	1.36bc	1.59bcd	1.34bc	1.20bcd
Provide[R]						
2.5 GA_{4+7}	1.70bcd	1.50cde	1.22bcde	1.18def	1.06cd	0.87ef
5.0 GA_{4+7}	1.88abc	1.60bcd	1.32bcd	1.22def	1.14cd	0.96def
7.5 GA_{4+7}	1.90abc	1.66bcd	1.38abc	1.27de	1.16cd	0.98def
10.0 GA_{4+7}	1.96abc	1.68bcd	1.49abc	1.49bcde	1.25cd	1.08cde
12.5 GA_{4+7}	-	-	-	1.38cde	1.17cd	1.02cdef
15.0 GA_{4+7}	-	-	-	1.32de	1.17cd	0.99def
Florissant 200	1.88abc	1.82abcd	1.19cde	1.34de	1.14cd	0.85ef
2.0 mM STS	1.27d	1.06e	0.72e	1.01ef	0.87de	0.78f
Significance	****	****	****	****	****	****
Tukey's HSD	0.51	0.52	0.55	0.52	0.43	0.31

*, **, ***, ****, ns, significance within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by Tukey's HSD (P=0.05); means with the same letter(s) within columns are not significantly different.

4.3 Dry weight of the leaves of *Alstroemeria*

Accel (75 or 100 mg/litre BA equivalent) significantly increased the leaf dry weight of *Alstroemeria* cut flowers (Table 9). In experiment one, 75 and 100 mg/litre BA equivalent (Accel) were significantly different, however, the difference between the two concentrations was not apparent, in the second experiment (Table 9), except at 14 days after harvest. In both experiments, 25 or 50 mg/litre BA equivalent (Accel) had no effect on leaf dry matter accumulation, except during the second experiment, 14 days after harvest, when there was a significant dry matter retention (Table 9). There was significant cultivar difference in terms of dry weight of the leaves, except 7 days after Accel treatment, in the first experiment, when there was no cultivar effect. `Yellow King' maintained higher dry weight than `Marina' at similar Accel levels, except 21 days after treatment, when the reverse was true, in the first experiment (Table 9).

In general, GA_{4+7} tended to decrease leaf dry weight (Table 10). In the first experiment, all GA_{4+7} treatments had no effect on the dry weight of the leaves, except 2.5 mg/litre GA_{4+7}, after 7 days significantly reduced the dry weight, compared to the control (Table 10). All the levels of GA_{4+7} were not significantly different from one another. The response to GA_{4+7} concentration was quadratic, 7 days after treatment. The cultivar difference was significant with `Yellow King' retaining higher dry weight than `Marina', 7 days, after GA_{4+7} treatment and nonsignificant, after 21 days.

In the second experiment, 10 mg/litre GA_{4+7} had the lowest dry weight of the leaves, except after 7 days, when it was not significantly different from the control (Table 10). The rest of GA_{4+7} treatments were not different from one another, except 2.5 mg/litre GA_{4+7}, 14 days after treatment. The response to GA_{4+7} concentration was cubic, 7 days after treatment.

Table 9: The effect of Accel on dry weight (g) of the leaves of *Alstroemeria* cut flowers

	First Experiment		Second Experiment		
Accel (BA equivalent mg/litre)	Dry weight(g) of leaves		Dry weight(g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	1.26cd	1.31cd	1.10c	3.28d	3.87c
25	1.17d	1.23d	1.42bc	3.95c	4.91bc
50	1.34c	1.42bc	1.50bc	4.10bc	5.70b
75	1.53b	1.60b	1.81ab	4.55b	7.10a
100	1.71a	2.01a	2.18a	5.83a	7.50a
Significance[z]	L^{*},Q^{**},C^{*}	****	***	L^{**},Q^{*},C^{**}	****
LSD	0.10	0.19	0.45	0.47	1.35
Cultivars					
Yellow King	1.41a	1.44b	1.94a	5.84a	6.89a
Marina	1.39a	1.59a	1.26b	2.84b	4.74b
Significance	ns	*	****	****	****
LSD	0.07	0.12	0.28	0.30	0.85

[z]The response was linear(L), quadratic(Q) or cubic(C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively.

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 10: The effect of GA_{4+7} (Provide) on dry weight(g) of the leaves of *Alstroemeria* cut flowers.

GA_{4+7} (mg/litre)	First Experiment		Second Experiment		
	Dry weight of the leaves(g)		Dry weight of the leaves(g)		
	7 days	21 days	7 days	14 days	21 days
0 (control)	1.26a	1.31a	1.10ab	3.28a	3.87a
2.5	1.14b	1.21a	1.13ab	2.14cd	2.44b
5.0	1.18ab	1.25a	1.37ab	2.77abc	2.94ab
7.5	1.19ab	1.29a	1.39a	2.90ab	3.04ab
10.0	1.22ab	1.31a	1.06b	1.85d	2.10b
12.5	-	-	1.10ab	2.37bcd	2.56b
15.0	-	-	1.21ab	2.67abc	2.93ab
Significance^{Z}	L*,Q*	ns	L**,Q*,C*	****	***
LSD/HSD^{Y}	0.09	0.15	0.32	0.69	1.01
Cultivars					
Yellow King	1.27a	1.32a	1.36a	3.54a	3.84a
Marina	1.12b	1.23a	1.03b	1.60b	1.84b
Significance	****	ns	****	****	****
LSD	0.06	0.09	0.11	0.24	0.35

^{Z}The response was linear(L), quadratic(Q) or cubic(C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively.

^{Y}Means separated by the protected LSD (P=0.05) and Tukey's HSD (P=0.05) in the first and second experiment respectively; means with the same letter(s) within columns are not significantly different.

The cultivar difference was highly significant with `Yellow King' maintaining higher dry weight than`Marina' for the 3 weeks (7, 14 and 21 days) (Table 10).

In the first experiment, holding *Alstroemeria* cut flowers in 25 mg/litre BA had no effect, on the leaf dry weight and it was not significantly different from 50 mg/litre BA (Table 11). Higher concentrations of BA (75 or 100 mg/litre) increased the leaf dry weight, with 100 mg/litre giving the highest values, since the two concentrations were significantly different.

In the second experiment, except 7 days after harvest, all the levels of BA increased the leaf dry weight, with higher levels (75 or 100 mg/litre BA) giving the highest values (Table 11). However, 25 or 50 mg/litre BA were not significantly different from the control, 7 days after treatment. BA (25, 50 or 75 mg/litre) were not different from one another, except 21 days after treatment, when 75 mg/litre was different from the rest (Table 11).

Accel (75 or 100 mg/litre BA equivalent) significantly increased the leaf dry weight of *Alstroemeria* cut flowers, in both experiments (Table 12). Florissant 200 treatment had no effect on the dry weight of the leaves, in both experiments (Table 12). Also, it was not different from 25, or 50 mg/litre BA equivalent (Accel) and most of GA_{4+7} concentrations, except 10 mg/litre, 14 and 21 days after treatment, in the second experiment. STS (2.0 mM) significantly increased the dry weight of the leaves, 14 and 21 days after treatment, in the second experiment. However, it had no effect on the rest of the days, in both experiments (Table 12). STS (2.0 mM) was not different from 25 or 50 mg/litre BA equivalent (Accel), except 21 days after treatment, in the second experiment, when it significantly increased the dry weight.

Table 11: The effect of Benzyladenine (Accel-GA_{4+7}) on the dry weight (g) of the leaves of *Alstroemeria* cut flowers.

BA (mg/litre)	First Experiment		Second Experiment		
	Dry weight (g) of the leaves		Dry weight (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	0.00c	0.00d	0.00c	0.00c	0.00d
25	0.02c	0.03cd	0.29bc	1.81b	2.47c
50	0.17bc	0.16c	0.14bc	1.33b	2.76c
75	0.32b	0.35b	0.42b	1.65b	4.06b
100	0.70a	0.50a	1.12a	3.99a	5.40a
Significance	****	****	****	****	****
LSD	0.25	0.14	0.41	0.68	1.30

****, ns, significant within columns at P=0.001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 12: The effect of plant growth regulators on dry weight(g) of the leaves of *Alstroemeria* cut flowers.

Concentration (mg/litre)	First Experiment Dry weight(g) of the leaves		Second Experiment Dry weight(g) of leaves		
	7 days	21 days	7 days	14 days	21 days
Accel					
0 BA (control)	1.26bc	1.31c	1.10c	3.28cde	3.87bcd
25 BA	1.17bc	1.23c	1.42bc	3.95bcd	4.91b
50 BA	1.34b	1.42bc	1.50bc	4.10bc	5.70ab
75 BA	1.53a	1.60b	1.81ab	4.55b	7.10a
100 BA	1.71a	2.01a	2.18a	5.83a	7.50a
Provide					
2.5 GA_{4+7}	1.14c	1.21c	1.13c	2.14fg	2.44cd
5.0 GA_{4+7}	1.18bc	1.25c	1.37bc	2.77efg	2.94cd
7.5 GA_{4+7}	1.19bc	1.29c	1.39bc	2.90ef	3.04cd
10.0 GA_{4+7}	1.22bc	1.31c	1.06c	1.85g	2.10d
12.5 GA_{4+7}	-	-	1.10c	2.37efg	2.56cd
15.0 GA_{4+7}	-	-	1.21c	2.67efg	2.93cd
Florissant 200	1.25bc	1.34bc	1.27bc	3.07def	4.03bc
2.0 mM STS	1.34b	1.39bc	1.22c	4.89ab	7.42a
Significance	****	****	****	****	****
Tukey's HSD	0.18	0.29	0.58	0.98	1.87

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by Tukey's HSD (P=0.05); means with the same letter(s) within columns are not significantly different.

4.4 Water content in the leaves of Alstroemeria

The water content of the leaves tended consistently to decrease with the increase in the levels of Accel, in the two experiments (Table 13). However, 25 and 50 mg/litre BA equivalent (Accel) were not different from the control, in the first experiment and at 7 days after harvest, in the second experiment. Also, 25 mg/litre BA equivalent (Accel) was not different from the control, 21 days after harvest, in the second experiment. In the first experiment, 25 mg/litre BA equivalent (Accel) had the highest water content while in the second experiment, it was not significantly different from the control, 7 and 21 days after harvest. The higher Accel concentrations (75 or 100 mg/litre BA) significantly decreased the leaf water content, in both experiments (Table 13). The response to Accel concentration was cubic, after 7 and 14 days,in the first and second experiments, respectively (Table 13). In both experiments, there was significant cultivar difference in respect to water content, except 7 days after harvest, in the first experiment. `Marina' maintained higher water content in the leaves than `Yellow King'. However, 21 days after Accel treatment, in the first experiment, `Yellow King' had higher water content than `Marina'(Table 13).

In the first experiment, all the GA_{4+7} treatments had no effect on the water content of the leaves, except 2.5 mg/litre, 7 days after treatment, which significantly increased the water content as compared to the control (Table 14). All the levels of GA_{4+7} were not significantly different from one another, in the first experiment. The response to GA_{4+7} concentration was quadratic, 7 days after treatment. The cultivar difference was only significant, 7 days after treatment, in the first experiment. `Marina' maintained high water content in the leaves than `Yellow King' (Table 14).

Table 13: The effect of Accel on water content (g) of the leaves of *Alstroemeria* cut flowers

	First Experiment		Second Experiment		
Accel (BA equivalent mg/litre)	Moisture content (g) of leaves		Moisture content (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	6.75ab	6.69ab	6.90a	4.72a	4.13a
25	6.83a	6.77a	6.58ab	4.05b	3.09ab
50	6.66b	6.58bc	6.50ab	3.90bc	2.30b
75	6.47c	6.40c	6.20bc	3.45c	0.91c
100	6.29d	5.99d	5.82c	2.17d	0.50c
Significance[Z]	L*,Q**,C*	****	***	L**,Q*,C**	****
LSD	0.10	0.19	0.45	0.47	1.35
Cultivars					
Yellow King	6.59a	6.56a	6.06b	2.16b	1.11b
Marina	6.61a	6.41b	6.74a	5.16a	3.26a
Significance	ns	*	****	****	****
LSD	0.07	0.12	0.28	0.30	0.85

[Z]The response was linear (L), quadratic (Q) or cubic (C).

*,**,***,****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001, or nonsignificant respectively.

Means separated by the protected LSD (P=0.05); means with the same letter(s) within the columns are not significantly different.

Table 14: The effect of GA_{4+7} (Provide) on water content (g) of the leaves of *Alstroemeria* cut flowers.

	First Experiment		Second Experiment		
GA_{4+7} (mg/litre)	Water content (g) of leaves		Water content (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	6.75b	6.69a	6.90ab	4.72d	4.13b
2.5	6.86a	6.79a	6.87ab	5.86ab	5.56a
5.0	6.82ab	6.75a	6.63ab	5.23bcd	5.06ab
7.5	6.81ab	6.71a	6.61b	5.10cd	4.97ab
10.0	6.79ab	6.69a	6.94a	6.16a	5.90a
12.5	-	-	6.90ab	5.63abc	5.44a
15.0	-	-	6.79ab	5.33bcd	5.07ab
Significance[Z]	L*,Q*	ns	L**,Q*,C*	****	****
LSD/HSD[Y]	0.09	0.15	0.32	0.69	1.01
Cultivars					
Yellow King	6.73b	6.68a	6.64b	4.46b	4.16b
Marina	6.88a	6.77a	6.97a	6.40a	6.17a
Significance	****	ns	****	****	****
LSD	0.06	0.09	0.11	0.24	0.35

[Z]The response was linear (L), quadratic (Q) or cubic (C).

*,**,***,****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001, or nonsignificant respectively.

[Y]Means separated by the protected LSD (P=0.05) and Tukey's HSD (P=0.05) in the first and second experiments respectively; means with the same letter(s) within columns are not significantly different.

In the second experiment, 10 mg/litre GA_{4+7} had the highest water content, except after 7 days, when it was not significantly different from the control (Table 14). The rest of GA_{4+7} concentrations were generally not different from one another, except 2.5 mg/litre, 14 days after treatment. The response to GA_{4+7} concentration was cubic, 7 days after treatment. Generally GA_{4+7} tended to increase the leaf water content of *Alstroemeria* cut flowers, in both experiments (Table 14). The cultivar difference was highly significant with `Marina' maintaining high water content than `Yellow King' for the 3 weeks (7, 14 and 21 days) (Table 14).

Generally, the BA levels decreased the water content in the *Alstroemeria* leaves, in both experiments. However, 25 or 50 mg/litre BA were not different from each other, and from the control, 7 days after treatment, in both experiments (Table 15). The higher BA concentrations (75 or 100 mg/litre) decreased the water content, with 100 mg/litre BA giving the lowest values, since the two concentrations were significantly different (Table 15).

The water content in the leaves consistently decreased with the increase in the levels of Accel, in both experiments (Table 16). GA_{4+7} treatments had no effect on the water content in the leaves, in the first experiment (Table 16). The same trend was observed 7 and 21 days after treatment, in the second experiment. However, 14 days after GA_{4+7} treatments, a slight increase in the water content of leaves was observed. All the levels of GA_{4+7} were not different from one another, except 10 mg/litre which resulted in the highest water content, in the second experiment (Table 16).

Table 15: The effect of Benzyladenine (Accel-GA_{4+7}) on water content (g) of the leaves of *Alstroemeria* cut flowers.

	First Experiment		Second Experiment		
BA (mg/litre)	Water content (g) of the leaves		Water content (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	0.00a	0.00a	0.00a	0.00a	0.00a
25	-0.02a	-0.03ab	-0.29ab	-1.81b	-2.47b
50	-0.17ab	-0.16b	-0.14ab	-1.33b	-2.76b
75	-0.32b	-0.35c	-0.42b	-1.65b	-4.06c
100	-0.70c	-0.50d	-1.12c	-3.99c	-5.40d
Significance	****	****	****	****	****
LSD	0.25	0.14	0.41	0.68	1.30

****, ns, significant within columns at P=0.0001 or nonsignificant respectively.

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Table 16: The effect of plant growth regulators on water content (g) of the leaves of *Alstroemeria* cut flowers.

	First Experiment		Second Experiment		
Concentration (mg/litre)	Water content (g) of leaves		Water content (g) of the leaves		
	7 days	21 days	7 days	14 days	21 days
Accel					
0 BA (control)	6.75ab	6.69a	6.90a	4.72cde	4.13abc
25 BA	6.83ab	6.77a	6.58ab	4.05def	3.09c
50 BA	6.66b	6.58ab	6.50ab	3.90ef	2.30cd
75 BA	6.47c	6.40b	6.20bc	3.45f	0.91d
100 BA	6.29c	5.99c	5.82c	2.17g	0.50d
Provide					
2.5 GA_{4-7}	6.86a	6.79a	6.87a	5.86ab	5.56ab
5.0 GA_{4+7}	6.82ab	6.75a	6.63ab	5.23abc	5.06ab
7.5 GA_{4+7}	6.81ab	6.71a	6.61ab	5.10bc	4.97ab
10.0 GA_{4+7}	6.79ab	6.69a	6.94a	6.16a	5.90a
12.5 GA_{4+7}	-	-	6.90a	5.63abc	5.44ab
15.0 GA_{4+7}	-	-	6.79a	5.33abc	5.07ab
Florissant 200	6.75ab	6.66ab	6.74ab	4.93bcd	3.97bc
2.0 mM STS	6.66b	6.61ab	6.78a	3.11fg	0.58d
Significance	****	****	****	****	****
Tukey's HSD	0.18	0.29	0.58	0.98	1.87

*,**,***,****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001, or nonsignificant respectively.

Means separated by Tukey's HSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Florissant 200, a commercial cut flower preservative, had no effect on the water content in the leaves compared to the control, in both experiments (Table 16). It was also not different from Accel (25 or 50 mg/litre BA equivalent). In the first experiment, Florissant 200 was not different from 2.0 mM STS, in respect to leaf water content of *Alstroemeria* cut flowers (Table 16). However, in the second experiment, Florissant 200 significantly increased leaf water content compared to 2.0 mM STS, 14 and 21 days after treatment (Table 16). STS (2.0 mM) significantly decreased the water content of the leaves, 14 and 21 days after treatment, in the second experiment, however, it had no effect on the rest of the days, in both experiments (Table 16). STS (2.0 mM) was not different from Accel (25 mg/litre BA equivalent), except 21 days after treatment, in the second experiment, when it significantly decreased the water content of the leaves (Table 16).

4.5 Leaf total nitrogen content (%)

The initial leaf total nitrogen content (%) of the cut flowers were: 3.52 and 3.73 % for `Yellow King' and 3.77 and 3.58 % for `Marina',in the first and second experiments, respectively.

Nitrogen content (%) retention in the leaves increased with increasing levels of Accel (Table 17). In the first experiment, Accel (50, 75 or 100 mg/litre BA equivalent) were not significantly different from one another, in respect of leaf nitrogen content (Table 17),but they were different from 25 mg/litre BA equivalent. The response to Accel concentration was cubic and linear, after 7 and 21 days, respectively. There was significant cultivar difference in the nitrogen content, 7 days after treatment, in the first experiment. `Marina' maintained higher nitrogen content in the leaves than `Yellow King' at similar Accel levels. However, there was no cultivar difference, 21 days after Accel treatment.

In the second experiment, a similar trend was observed. Accel (75 mg/litre BA) was not different from 100 mg/litre BA, except after 14 days, when they were significantly different. The same relationship was true for Accel (50 and 75 mg/litre BA) (Table 17). The response to Accel concentration was cubic for the 3 weeks (7, 14 and 21 days). `Marina' maintained higher leaf nitrogen content than "Yellow King", even though it was not significantly different, 21 days after treatment (Table 17).

Table 17: The effect of Accel on total nitrogen content (%) of the leaves of Alstroemeria cut flowers.

Accel (BA equivalent mg/litre)	First Experiment		Second Experiment		
	Total nitrogen (%) of the leaves		Total nitrogen (%) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	2.93c	2.26c	1.91c	1.70e	1.60d
25	3.36b	2.77b	3.07b	2.71d	2.66c
50	3.39ab	3.19a	3.24ab	2.96c	2.77bc
75	3.47ab	3.22a	3.30a	3.07b	2.87ab
100	3.53a	3.24a	3.35a	3.15a	3.01a
significance[z]	L***,Q**,C*	L**	L****,Q****,C****	L****,Q****,C****	L****,Q****,C****
LSD	0.16	0.26	0.18	0.08	0.15
Cultivars					
Yellow King	3.15b	2.89a	2.77b	2.66b	2.56a
Marina	3.52a	2.98a	3.17a	2.77a	2.60a
Significance	****	ns	****	****	ns
LSD	0.10	0.16	0.11	0.05	0.09

[z]The response was linear (L), Quadratic(Q) or Cubic(C).

*, **, ***, ****, ns, significance within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificance respectively.

Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different.

Alstroemeria cut flowers treated with GA_{4+7} had high leaf nitrogen content (%), that reached a peak at GA_{4+7} (10 mg/litre), in both experiments (Table 18), except 7 days after treatment, in the first experiment, when only 10 mg/litre GA_{4+7} had significantly higher leaf nitrogen content (Table 18). In the first experiment, the response to GA_{4+7} treatment was quadratic and linear, after 7 and 21 days, respectively. Further, the response to GA_{4+7} levels was quadratic, cubic and linear, after 7, 14 and 21 days, respectively, in the second experiment. There was cultivar difference in nitrogen content, 7 days after GA_{4+7} treatment, with 'Marina' having higher nitrogen content than 'Yellow King', in the first experiment (Table 18). In the second experiment, however, 'Yellow King' maintained higher nitrogen content (%) in the leaves than 'Marina' over the 3 weeks. There was no significant cultivar difference, 21 and 14 days after GA_{4+7} treatments, in the first and second experiments, respectively (Table 18).

Generally, treating *Alstroemeria* cut flowers with BA resulted in high leaf nitrogen content. However, in the first experiment, 25 and 100 mg/litre BA, were not significantly different from the control, 21 days after treatment (Table 19). Also, 100 mg/litre BA, was not different from the control, 7 days after treatment, in the second experiment. BA at 25, 50 or 75 mg/litre, were not different from one another, in both experiments, except in the second experiment, 21 days after treatment, when 25 mg/litre BA was different from 50 mg/litre BA (Table 19).

Florissant 200, 2.0 mM STS, 10 mg/litre GA_{4+7} and Accel (all levels, except 100 mg/litre BA equivalent, 7 days after treatment), had comparably high leaf nitrogen content, in the first experiment (Table 20). However, cut flowers treated with Florissant 200 and 2.0 mM STS had significantly higher leaf nitrogen content (%) than those treated with 2.5 or 5.0 mg/litre GA_{4+7}, in experiment one (Table 20).

Table 18: The effect of GA_{4+7} (Provide) on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers.

Provide (GA_{4+7} mg/litre)	First Experiment: Total nitrogen (%) of the leaves		Second Experiment: Total nitrogen (%) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	2.93bc	2.26d	1.91d	1.70d	1.60d
2.5	2.81c	2.51c	2.66c	2.42c	2.14c
5.0	2.88bc	2.68bc	2.71c	2.54bc	2.18bc
7.5	3.02b	2.75ab	3.02b	2.63bc	2.31abc
10.0	3.26a	2.92a	3.25a	2.89a	2.47a
12.5	-	-	3.06ab	2.75ab	2.42ab
15.0	-	-	2.93b	2.73ab	2.25abc
Significance[Z]	Q**	L**	L****,Q*	L****,Q***,C*	L****
LSD/HSD[Y]	0.20	0.18	0.20	0.22	0.26
Cultivars					
Yellow King	2.81b	2.61a	2.90a	2.55a	2.26a
Marina	3.14a	2.64a	2.68b	2.49a	2.12b
Significance	****	ns	****	ns	**
LSD	0.13	0.11	0.07	0.08	0.09

[Z]The response was linear (L), quadratic(Q) or Cubic(C).

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively.

[Y]Means separated by the protected LSD (P=0.05) and Tukey's HSD (P=0.05) in the first and second experiment respectively; means with the same letter(s) within columns are not significantly different.

Table 19: The effect of Benzyladenine (Accel-GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers.

BA (mg/litre)	First Experiment		Second Experiment		
	Total nitrogen (%) of the leaves		Total nitrogen (%) of the leaves		
	7 days	21 days	7 days	14 days	21 days
0 (control)	0.00c	0.00b	0.00c	0.00c	0.00c
25	0.56a	0.27ab	0.42a	0.29ab	0.52b
50	0.51a	0.50a	0.53a	0.42ab	0.59a
75	0.45a	0.46a	0.28ab	0.44a	0.56ab
100	0.27b	0.32ab	0.11bc	0.26b	0.54ab
Significance	****	*	**	****	****
LSD	0.15	0.34	0.26	0.17	0.06

*, **, ****, ns, significant within columns at P=0.05, 0.01, 0.0001 or nonsignificant respectively. Means separated by the protected LSD (P=0.05); means with the same letter(s) within columns are not significantly different

Table 20: The effect of plant growth regulators on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers.

Concentration (mg/litre)	First Experiment		Second Experiment		
	Total nitrogen (%) of the leaves		Total nitrogen (%) of the leaves		
	7 days	21 days	7 days	14 days	21 days
Accel					
0 BA (control)	2.93d	2.26e	1.91d	1.70h	1.60h
25 BA	3.36ab	2.77bcd	3.07abc	2.71def	2.66bc
50 BA	3.39ab	3.19a	3.24ab	2.96abc	2.77b
75 BA	3.47ab	3.22a	3.30ab	3.07ab	2.87ab
100 BA	3.53a	3.24a	3.35a	3.15a	3.01a
Provide					
2.5 GA_{4+7}	2.81d	2.51de	2.66c	2.42g	2.14g
5.0 GA_{4+7}	2.88d	2.68cd	2.71c	2.54fg	2.18fg
7.5 GA_{4+7}	3.02cd	2.75bcd	3.02abc	2.63efg	2.31defg
10.0 GA_{4+7}	3.26bc	2.92abc	3.25ab	2.89bcd	2.47cde
12.5 GA_{4+7}	-	-	3.06abc	2.75cdef	2.42de
15.0 GA_{4+7}	-	-	2.93abc	2.73def	2.25efg
Florissant 200	3.21bc	3.05ab	2.88bc	2.97cde	2.48cd
2.0 mM STS	3.33ab	3.09ab	2.88bc	2.76cdef	2.38def
Significance	****	****	****	****	****
Tukey's HSD	0.26	0.36	0.44	0.23	0.23

*, **, ***, ****, ns, significant within columns at P=0.05, 0.01, 0.001, 0.0001 or nonsignificant respectively. Means separated by Tukey's HSD (P=0.05); means with the same letter(s) within columns are not significantly dufferent.

In the second experiment, Florissant 200, 2.0 mM STS, all GA_{4+7} concentrations and Accel (25, 50 or 75 mg/litre BA equivalent), had comparably high leaf nitrogen content, 7 days after treatment (Table 20). Accel at 25 mg/litre BA equivalent was not different from Florissant 200, 2.0 mM STS and GA_{4+7} (10, 12.5 or 15 mg/litre), 14 days after treatment. Florissant 200 and 2.0 mM STS, significantly reduced nitrogen content of the *Alstroemeria* leaves as compared to all levels of Accel, 21 days after treatment. However, Florissant 200, 2.0 mM STS and GA_{4+7} (7.5, 10, or 12.5 mg/litre) were not different, in respect of leaf nitrogen content, 21 days after treatment (Table 20).

CHAPTER 5
DISCUSSION

5.1 Vase life of *Alstroemeria* cut flowers

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(a) Accel

Accel at 25, 50, or 75 mg/litre BA equivalent increased the mean number of days to full opening of primary florets. Accel may have delayed primary floret opening because cytokinins and gibberellins are documented to delaying senescence of cut flowers (Salisbury and Ross, 1986). Hicklenton (1991) reported that 50 mg/litre BA significantly increased the vase life of *Alstroemeria* cut flowers, by allowing full opening of primary and tertiary florets.

Accel (25 mg/litre BA) significantly increased the number of days to 50 % petal fall, thus increasing the vase life of *Alstroemeria*. Results with external applications of cytokinins which delay senescence of various flowers (Heide and Oydvin, 1969; Mayak and Halevy, 1970; Mayak and Dilley, 1976) support the possibility that, the diminishing of internal levels of the growth regulators is associated with senescence processes in plants. Further support for this view may be drawn from experiments in which, applied kinetin slowed down the reduction in dry weight of aging rose petals (Mayak and Halevy, 1974). Halevy and Kofranek (1976) found cytokinin to be a better inhibitor of flower abscission in roses. Probably this might be the reason for increased number of days to 50 % petal fall observed in this study, after Accel treatment.

Higher Accel concentrations (50, 75, or 100 mg/litre BA) tended to decrease the number of days to 50 % petal fall. The acceleration of flower senescence by higher Accel concentrations greater than 25 mg/litre could be explained by the role of BA (cytokinin) in ethylene biogenesis. Cytokinins have been shown to promote the synthesis of ACC synthase, an enzyme that catalyses the conversion of SAM to ACC (Yang, 1987). Hence BA (cytokinin) may have promoted flower senesence indirectly through enhanced ethylene production (Yang, 1987). Heide and Oydvin (1969) observed that too high a concentration or too long a BA treatment may be detrimental to cut flower vase life.

Premature leaf yellowing of cut flowers has been reported to be caused by hormonal imbalance in plants when the supply of hormones from the roots is cut off after they are harvested (Hofman, 1988). Accel (25 and 50 mg/litre BA) consistently increased the number of days to 50 % leaf yellowing. The lower levels of Accel may have delayed leaf yellowing probably because BA and GA_{4+7} present in Accel delayed chlorophyll degradation, and can also be explained by the significant retention of the total leaf nitrogen content, in Accel treated plants since nitrogen is used in protein and chlorophyll synthesis (Salisbury and Ross, 1986). In cut flowers, including *Alstroemeria*, discolouration has been reduced by foliar sprays of the cytokinin (BA) (Halevy and Mayak, 1981; Healy and Lang, 1989). Yellowing of leaves is due to the breakdown of chlorophyll which exposes other plant pigments such as xanthophylls and carotenoids (Leopold, *et al.*, 1959; Back and Richmond, 1971).

Senescence in detached leaves is reported to be delayed by the application of cytokinins (Osborne, 1967; Wareing and Phillips, 1970; Nooden and Leopold, 1980). Mothes and Engelbrecht (1961) demonstrated that if a small area of basal leaf was treated with kinetin solution, only that area remained green while the rest of the plant turned yellow. Osborne

(1962) reported that kinetin retarded chlorophyll disappearance of isolated discs of *Xanthium* leaves. Leopold and Kawase (1964) reported a retarded senescence of bean leaves (*Phaseolus vulgaris*) when treated with cytokinins. Richmond and Lang (1957) reported that cytokinin treatment extends the life span of detached leaves by delaying protein degradation and loss of chlorophyll. Ethylene is known to be involved in the control of senescence in a number of flowers (Halevy and Mayak, 1981). Delay of senescence and decrease in the sensitivity to ethylene by application of Kinetin (cytokinin) to carnations have been reported (Eisinger, 1977). Eisinger (1977) suggested that endogenous cytokinins are the natural anti-senescence factors in carnation cut flowers.

(b) Gibberellins (GA)

Generally, GA_{4+7} treatments had no effect in the opening of primary florets of *Alstroemeria*. The probable explanation for this observation, could be due to the low concentrations of GA_{4+7} (2.5 to 15 mg/litre) used in this study,which possibly were not effective in opening of the primary florets. Arditti (1979) reported that GA had little or no effect on orchids and most other flowers he tested. Other reports found high levels of GA_3 to be effective in promoting bud opening (Cywinska-Smoter *et al.*, 1978). GA_3 (100 to 400 mg/litre) in opening solution of carnations promoted bud opening but decreased longevity and caused discolouration of flowers (Cywinska-Smoter *et al.*, 1978). GA (20 to 35 mg/litre) accelerated bud opening of carnations (Goszczynska and Nowak, 1979) and gladiolus (Ramanuja Rao and Mohan Ram, 1979) after storage.

Gibberellins have been extracted from carnation flowers and have been shown to play a role in the control of carnation petal growth (Jeffcoat and Harris, 1972). GA_{4+7} treatment increased the number of days to 50 % petal fall in *Alstroemeria*. The delay in the onset of 50 % petal fall

caused by GA_{4+7} may be attributed, to the role of gibberellins in delaying senescence in cut flowers (Fletcher and Osborne, 1966). Results with exogenous application of GA to isolated carnation petals delayed their senescence (Garrod and Harris, 1978),which further supports this view. Dai and Paull (1991) showed that Oasis Floral Preservative plus GA_3 solutions increased flower longevity by 1 day, after 2 days in packaging.

Gibberellins are reported to delay leaf senescence of a few species, but the physiological effects of GA_3 on leaf senescence are not clear. In this study, all GA_{4+7} treatment delayed the onset of 50 % leaf yellowing. The delay in the onset of leaf yellowing in *Alstroemeria* cut flowers caused by GA_{4+7}, may be attributed to the role of gibberellins as a juvenile hormone. Further support for this view, may be drawn from the work of Chin and Beevers (1970), in which, GA_3 content decreased steadily as *Tropaeolum* leaves senesced. Addition of GA_3 (50 mg/litre) to vase solutions significantly delayed leaf yellowing of *Alstroemeria* (Hicklenton, 1991). Results of Dai and Paull (1991) further confirmed the effectiveness of GA_3 in delaying leaf yellowing. Studies on nasturtium (*Tropaeolum majus* L.) suggested a relationship between GA_3 content in leaves and the onset of senescence (Beevers, 1966). Dai and Paull (1991) concluded that premature leaf yellowing was possibly associated with a low level of GA_3 in the leaves of certain cultivars of *Alstroemeria.*

(c) Benzyladenine (BA)

BA (100 mg/litre) had no effect on the opening of the primary florets, however it accelerated senescence as evidenced by early onset of 50 % leaf yellowing and 50 % petal fall. Heide and Oydvin (1969) observed that too high cytokinin concentration may be detrimental to cut flowers, through their interaction with ethylene (Eisinger, 1977). Cytokinins have been shown to promote the synthesis of ACC synthase, which is an enzyme that catalyses the conversion of

S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang, 1987), hence increased ethylene production. Therefore, high levels of BA (cytokinin) may have promoted senescence indirectly via enhanced ethylene production, and ethylene has been shown to promote flower senescence (Halevy and Mayak, 1981; Kader, 1985).

Higher BA concentrations (75 or 100 mg/litre) accelerated the onset of 50 % petal fall. Ethylene is known to cause floret abscission in *Alstroemeria* and snapdragons (Halevy and Mayak, 1981). Cook *et al.* (1985) reported that BA treated carnation flowers produced high levels of ethylene in response to exogenous ethylene without affecting the timing of the onset of senescence. As explained above the accelerated senescence of *Alstroemeria* cut flowers caused by high BA concentrations (75 or 100 mg/litre) may be due to cytokinin-induced ethylene production.

Holding *Alstroemeria* cut flowers in 75 or 100 mg/litre BA enhanced the onset of 50 % leaf yellowing while lower BA concentrations (25 or 50 mg/litre) had no effect. The earlier senescence of cold stored roses appears to be caused, at least in part, by earlier and increased ethylene production (Faragher *et al.*, 1986). BA treated carnation flowers produced high levels of ethylene without affecting the timing of the onset of senescence (Cook *et al.*, 1985), which probably explains enhanced onset of 50 % leaf yellowing in *Alstroemeria* treated with high BA levels (75 or 100 mg/litre) in this study.

(d) Florissant 200

Florissant 200, is used to prevent leaves from yellowing, produces full bloom and prolongs flower life, as per manufacturer's label. Florissant 200, increased the days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing. Probably, Florissant 200, contains

cytokinins and/or gibberellins. This is because the response of *Alstroemeria* flowers to Florissant 200, with respect to opening of primary florets, was not different from Accel and GA_{4+7} levels, in the second experiment. Further, Florissant 200, all GA_{4+7} levels and Accel (25 or 50 mg/litre BA) increased the number of days to 50 % petal fall and 50 % leaf yellowing comparably, in both experiments. This shows Accel (25 mg/litre BA) and GA_{4+7} (10.0 mg/litre), are effective as Florissant 200, in delaying cut flower senescence, hence either of the chemicals can be used in cut flower preservative solutions. Pulse treatments of GA_3 and BAP effectively reduced or eliminated foliage yellowing over 14 days and maintained colour intensity in *Alstroemeria* cultivars (Hicklenton, 1991). Treatment of intact plants with GA_3, BA, or the combination of both, before cold storage, significantly delayed post-storage development of leaf chlorosis in Easter lilies (Han, 1995).

The mechanism by which GA_3 delays senescence of excised leaves is not known. Postharvest treatment of GA markedly retarded the ripening of tomatoes by lowering respiratory rate and retarded climacteric. Han (1995) reported that respiration rates of Easter lily leaves, treated with GA_3 declined rapidly not long after the chemical treatments. The lower respiration rate of the leaves, indicated that carbohydrate depletion was not as rapid as in those treated with water. This in turn, suggested that the postproduction development of foliar chlorosis in Easter lily leaves, may be associated with the carbohydrate status of the leaves (Han, 1995).

(e) 2.0 mM STS

Silver thiosulphate is very mobile and travels from the base of the cut stem to the flower, blocking ethylene action and extending longevity of carnation (Veen and Van de Geijn, 1978). In this study, 2.0 mM STS had no effect on the opening of primary florets in *Alstroemeria*. Behe and Krentz (1995) reported that no STS treatment was more effective in increasing floret

development in Buddleia. However, STS applied before storage inhibited flower opening after storage in 'Gabriella', 'Mercedes' and 'Sonia' roses (Mor *et al.*, 1989; Faragher, 1985). It is possible that ethylene is required for petal growth, as it was found in carnations (Camprubi and Nichols, 1979).

In this study, 2.0 mM STS increased the vase life of petals by 5 and 1.5 days, in the first and second experiments, respectively. This indicates that endogenous and presumably exogenous ethylene is important in senescence of *Alstroemeria* cut flowers and that inhibitors of ethylene action can extend the vase life of *Alstroemeria.* Ethylene causes floret abscission in cut snapdragons and STS pretreatment prevented this floret abscission (Lee *et al.*, 1995). STS prevents normal and ethylene-induced floret shattering in snapdragon (Farnham *et al.*, 1981), *Delphinium*, and sweet peas (Mor *et al.*, 1980; Shillo *et al.*, 1980). Mor *et al.* (1989) reported that STS increased the vase life of roses that had been cold-stored for 3 weeks by 3.2 to 3.4 days, again suggesting the greater importance of ethylene in senescence of cold-stored roses than in fresh ones.

Silver thiosulphate (2.0 mM) accelerated the onset of 50 % leaf yellowing in this study. Dai and Paull (1991) reported similar results in that, pulsing for 24 hours with 4 mM STS accelerated leaf yellowing. STS pretreatment increased *Alstroemeria* flower vase life (Staby and Naegele, 1984; Chepkairor, 1986; Vermeulen, 1986) by reducing leaf yellowing but was toxic to gerbera (Nowak, 1979). Silver uptake estimations indicated that a minimum of 0.5 μmol Ag^+ was required per stem for maximum vase life of carnations and that more than 5 μmol Ag^+ per stem was toxic. Excess concentration or time of STS treatment may damage the foliage (Halevy and Mayak, 1981), which is the probable cause of accelerated leaf yellowing of *Alstroemeria* flowers, in the present study.

5.2 Leaf Chlorophyll Content

Accel or BA generally had high leaf chlorophyll content compared to the control, over the 3 weeks (7, 14 and 21 days). However, chlorophyll content decreased as the leaves aged. Cytokinins have been known to prevent leaf senescence by arresting degradation of protein and chlorophyll (Sacher, 1973). Several studies have reported that, cytokinins maintain protein by retarding the rate of breakdown rather than enhancing the rate of synthesis (Sacher, 1973). Therefore, in the present study, Accel or Benzyladenine delayed the degradation of chlorophyll by probably delaying the breakdown of protein used in the synthesis of chlorophyll, as evidenced by the high retention of nitrogen in the leaves of *Alstroemeria* throughout the experimental period.

Cytokinin applied to yellowing leaves of cocklebur (*Xanthium Pennsylvanicum* L.) reversed the breakdown of aged chloroplasts, resulting in regreening of leaves (Leshem, 1986). The gradual disorganization of grana in the chloroplasts of senescing cocklebur leaves was actually reversed back to orderly pattern during the regreening process. Cytokinin have been reported to promote chloroplast development and chlorophyll synthesis (Salisbury and Ross, 1986). Cytokinin enhances the subsequent (in light) development of etioplasts into chloroplasts, especially by promoting grana formation, and it increases the rate of chlorophyll formation (Lew and Tsuji, 1982). BA has been shown to activate synthesis of two proteins of the chloroplasts namely: RUBP carboxylase and the chlorophyll a/b protein complex (Funckees-Shippy and Levine, 1985). Cytokinins retarded the breakdown of chlorophyll and proteins in excised oat leaves and delayed the onset of rising respiration associated with leaf senescence (Thimann, 1987). Paulin and Muloway (1979) concluded that the effects of cytokinin on the vase life seemed to be associated with a decrease in water loss, dry matter loss and proteolysis. Richmond and Lang (1957) reported that kinetin can reduce or prevent the accelerated chlorophyll loss that is typical

of detached leaves and extends their vase life.

GA_{4+7} had significantly higher leaf chlorophyll content, over the 3 weeks (7, 14 and 21 days). However, the chlorophyll content was not dependent on the concentration of GA_{4+7}, suggesting that GAs are not universal chlorophyll breakdown retardants or inhibitors. The physiological effects of GA on leaf senescence are not clear. However, gibberellins have been shown to interfere with the degradation of chlorophyll, as well as with the biosynthesis of carotenoids and anthocyanins (Dostal and Leopold, 1967). Gibberellins also enhance the regreening of Valencia oranges, indicating that gibberellins act as inducers of green chloroplast development and not merely as inhibitors of senescence (Goldschmidt, 1974). Further, the delay in senescence of excised leaves following treatment with GA_3 was evidenced by the retention of chlorophyll (Han, 1995). Han (1995) recommended microscopy studies to determine if application of GA_3 improves the longevity of cold-stored excised leaves by reversing, stopping or delaying the disorganization process in the chloroplasts.

Florissant 200, had high leaf chlorophyll content compared to the control, over the 3 weeks, in the second experiment. Florissant 200, was not different from all the levels of GA_{4+7} in reducing leaf chlorophyll breakdown, in the first experiment. However, Accel (25 mg/litre BA) had significantly high leaf chlorophyll content compared to GA_{4+7} and Florissant 200, in the second experiment. Accel (25 mg/litre BA) may have delayed chlorophyll degradation by possibly delaying the breakdown of protein used in the synthesis of chlorophyll. This is evidenced by the high retention of leaf nitrogen content in *Alstroemeria*. Probably Florissant 200, had very low levels of cytokinins and gibberellins, which were not effective in delaying chlorophyll breakdown.

STS (2.0 mM) treatment resulted in low chlorophyll content of the leaves over the 3 weeks (7, 14 and 21 days). Foliar application of 0.2 mM STS did not prevent the post-storage development of foliar chlorosis in potted Easter lilies (Prince et al., 1987). Probably, the long period of STS treatment in this study, might have damaged the *Alstroemeria* foliage by being phytotoxic, hence accelerating chlorophyll breakdown. Toxicity is a form of stress, and water stress commonly cause leaf yellowing due to increased ethylene production, which is known to promote chlorophyll loss (Thimann, 1980).

5.3 Dry weight of the leaves

Because of problems existing from the variable water contents, agronomists prefer to use the increase in dry weight of plant or plant part as a measure of its growth, since it gives a more valid estimate than fresh weight (Salisbury and Ross, 1986). The dry weight increase induced by Accel (75 and 100 mg/litre BA) was a BA response and not due to gibberellins. This is because GA_{4+7} had no effect, apart from 2.5 and 10.0 mg/litre, which significantly reduced the dry weight. GA promotes fresh weights but not dry weights (Salisbury and Ross, 1986), as confirmed by the results of Eid and Ahmed (1976) on sweet basil (*Ocimum basilicum* L.).

Weaver and Johnson (1985) and Clifford *et al.* (1986) reported increased loading and unloading of assimilates across the membrane boundaries of the vascular tissues of plant sprayed with cytokinins, leading to enhanced crop growth and dry matter production. The increase in dry matter caused by Accel or high levels of BA treatment may be attributed to cytokinins ability to promote carbohydrate metabolism and create new source-sink relationship (Mothes and Engelbretcht, 1961; Dyer *et al.*, 1990), thus leading to increased dry matter accumulation in the sink.

Treating *Alstroemeria* with Accel and BA resulted in higher leaf chlorophyll content. Cytokinins have been shown to enhance the subsequent (in light) development of etioplasts into chloroplasts, especially by promoting grana formation thus increasing the rate of chlorophyll formation (Lew and Tsuji, 1982). The main storage products accumulating in the leaves during photosynthesis are usually starch, fructans and sucrose, which in turn are the principal components of dry weight (Salisbury and Ross, 1986). This could explain the increase in the dry weight of the leaves observed in this study.

GA_{4+7} reduced the dry weight of the leaves. The principal leaf storage products accumulating in light are usually starch, fructans and sucrose, which are the principal components of dry weight (Salisbury and Ross, 1986). Gibberellins have been shown to increase hydrolysis of starch, fructans and sucrose into glucose and fructose molecules (Salisbury and Ross, 1986), which could explain the decrease in the dry weight of the leaves, observed in the present study. Eid and Ahmed (1976) and Sadowska *et al.* (1983) reported that gibberellic acid lowered the dry weight of sweet basil (*Ocimum basilicum* L.) and *Catharanthus roseus* L. plants.

Florissant 200, had no effect, on the dry weight of the leaves and was not different from Accel (25 mg/litre BA equivalent). Cytokinins are known to decrease dry matter loss, hence increasing vase life of cut flowers (Paulin and Muloway, 1979). It is suggested that, probably Florissant 200, contained high levels of GAs and low levels of cytokinins, which had no profound effect in promoting carbohydrate metabolism and in creating new source-sink relationship for increased dry matter accumulation (Dyer *et al.*, 1990).

STS (2.0 mM) significantly increased the dry weight of the leaves in this study. It is suggested that, the silver ion reduced ethylene action resulting in reduced respiration rate, hence the

observed increase in the dry weight. Pratt and Goeschl (1969) reported that the main physiological effect of ethylene on plants is increased respiratory activity. A number of researchers have found that STS acts as an ethylene antagonist, hence it reduces ethylene production (Veen, 1979a) and respiration (Veen, 1979b). Nichols (1976) found that ethylene promotes accumulation of sugars and inorganic materials in the ovary, accompanied by the loss of dry weight of the petals, probably due to increased respiration rate.

5.4 Water content in the leaves

One of the most important factors determining cut flower longevity, is the ability of the flower to maintain turgidity. Turgidity in cut flowers is dependent upon a balance between the rate of water loss or utilization and of water supply (Hardenburg *et al.*, 1986; Rogers, 1973). A high level of turgidity is necessary for development of lower buds to full-bloom maturity. It is also necessary for the continuance of normal metabolic activities in the cut flowers (Roger, 1973). Water relations in cut flowers is influenced by plant growth regulators (Mayak and Halevy, 1974) and other chemicals such as antimicrobial compounds (Baker, 1983).

Higher Accel levels (75 or 100 mg/litre), significantly decreased *Alstroemeria* leaf water content, while GA_{4+7} increased the leaf water content. This suggests, the increase in leaf water content, in lower levels of Accel is attributable to GA_{4+7}. This is confirmed because benzyladenine (cytokinin) decreased the leaf water content (Table 15). The BA present in Accel, possibly increased water deficit by inducing stomatal opening and increasing water loss, leading to a decrease in leaf water content in *Alstroemeria*. Arad *et al.* (1973) reported similar results in barley.

The main initial effect of kinetin on water balance was the enhancement of water uptake (Mayak and Halevy, 1974), by enhancing the opening of stomata in leaves of cut rose flower shoots. Such an effect should increase transpiration leading to the development of water stress and enhanced wilting (Tal and Imber 1971; Arad *et al.*, 1973). However, in cut flower shoots, kinetin also promoted water uptake which more than compensated for the increased water loss and therefore delayed wilting (Mayak and Halevy, 1974). They suggested the above observation was achieved by maintenance of cell integrity. This is supported by the finding that benzylaminopurine (BAP) delayed the decrease in water content and the increase in ion leakage associated with senescence of gerbera flowers (Van Meeteren, 1979). However, this was not the case in this study, because benzyladenine decreased leaf water content and increased leaf dry matter content and postharvest shelf life of *Alstroemeria* flowers.

Gibberellins sometimes promote cell growth because they increase hydrolysis of starch, fructans and sucrose into glucose and fructose molecules (Salisbury and Ross, 1986). These hexoses make the cell's water potential momentarily more negative. As a result of the decrease in water potential, water enters more rapidly, causing cell expansion but diluting the sugars (Salisbury and Ross, 1986). This could explain why GA_{4+7} treatments in this study increased the leaf water content of *Alstroemeria* cut flowers.

Silver thiosulphate (2.0 mM) significantly decreased the water content in the leaves, 14 and 21 days after treatment, in the second experiment, but had no effect on the rest of the days. Behe and Krentz (1995) reported similar results, in that, no STS treatment was effective in increasing stem fresh weight in Buddleia. Therefore, it is suggested that physiological blockage caused by ethylene induced wound gums did not occur in this study. However, Paull and Goo (1985) found the major response to STS in cut *Anthurium* was increased rate of water uptake. Similar

patterns were reported in roses and carnations (Camprubi and Fontarnau, 1977; Durckin and Kuc, 1966; Mayak *et al*., 1974).

5.5 Total nitrogen content in the leaves

In this study, Accel or BA decreased the rate of nitrogen degradation in the leaves over, the 3 weeks (7, 14 and 21 days), compared to the control. Cytokinins are known to delay senescence by retarding the rate of breakdown of protein rather than enhancing the rate of protein synthesis (Sacher, 1973),which explains the observed results. Richmond and Lang (1957) reported that kinetin can reduce or prevent the accelerated protein loss that is typical of detached leaves.

Salunke *et al*. (1962) explained that the primary step in the degradation of the soluble type ribonucleic acid is thought to involve the loss of the end adenine. A treatment with BA, therefore should provide the necessary adenine and restore the soluble ribonucleic acid molecule. Thus protein breakdown would be retarded and the treated produce would stay fresh for a longer time, as evidenced by high retention of nitrogen in the leaves of *Alstroemeria*. Cytokinins apparently are involved in the regulation of flower senescence (Mayak and Halevy, 1974), possibly by maintaining high RNA and protein levels (Ballantyne, 1966). Fosket (1977) concluded that, cytokinins promote cell division by increasing the transition of the cells from G_2 to mitosis and that they do this by increasing the rate of protein synthesis. The cytokinin effect seems to be specifically on translation and one of the several evidences for this is that, the ribosomes in treated cells are frequently grouped in large protein-synthesizing polysomes rather than in smaller polysomes or as free monoribosomes characteristic of slowly dividing untreated cells.

GA_{4+7} treatment resulted in high leaf nitrogen content. The effect of GA_{4+7}, on nitrogen metabolism is associated with DNA dependent RNA and protein synthesis (Fletcher and Osborne, 1966). It is suggested that, GA_{4+7} treatment increased protein synthesis leading to high nitrogen content in the leaves of *Alstroemeria* leaves, in this study. Beevers and Guernsey (1967) observed that, the response of leaf protein to GA treatment is inconsistent and only in a few cases it has been effective.

Florissant 200, treatment had high leaf nitrogen content, compared to the control. Probably, cytokinin maintained the RNA and protein levels (Ballantyne, 1966), while GA possibly affected the integrity of mitochondrial membranes (Lewis *et al.*, 1967), leading to high nitrogen content in the leaves, since Florissant 200, possibly contains gibberellins and/or cytokinins.

STS (2.0 mM) had high leaf nitrogen content. In tomato leaf segments, the rise in respiration is paralleled by a rise in ethylene production (McGlasson *et al.*, 1975). STS, an inhibitor of ethylene action, could have reduced protein degradation of the leaves leading to high nitrogen content, as compared to the control via inhibiting ethylene action . Ethylene promotes protein and chlorophyll degradation in leaves of leafy vegetables and cut flowers (Kader, 1985).

The increase in dry weight and nitrogen content, stems from the inhibition of ethylene action by STS as discussed earlier. It can be concluded that, the advantage of using STS over other chemicals that inhibits ethylene biosynthesis is, the former provides protection against exposure to ethylene in the environment (Baker, 1983). However, due to the risk of heavy metal pollution to the environment, other chemicals such as 1- methyl cyclopropane (Serek *et al.*, 1994), which have similar effects as STS, and whose disposal does not pose an environmental threat, have been provided (Serek *et al.*, 1994).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Numerous reports have been published, addressing the problem of postharvest foliage yellowing in a range of horticultural crops, including *Alstroemeria* (Beevers, 1966; Halevy and Mayak, 1981; Leshem, 1986; Thimann, 1987; Hicklenton, 1991; Dai and Paull, 1991; Han, 1995). However, the effects of growth regulators on leaf senescence varies greatly among plant species.

The application of GA_{4+7} on its own at low concentrations (2.5, 5.0 or 7.5 mg/litre), although having no influence on the number of days to full opening of the primary florets, did improve other parameters of vase life, that is, increased the period to the onset of 50 % petal fall and 50 % leaf yellowing. Higher concentrations of GA_{4+7} (10 mg/litre), however increased the number of days to full opening of the primary florets in one experiment, in addition to improving the other parameters of vase life named above. It is therefore concluded that low concentration of GA_{4+7} (2.5 to 15 mg/litre) used in this study, were not effective in opening primary florets of *Alstroemeria*. The author recommends further study, using higher levels of GA_{4+7}.

Assuming additive effects of BA and GA_{4+7} in Accel, BA alone at 25 mg/litre increased the number of days to full opening of the primary florets, but had no effect on the number of days to 50 % petal fall and 50 % leaf yellowing. However, at higher levels (75 or 100 mg/litre), it hastened the onset of senescence, by reducing the number of days to 50 % petal fall and accelerating the onset of 50 % leaf yellowing. It can be concluded, that too high concentration of BA has detrimental effects on the vase life of cut flowers. The author recommends further studies using low levels of pure BA, to see whether it will have any beneficial effects, as observed in Accel treatment.

The application of Accel at lower levels (25 or 50 mg/litre BA equivalent) increased the number of days to full opening of primary florets, days to 50 % petal fall and delayed the onset of 50 % leaf yellowing. The trend was similar at higher concentrations, although not statistically different within the concentration ranges tested. Accel (25 mg/litre BA) was the most effective in this regard. The author recommends the use of Accel (25 mg/litre BA) in postharvest treatment of *Alstroemeria.* However, further study on the effect of low levels of Accel (5, 10, 15, 20 or 25 mg/litre BA) on the above parameters should be done to determine the best treatment for *Alstroemeria.*

Florissant 200, a commercial cut flower preservative, increased the number of days to full opening of primary florets, days to 50 % petal fall and delayed the onset of 50 % leaf yellowing. These findings demonstrated that, Accel (25 mg/litre BA) is effective and can be used instead of Florissant 200, since they were not significantly different. However, Accel at 25 mg/litre BA equivalent, retarded leaf chlorophyll degradation, which is an added advantage.

STS (2.0 mM) had no effect on the number of days to opening of primary florets, increased days to 50 % petal fall and accelerated the onset of 50 % leaf yellowing. As regards petal fall, it can be concluded that, the silver ion effectively inhibited ethylene action, hence reducing ethylene induced floret abscission. As to why 2.0 mM STS accelerated the onset of 50 % leaf yellowing, the author suggests continuous holding of *Alstroemeria* cut flowers in STS solution led to phytotoxicity to *Alstroemeria* cut flowers. It is recommended that further research should be carried out to determine the precise concentration and pulsing time of STS, in order to increase the vase life of *Alstroemeria* cut flowers.

Accel (25 mg/litre BA) was most effective, in increasing both chlorophyll and water content of the *Alstroemeria* leaves. However, high levels of Accel (100 mg/litre BA) increased both dry weight and nitrogen content of the leaves. As far as the postharvest quality of cut flowers is concerned, green foliage implying high chlorophyll content and high water content are of prime importance, as opposed to dry weight and nitrogen content. Therefore, the author recommends the use of Accel (25 mg/litre BA) to improve the postharvest quality of *Alstroemeria*. In order to determine the precise concentration of Accel, which will give the best result for most of the parameters determined, it is recommended to use low levels of Accel (5, 10, 15, 20 or 25 mg/litre BA), in further studies.

Lower BA levels (25 or 50 mg/litre), increased both leaf chlorophyll and nitrogen content. BA at higher levels (75 or 100 mg/litre), unexpectedly tended to decrease the water content with a corresponding increase in leaf dry weight. The author recommends the use of pure BA to see how it compares with corresponding BA equivalent in Accel, for the parameters determined.

GA_{4+7} (10.0 mg/litre) significantly increased chlorophyll content, water and nitrogen content of the leaves. However, it significantly reduced the dry weight of the leaves. Hence, the major factors limiting the commercial use of gibberellins have been their cost and their frequent promotion of fresh weights but not of dry weights (Salisbury and Ross, 1986). The author recommends further studies to determine, if application of GA_{4+7} improves the longevity of *Alstroemeria* leaves by reversing, stopping or delaying the disorganization process in the chloroplasts.

Florissant 200 and Accel (25 mg/litre BA) increased all the parameters of vase life and nitrogen content of the leaves comparably. Although, both treatments had no effect on the dry weight

and water content of the leaves, Accel (25 mg/litre BA) had significantly higher leaf chlorophyll content compared to Florissant 200. It can be concluded that Accel (25 mg/litre BA) has the potential to substitute for the use of the Florissant 200 to prolong the vase life and prevent premature leaf yellowing in *Alstroemeria* cut flowers.

STS (2.0 mM), increased the dry weight and nitrogen content of the leaves. However, it decreased leaf chlorophyll and water content. It can be concluded that to avoid Ag^+ toxicity in *Alstroemeria* cut flowers either use lower concentrations of STS or reduce the pulsing time of STS to *Alstroemeria* flowers.

In terms of aesthetics, 'Yellow King' was better than 'Marina', when both flower longevity and leaf quality were considered together. The cultivar difference could be due to differences in hormonal levels.

BA in combination with GA_{4+7} applied in the form of Accel increased the number of days to the full opening of the primary florets. Accel improved vase life of *Alstroemeria* cut flowers as measured by the number of days to the onset of 50 % petal fall and 50 % leaf yellowing. The mode of action was unclear but results of present study suggest that BA (especially at low concentrations) could have improved the longevity of *Alstroemeria* by delaying the breakdown of protein used in the synthesis of chlorophyll, as evidenced by the high retention of nitrogen in the leaves of *Alstroemeria*, throughout the experimental period and significant dry weight retention, in one experiment. Overall, the results of the present study indicate that, BA and / or GA_{4+7} applied together in the form of Accel have the potential to delay leaf senescence and prolong the vase life of *Alstroemeria* cut flowers.

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7.0 APPENDICES

A1: ANOVA table for the effect of Accel on the number of days to opening of Primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1)

		Mean sum of squares		
Source of variation	df	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.03	0.10	1.30
Variety	1	16.13****	38.53****	4.03**
Treatment	4	1.88***	28.33****	15.13****
Error	22	0.28	0.99	0.52
Total	29			
CV		10.03	5.87	3.79

df = degree of freedom

CV = % coefficient of variation

, *, ****, ns = significant at P=0.01, 0.001, 0.0001 or nonsignificant respectively.

A2: ANOVA table for the effect of Accel on the number of days to opening of Primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2)

		Mean sum of squares		
Source of variation	df	Days to opening of Primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.23	0.10	2.03
Variety	1	2.13*	0.53ns	9.63***
Treatment	4	1.45*	3.12***	30.20****
Error	22	0.41	0.35	0.66
Total	29			
CV		13.19	3.87	4.60

df = degree of freedom

CV = % coefficient of variation

*, ***, ****, ns = significant at P=0.05, 0.001, 0.0001 or nonsignificant respectively.

A3: ANOVA table for the effect of GA_{4+7} (Provide) on the number of days to opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1).

Source of variation	df	Mean sum of squares		
		Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.43	4.23	0.43
Variety	1	12.03****	20.83****	1.63ns
Treatment	4	0.67*	27.78****	11.55****
Error	22	0.21	0.84	0.85
Total	29			
CV		9.46	5.11	4.44

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A4: ANOVA table for the effect of GA_{4+7} (Provide) on the number of days to opening of Primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2).

Source of variation	df	Mean sum of squares		
		Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.17	0.45	4.31
Variety	1	6.88***	0.86ns	44.02****
Treatment	6	1.32*	2.82****	35.19****
Error	32	0.54	0.35	2.04
Total	41			
CV		17.84	3.79	7.25

df = degree of freedom

CV =% coefficient of variation

*, ***, ****, ns = significant at P=0.05, 0.001, 0.0001 or nonsignificant respectively.

A5: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares		
		Days to opening of primary florets	Days to 50 % petal fall	Days to 50 % leaf yellowing
Replication	2	0.53	5.63	0.93
Variety	1	0.30ns	2.70ns	0.53ns
Treatment	4	2.63**	21.62****	30.25****
Error	22	0.52	1.75	1.51
Total	29			
CV		166.60	-136.91	-73.78

df = degree of freedom

CV = % coefficient of variation

, **, ns = significant at P = 0.01, 0.0001 or nonsignificant respectively.

A6: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the number of days to full opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2).

Source of variation	df	Mean sum of squares		
		Days to opening of primary florets	Days to 50 % petal fall	Days to 50 % leaf yellowing
Replication	2	0.53	0.43	3.63
Variety	1	0.00ns	0.03ns	2.13ns
Treatment	4	5.22****	5.78****	38.58****
Error	22	0.54	0.50	2.50
Total	29			
CV		100.43	-192.26	-94.81

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P= 0.0001 or nonsignificant respectively.

A7: ANOVA table for the effect of plant growth regulators on the number of days to opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 1).

		Mean sum of squares		
Source of variation	df	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.14	1.77	2.74
Variety	1	29.33****	72.14****	6.06**
Treatment	10	1.38****	17.50****	46.78****
Error	52	0.28	1.39	0.68
Total	65			
CV		10.15	6.56	4.21

df = degree of freedom

CV = % coefficient of variation

,**, ns = significant at P=0.01, 0.0001 or nonsignificant respectively

A8: ANOVA table for the effect of plant growth regulators on the number of days to opening of primary florets, 50 % petal fall and 50 % leaf yellowing of *Alstroemeria* cut flowers (Experiment 2).

		Mean sum of squares		
Source of variation	df	Days to opening of primary florets	Days to 50% petal fall	Days to 50% leaf yellowing
Replication	2	0.24	0.09	6.50
Variety	1	8.67****	0.63ns	54.17****
Treatment	12	1.93****	2.15****	44.65****
Error	62	0.47	0.42	1.78
Total	77			
CV		15.38	4.15	7.12

df = degree of freedom

CV =% coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A9: ANOVA table for the effect of Accel on chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares		
		Chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.14	0.06	0.01
Variety	1	0.14ns	0.33ns	2.37***
Treatment	4	0.58***	0.77***	1.16****
Error	22	0.07	0.09	0.12
Total	29			
CV		13.35	15.44	22.76

df=degree of freedom

CV = % coefficient of variation

, *, ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A10: ANOVA table for the effect of Accel on chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.01	0.07	0.04
Variety	1	0.44ns	0.08ns	0.01ns
Treatment	4	2.07****	2.15****	1.44****
Error	22	0.13	0.09	0.02
Total	29			
CV		21.48	20.50	11.79

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A11: ANOVA table for the effect of GA_{4+7} (Provide) on chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares		
		Chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.05	0.02	0.03
Variety	1	0.09ns	0.16ns	0.50***
Treatment	4	0.20ns	0.11ns	0.42****
Error	22	0.08	0.06	0.03
Total	29			
CV		15.34	16.12	14.72

df = degree of freedom

CV = % coefficient of variation

, *,ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A12: ANOVA table for the effect of GA_{4+7} (Provide) on chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.01	0.02	0.02
Variety	1	0.01ns	0.01ns	0.11*
Treatment	6	0.35****	0.34****	0.34****
Error	32	0.03	0.02	0.02
Total	41			
CV		14.19	12.27	15.11

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A13: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares		
		chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.03	0.14	0.06
Variety	1	0.01ns	0.03ns	0.69*
Treatment	4	0.30*	0.44**	0.62**
Error	22	0.11	0.08	0.15
Total	29			
CV		149.64	83.46	153.28

df = degree of freedom

CV = % coefficient of variation

*, **, ns = significant at P = 0.05, 0.01 or nonsignificant respectively.

A14: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the chlorophyll content (mg/cm^2) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		chlorophyll content (mg/cm^2) of the leaves		
		7 days	14 days	21 days
Replication	2	0.06	0.13	0.01
Variety	1	0.53ns	0.16ns	0.03ns
Treatment	4	1.28***	1.22****	0.60****
Error	22	0.20	0.07	0.04
Total	29			
CV		87.10	60.81	55.51

df = degree of freedom

CV = % coefficient of variation

, *, ns = significant at P = 0.05, 0.0001 or nonsignificant respectively.

A15: ANOVA table for the effect of plant growth regulators on chlorophyll content (mg/cm²) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares Chlorophyll content (mg/cm²) of the leaves		
		7 days	14 days	21 days
Replication	2	0.25	0.01	0.002
Variety	1	0.28*	0.45*	2.20****
Treatment	10	0.53****	0.72****	0.81****
Error	52	0.07	0.07	0.08
Total	65			
CV		14.02	15.74	21.13

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A16: ANOVA table for the effect of plant growth regulators on chlorophyll content (mg/cm²) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares Chlorophyll content (mg/cm²) of the leaves		
		7 days	14 days	21 days
Replication	2	0.002	0.02	0.07
Variety	1	0.31*	0.12ns	0.14*
Treatment	12	1.03****	0.93****	0.62****
Error	62	0.07	0.05	0.02
Total	77			
CV		18.38	17.41	14.86

df = degree of freedom

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CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A17: ANOVA table for the effect of Accel on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares Dry weight (g) of the leaves 7 days	 21 days
Replication	2	0.02	0.0007
Variety	1	0.003ns	0.15*
Treatment	4	0.29****	0.58****
Error	22	0.008	0.02
Total	29		
CV		6.24	10.30

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A18: ANOVA table for the effect of Accel on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares Dry weight (g) of the leaves 7 days	 14 days	 21 days
Replication	2	0.05	0.05	0.22
Variety	1	3.50****	67.50****	34.48****
Treatment	4	0.99***	5.39****	13.62****
Error	22	0.14	0.16	1.27
Total	29			
CV		23.34	9.12	19.36

df = degree of freedom

CV = % coefficient of variation

, *, ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A19: ANOVA table for the effect of GA_{4+7} (Provide) on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares Dry weight (g) of the leaves 7 days	 21 days
Replication	2	0.01	0.03
Variety	1	0.19****	0.05ns
Treatment	4	0.01ns	0.01ns
Error	22	0.005	0.02
Total	29		
CV		6.41	9.73

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A20: ANOVA table for the effect of GA_{4+7} (Provide) on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares Dry weight (g) of the leaves 7 days	 14 days	 21 days
Replication	2	0.05	0.06	0.17
Variety	1	1.09****	39.67****	42.36****
Treatment	6	0.11**	1.42****	1.90**
Error	32	0.03	0.14	0.31
Total	41			
CV		14.97	14.78	19.52

df =degree of freedom

CV = % coefficient of variation

, *, ****, ns = significant at P=0.01, 0.001, 0.0001 or nonsignificant respectively.

A21: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the dry weight(g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares	
		Dry weight (g) of the leaves	
		7 days	21 days
Replication	2	0.01	0.01
Variety	1	0.38**	0.14**
Treatment	4	0.49****	0.27****
Error	22	0.04	0.01
Total	29		
CV		86.87	54.67

df = degree of freedom

CV = % coefficient of variation

, **, = significant at P = 0.01, or 0.0001 respectively.

A22: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the dry weight(g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Dry weight (g) of the leaves		
		7 days	14 days	21 days
Replication	2	0.11	0.02	0.11
Variety	1	0.96**	8.64****	0.03ns
Treatment	4	1.14****	12.37****	24.29****
Error	22	0.12	0.32	1.18
Total	29			
CV		87.93	32.12	36.93

df = degree of freedom

CV = % coefficient of variation

, **, ns = significant at P = 0.01, 0.0001 or nonsignificant respectively.

A23: ANOVA table for the effect of plant growth regulators on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares	
		Dry weight (g) of the leaves	
		7 days	21 days
Replication	2	0.03	0.02
Variety	1	0.13***	0.02ns
Treatment	10	0.19****	0.32****
Error	52	0.01	0.02
Total	65		
CV		7.23	10.59

df = degree of freedom

CV = % coefficient of variation

, *,ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A24: ANOVA table for the effect of plant growth regulators on dry weight (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Dry weight (g) of the leaves		
		7 days	14 days	21 days
Replication	2	0.04	0.07	0.16
Variety	1	4.28****	113.14****	84.49****
Treatment	12	0.61****	8.31****	23.49****
Error	62	0.09	0.24	0.88
Total	77			
CV		21.37	14.42	21.57

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A25: ANOVA table for the effect of Accel on water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Means sum of squares: Water content (g) of the leaves	
		7 days	21 days
Replication	2	0.02	0.0007
Variety	1	0.003ns	0.15*
Treatment	4	0.29****	0.58****
Error	22	0.008	0.02
Total	29		
CV		1.33	2.41

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P=0.05, 0.0001 or nonsignificant respectively.

A26: ANOVA table for the effect of Accel on water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares: Water content (g) of the leaves		
		7 days	14 days	21 days
Replication	2	0.05	0.05	0.22
Variety	1	3.50****	67.50****	34.48****
Treatment	4	0.99***	5.39****	13.62****
Error	22	0.14	0.16	1.27
Total	29			
CV		5.84	10.83	51.49

df = degree of freedom

CV = % coefficient of variation

, *, ns =significant at P=0.001, 0.0001 or nonsignificant respectively.

A27: ANOVA table for the effect of GA_{4+7} (Provide) on water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares	
		Water content (g) of the leaves	
		7 days	21 days
Replication	2	0.01	0.003
Variety	1	0.19****	0.05ns
Treatment	4	0.01ns	0.01ns
Error	22	0.006	0.02
Total	29		
CV		1.12	1.84

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A28: ANOVA table for the effect of GA_{4+7} (Provide) on water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Water content (g) of the leaves		
		7 days	14 days	21 days
Replication	2	0.05	0.06	0.17
Variety	1	1.09****	39.67****	42.36****
Treatment	6	0.11**	1.42****	1.89***
Error	32	0.03	0.14	0.31
Total	41			
CV		2.63	6.99	10.74

df = degree of freedom

CV = % coefficient of variation

, *, ****, ns = significant at P=0.01, 0.001, 0.0001 or nonsignificant respectively.

A29: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares Water content (g) of the leaves 7 days	 21 days
Replication	2	0.01	0.01
Variety	1	0.38**	0.14**
Treatment	4	0.49****	0.27****
Error	22	0.04	0.01
Total	29		
CV		-86.87	-54.67

df = degree of freedom

CV = % coefficient of variation

, **, = significant at P = 0.01, or 0.0001 respectively.

A30: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the water content of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares Water content (g) of the leaves 7 days	 14 days	 21 days
Replication	2	0.11	0.02	0.11
Variety	1	0.96**	8.66****	0.03ns
Treatment	4	1.14****	12.37****	24.29****
Error	22	0.12	0.32	1.18
Total	29			
CV		-87.93	-32.12	-36.93

df = degree of freedom

CV = % coefficient of variation

, **, ns = significant at P = 0.01, or 0.0001 nonsignificant respectively.

A31: ANOVA table for the effect of plant growth regulators on water content (g) of the leaves of *Astroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares: Water content (g) of the leaves	
		7 days	21 days
Replication	2	0.03	0.02
Variety	1	0.13***	0.02ns
Treatment	10	0.19****	.32****
Error	52	0.009	0.02
Total	65		
CV		1.40	2.24

df = degree of freedom

CV = % coefficient of variation

, *, ns = significant at P=0.001, 0.0001 or nonsignificant respectively

A32: ANOVA table for the effect of plant growth regulators on water content (g) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares: Water content (g) of the leaves		
		7 days	14 days	21 days
Replication	2	0.04	0.07	0.16
Variety	1	4.28****	113.14****	84.49****
Treatment	12	0.61****	8.31****	23.49****
Error	62	0.09	0.24	0.88
Total	77			
CV		4.40	10.73	25.69

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A33: ANOVA table for the effect of Accel on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares	
		Total nitrogen (%) of the leaves	
		7 days	21 days
Replication	2	0.001	0.02
Variety	1	1.06****	0.07ns
Treatment	4	0.34****	1.07****
Error	22	0.02	0.05
Total	29		
CV		3.90	7.37

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A34: ANOVA table for the effect of Accel on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Total nitrogen (%) of the leaves		
		7 days	14 days	21 days
Replication	2	0.003	0.003	0.0007
Variety	1	1.22****	0.09****	0.008ns
Treatment	4	2.19****	2.11****	1.92****
Error	22	0.02	0.004	0.01
Total	29			
CV		5.06	2.40	4.74

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.

A35: ANOVA table for the effect of GA_{4+7} (Provide) on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares: Total nitrogen (%) of the leaves	
		7 days	21 days
Replication	2	0.0006	0.03
Variety	1	0.79****	0.01ns
Treatment	4	0.18***	0.38****
Error	22	0.03	0.02
Total	29		
CV		5.61	5.61

df = degree of freedom

CV = % coefficient of variation

, *, ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A36: ANOVA table for the effect of GA_{4+7} (Provide) on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Exeriment 2)

Source of variation	df	Mean sum of squares: Total nitrogen (%) of the leaves		
		7 days	14 days	21 days
Replication	2	0.00003	0.0002	0.0004
Variety	1	0.51****	0.04ns	0.21**
Treatment	6	1.16****	0.93****	0.50****
Error	32	0.01	0.01	0.02
Total	41			
CV		3.91	4.75	6.53

df = degree of freedom

CV =% coefficient of variation

, **, ns = significant at P=0.01, 0.0001 or nonsignificant respectively.

A37: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of squares	
		Total nitrogen (%) of the leaves	
		7 days	21 days
Replication	2	0.003	0.09
Variety	1	0.02ns	0.03ns
Treatment	4	0.31****	0.24*
Error	22	0.01	0.08
Total	29		
CV		34.29	91.27

df = degree of freedom

CV = % coefficient of variation

*, ****, ns = significant at P = 0.05, 0.0001 or nonsignificant respectively.

A38: ANOVA table for the effect of Benzyladenine (Accel - GA_{4+7}) on the total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares		
		Total nitrogen (%) of the leaves		
		7 days	14 days	21 days
Replication	2	0.003	0.002	0.0007
Variety	1	3.38****	0.27***	0.09****
Treatment	4	0.28**	0.19****	0.37****
Error	22	0.05	0.02	0.002
Total	29			
CV		81.17	49.33	10.87

df = degree of freedom

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CV = % coefficient of variation

, *, ****, = significant at P = 0.01, 0.001 or 0.0001 respectively.

A39: ANOVA table for the effect of plant growth regulators on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 1)

Source of variation	df	Mean sum of sqaures Total nitrogen (%) of the leaves 7 days	 21 days
Replication	2	0.01	0.002
Variety	1	2.47****	0.40***
Treatment	10	0.38****	0.60****
Error	52	0.02	0.03
Total	65		
CV		4.12	6.49

df = degree of freedom

CV = % coefficient of variation

, *, ns = significant at P=0.001, 0.0001 or nonsignificant respectively.

A40: ANOVA table for the effect of plant growth regulators on total nitrogen content (%) of the leaves of *Alstroemeria* cut flowers (Experiment 2)

Source of variation	df	Mean sum of squares Total nitrogen (%) of the leaves 7 days	 14 days	 21 days
Replication	2	0.001	0.002	0.0007
Variety	1	0.003ns	0.04ns	0.26****
Treatment	12	0.86****	0.78****	0.80****
Error	62	0.05	0.01	0.01
Total	77			
CV		7.49	4.31	4.75

df = degree of freedom

CV = % coefficient of variation

****, ns = significant at P=0.0001 or nonsignificant respectively.