GLADIOLUS SCABRIDUS - THE ROAD TO CONSERVATION AND COMMERCIALISATION

By

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Agriculture in

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December 2005

DECLARATION

I hereby declare that the research work reported in this thesis is the result of my own investigation, except where acknowledged.

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We certify that the above statement is correct.

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To see the world in a grain of sand
And a Heaven in a wild flower,
Hold infinity in the palm of your hand
And eternity in an hour

William Blake (1757-1827)

Then God said, "Let the land produce vegetation: seed bearing plants and trees on the land that bear fruit with seed in it, according to their various kinds". And it was so.
The land produced vegetation: plants bearing seed according to their kinds and trees bearing fruit with seed in it according to their kinds. And God saw that it was good.

Genesis 1: 11-12

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to the following people and organisations:

Prof John Bower for his supervision, guidance and encouragement.

Renate van Niekerk for her valuable comments and suggestions.

The John Travis Bursary Fund and Midland's Flower Initiative for financial assistance.

Dr Paul Shanahan, Prof Peter Greenfield, Dr Albert Modi, Ms Colleen Carlson, Dr Isa Bertling and all the staff in the Plant Sciences Department for their help and advice.

Special thanks to Paul Hildyard, Teri Dennison, Lowie de Klerk, Selwyn Sampson, Dave Gregory, Barry Hunkin, Quenton Tharrot, Gail Papli, Kogie Pillay, Amen Ngidi and Thomas Zuma for their cheerful and willing assistance in the ordering of chemicals, glasshouse production, lab work and sorting out any crises that occurred.

Dr Jeff Finnie, Prof Dick Haynes and Dr Ed Granger for advice in their areas of expertise.

Rob Scott-Shaw and Brigitte Church at KwaZulu-Natal Wildlife for being so willing to give of their time and for supplying plant material.

My fellow postgraduates, Zelda van Rooyen, Andrew Dominy, Thabiso Lebese, Molipa Mosoeunyane (Boetie), Bob Kalala, Nicky Taylor, Vivienne Deigna-Mockey, Delia Thompson, Julius Ochuodho, Alfred Odindo, Dambuza Ngomane, Mireille Asanzi, Rendani Nemakanga, Samson Tesfay, Nathan Phiri, Rorisang Mare and Mangani Katundu, for advice, friendship, laughter and encouragement. A special thank you to Ryan Cripps for assistance with the HPLC work.

Jennifer Mander at The Institute for Natural Resources, Barry Killian, the dam operator at Bivane Dam, and Impala Water User Association for making the trip to Bivane Dam possible.

Grateful thanks and appreciation to all the garden centres and florists who so willingly gave of their time to fill in the market research questionnaire. Thanks go to the customers of 'Our Secret Garden' in Hilton who also gave of their time in this regards.

Johannes Maree at Hosgro for information and advice.

Mr Nevil Tyler and NET Projects for the use of computer facilities.

Jonathan Tyler for his encouragement and assistance.

Janet Fuller and Neil Horner for making the trip to Bivane Dam such an adventure. Your help was greatly appreciated. Neil, you are an inspiration!

Ian and Lisa Hobson for their support and understanding and above all for being such good friends. Thank you for giving me the opportunity to learn so much about plants!

My precious family for their endless patience, love and support and for putting up with months of long, stressful working hours.

My heavenly Father: 'You are my God and I will give you thanks; you are my God and I will exalt you.' Psalm 118:28.

ABSTRACT

There is at present a growing concern and awareness of the endangered status of many indigenous South African plants in the wild, a number of which have potential for commercial production. One such example is *Gladiolus scabridus*, a vulnerable species endemic to the mountains of northern KwaZulu-Natal and southern Swaziland. It has considerable potential due to its floral characteristics. However, little is known about its horticultural requirements. Thus propagation and cultural practices were investigated with the aim of both conservation and commercialisation of the species.

The ideal conditions for *G. scabridus* seed germination were determined. The presence or absence of light had no significant effect. Optimum germination was achieved at 20 °C of both winged and dewinged seeds. Higher temperatures appear to have a negative effect on germination and none of the winged seeds germinated at 30 °C. Under ideal conditions, fresh seed showed significantly higher vigour and viability than stored seed although the germination of stored seed was hampered by a higher internal fungal content.

Successful tissue culture protocol was established for *G. scabridus* axillary bud and cormel halve explants. During the initial shoot initiation and proliferation stages (stage I and II), the significantly higher shoot numbers occurred in the absence of growth regulators in both explant types. However, shoots with 1.0 mg l^{-1} 6-benzyl-amino-purine (BAP) and 0.5 mg l^{-1} 1-naphthalene-acetic-acid (NAA) were healthier in appearance. Higher levels of 5.0 mg l^{-1} NAA inhibited shoot production and encouraged root development in cormel halve explants. During stage II, axillary bud explants showed root and cormlet development. More roots were initiated without growth regulators, whilst 5.0 mg l^{-1} NAA resulted in significantly better cormlet development. Shoot and cormlet growth of cormel halve explants during stage II was not significantly affected by the presence or absence of NAA and BAP. Significantly more roots were produced with 5.0 mg l^{-1} NAA. During stage III (rooting), the presence of activated charcoal (AC) was essential for the initiation and development of roots *in vitro*. Root and cormlet development in all explant types was significantly affected by the interaction between the previous treatments from stages I and II and the new treatments. During stage IV (hardening off) , most explants died down in the hardening off media leaving resting cormlets. There was a significant interaction between 1.0 mg l^{-1} NAA and 0.3 % AC from stage III, resulting in significantly more cormlets in both axillary bud and cormel halve explants. There was successful cormlet growth after cold storage which is advantageous in reducing the need for acclimatization.

G. scabridus corms were successfully forced out of their normal flowering period. Temperatures of 10, 5.5 and 2 °C showed successful corm initiation although the corms need to be stored for longer at warmer temperatures. It is suggested that a treatment of 2 °C for 6 weeks is the optimal condition for forcing *G. scabridus* corms.

Although originating from a stressful environment, *G. scabridus* appears to show capacities for improved growth under controlled conditions. Fertilizer applications enhanced growth and reduced the time to flowering. Nitrogen (N) was found to be important for vegetative growth, flowering and daughter corm development, whereas potassium (K) influenced cormel production. Fertilizer with higher N and lower K is appropriate for the beginning and middle of the growing season and then adjusted to a lower N and higher K fertilizer to promote cormel formation. *G. scabridus* was found to produce prolific numbers of cormels which is an important source of plant material. Mineral leaf analysis showed that optimum levels for wild species are lower than those for hybrid gladioli with the optimum levels affected by physiological corm maturity and subsequent plant growth.

Norms for postharvest handling of cut *G. scabridus* spikes have been developed. Spikes held in 2 % sucrose had a longer vase life and better floret opening and quality than those kept in distilled water, Prolong®, Chrysal®, 2 % sucrose and 2 % ethanol, 1 % sucrose, 4 % sucrose, 1 % sucrose and 0.5 % JIK®, 2 % sucrose and 0.5 % JIK®and 4 % sucrose and 0.5 % JIK®, 2 % fructose and 2 % glucose. The use of commercially available solutions should be used with caution. Florets produced a climacteric-like CO_2 peak, but levels of ethylene were unmeasurable. Cold storage and the use of polypropylene sleeves delays senescence. *G. scabridus* spikes secrete droplets of a sticky substance which was confirmed to be extra floral nectar through HPLC analysis. Market research revealed a positive response to the species from consumers and retailers alike with potential for cultivation as a cut flower and bedding plant. However, the cost will determine supply and demand.

A field study conducted at Bivane Dam, northern KwaZulu-Natal, confirmed that *G. scabridus* colonies prevail in rocky, quartzite outcrops where they become wedged between the rocks. Plants were found at different stages of development with populations of not more than 108 plants per colony. Soil data of *G. scabridus* sites was compared to that of two sites nearby. It was found that *G. scabridus* soils are higher in phosphorus (P), zinc (Zn) and organic carbon. Leaf analysis confirmed that they have adapted their growth to low nutrient levels.

The *G. scabridus* studies have clearly shown that the species can be successfully moved from a wild plant to a commercially viable one and in so doing its conservation status can also be improved.

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LIST OF ABBREVIATIONS

ABA	abscisic acid
AC:	activated charcoal
ANOVA:	analysis of variance
ASL:	above sea level
B:	boron
BA:	benzyladenine
BAP:	6-benzyl-amino-purine
Ca:	calcium
$CaClO_4$:	calcium hypochlorite
CBD:	Convention on Biological Diversity
cobalt:	Co
Cu:	copper
cv:	cultivar
ESEM:	Environmental Scanning Electron Microscope
ETOH:	ethanol
Fe:	iron
GA:	gibberellic acid
GC:	gas chromatograph
GPS:	global positioning system
ha:	hectare
HCl:	hydrochloric acid
$HgCl_2$:	mercurc chloride
HPLC:	high performance liquid chromatography
IAA:	indole-3-acetic acid
IBA:	3-indolebutyric acid
K:	kinetin
K:	potassium
KZN:	KwaZulu-Natal
LD:	long days
LSD:	least significant difference
m:	metre

M:	Molar
Mg:	magnesium
Mn:	manganese
MS:	Murashige and Skoog
N:	nitrogen
Na:	sodium
NAA:	1-naphthalene-acetic-acid
NaOCl:	sodium hypochlorite
NaOH:	sodium hydroxide
NBI:	National Botanical Institute
O:	phosphorus
PEA:	Photosynthetic Efficiency Analyser
ppm:	parts per million
SD:	short days
STS:	silver thiosulphate
suc:	sucrose
UV:	ultra violet
Zn:	zinc
2, 4-D:	2, 4-dichlorophenoxyacetic acid
8-HQC:	8-hydroxyquinoline citrate
8-HQS:	8-hydroxyquinoline sulphate

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INTRODUCTION

Southern Africa has the richest temperate flora of any area of comparable size in the world (Meyer *et al.*, 1997; Smith *et al.*, 1996). It is home to a third of the world's six floristic regions, with almost 10 % of all flowering plants occurring here on less than 2 % of the global land surface area (Smith *et al.*, 1999). These diverse and interesting species have inspired botanical artists, writers and plant lovers alike for over 400 years and they form an integral part of our natural heritage, hence the importance and value placed on each one (Huntley, 1997).

The progressive distribution of South African blooms abroad, reflects not only the history of botanical endeavour in South Africa and in other parts of the world, but also the cyclical whims and trends of horticulture and gardening in general (Stirton, 1980). Some consider South Africa the 'cradle of modern floriculture' because it has provided the world with some of the more beautiful products of the trade (Pizano, 2003). However, despite this resource, South Africa remains an untapped reservoir of horticultural importance and little has been done to realize the commercial potential of many of these endemic species (Delpierre & du Plessis, 1973; Stirton, 1980; Pooley, 1998).

'The fascination with South African plants is currently undergoing a renewed interest that has been stimulated by a growing domestic concern and awareness of the endangered status of many of the plants in the wild and by an appreciation of their undoubted economic value' (Stirton, 1980). Endemic plants are under threat due to changes in natural fire regimes, invasive species, habitat conversion, foreign pathogens, unsustainable levels of subsistence farming and overgrazing and the commercial and illegal harvesting of wild plants, all leading to a rapid breakdown of fragile natural resources (Winter & Botha, 1994; Armstrong *et al.*, 1998; Scott-Shaw, 1999).

Among the most beautiful representatives of South African flora, and certainly a group of plants barely exploited commercially, are the petaloid monocotyledons. They occur throughout southern Africa and species can be found in almost every type of habitat (Stirton, 1980). Many of these bulbous plants are endangered, but often prove easy to cultivate and propagate. Such plants can be released into the horticultural trade for commercial production as a means of aiding the conservation process (Winter & Botha, 1994).

One such example, is *Gladiolus scabridus*, a vulnerable, indigenous species with possible commercial potential due to its floral characteristics (Scott-Shaw, 1999). Floriculture is always receptive to new crops (Horn *et al.*, 1989; Shillo, 1997) and *G. scabridus* would make an attractive cut flower to satisfy ever increasing consumer demands for new products. As part of their programme to propagate threatened plants, the Threatened Plant Conservation Unit at KwaZulu-Natal Wildlife has designated sections of their propagation area to the cultivation of *G. scabridus ex-situ*. However, it is not a high priority species of which there are about 600. Hence, not many resources are allocated to this species in terms of research and little is known about its propagation methods and growth requirements. In the wild, the species does not reach its full genetic potential but if more were known about its horticultural requirements, considerable opportunity for commercial development may be possible.

The objectives of this study were therefore to investigate propagation and cultural practices suited to *G. scabridus*. This information, together with knowledge of its ecology, will allow for the promotion of its commercial production as well as aid the enhancement of its conservation status.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The *Gladiolus*, or Sword Lily (Hessayon, 1995), is the largest genus of the petaloid monocotyledonous family, Iridaceae (Lewis *et al.*, 1972; Goldblatt, 1996; Takemura *et al.*, 2005) and comprises some 260 species and many named hybrids (Goldblatt *et al.*, 2001; Bryan, 2002). The genus is centralized in southern Africa where there are at least 165 species, with a pronounced concentration in the south western Cape region of South Africa, but it ranges through all of tropical Africa, Madagascar, into the Mediterranean basin, southern Europe, the Middle East and as far east as Afghanistan (Anderton & Park, 1989; Goldblatt, 1996; Goldblatt *et al.*, 2001; Bryan, 2002). The *Gladiolus* has certainly shown a remarkable aptitude for colonizing a wide spectrum of environments (Delpierre & du Plessis, 1973).

Gladioli are one of the world's most important horticultural plants today, both as an ornamental garden subject used in herbaceous borders, beddings, rockeries or pots, and as a cut flower crop (Salinger, 1985; Bryan, 1989; Rajasekharan *et al.*, 1994; Goldblatt & Manning, 1998; Mangal *et al.*, 2003). Many are hybrids, although a number of original species are grown commercially as well (Bryan, 1989; 2002). Gladioli hybridisation began some 200 years - it is believed that less than 20 species were used in the development of the existing cultivars (Ferreira & Hanke, 1985). *Gladiolus* cultivation dates back to the 1st century, to the days of ancient Greece and Rome (Buschman, 1998b). The first indigenous South African gladioli were taken to Europe between 1739 and 1745 by Dutch and English spice traders en route to India (Buch, 1972; Rees, 1992).

Gladioli are prized by florists for their showy stems and by growers for their relative ease of production and make up a substantial portion of the cut flower trade worldwide (Serek *et al.*, 1994; Gonzalez *et al.*, 1998). In 1992, it was estimated that the world production of gladioli made up about 26 % of the total flower bulb cultivation (De Hertogh *et al.*, 1992). The *Gladiolus* is an important cut flower crop in South Africa valued at R 7.7 million (Pizano, 2003). Another separate industry is corm production and sales, where millions of corms are

sold each year for cut flower and landscape use (Wilfret, 1992). In 1999, gladioli made up about 8.4% of the total world production of gladioli corms (Anon, 2000).

The vast majority of the literature available on the genus *Gladiolus* applies to cultivated hybrids, with little known or published on wild species. However, this research will provide an important background and guide to studies on *G. scabridus*.

1.2 GENERAL ASPECTS OF GLADIOLI

Gladioli are herbaceous perennials (du Plessis & Duncan, 1989; Wilfret, 1992; De Hertogh & Le Nard, 1993) and are grouped into winter and summer flowering cultivars (du Plessis & Delpierre, 1977; Duncan, 1996). They are additionally grouped by flower shape, size and characteristics. They can be divided into 5 groups, namely the *Species, Miniature, Butterfly, Primulinus and Large-flowered* hybrids (Hessayon, 1995).The flowers are available in a wide variety of colours except true blue (De Hertogh & Le Nard, 1993; Dole & Wilkins, 1999; Takemura *et al.*, 2005).

Gladiolus corms are bitter in taste and some are reported to be poisonous (McLean, 1941). The corms of *G. permeabilis* subsp. *edulis* are known to be eaten by the Tswanas (Fox & Norwood Young, 1982; du Plessis & Duncan, 1989). They apparently taste like chestnuts when roasted and are also commonly eaten by baboons (Bryan, 1989; 2002). The corms of *G. dalenii* and *G. saundersii* are used medicinally by the southern Sothos (du Plessis & Duncan, 1989). Flowers of *G. dalenii*, *G. cruentus* and *G. eckloni* are eaten by the indigenous peoples of southern Africa, either raw as a salad or cooked as a pot-herb. Children are known to suck the nectar from the flowers (Fox & Norwood Young, 1982).

The South African species have a diploid chromosome number (2n = 30), whereas the European species and most of the commercial varieties are polyploids (2n = 60 to 130) (Ferreira & Hanke, 1985; Wilfret, 1992).

1.2.1 Morphology and anatomy (Figure 1.1)

The corm: The storage and perennating rootstock is a corm (Salinger, 1985; Goldblatt & Manning, 1998). This nutrient-holding body is a compressed, swollen, underground stem base, derived from the swollen lower internodes of the underground portion of the stem and which is replaced annually (Delpierre & du Plessis, 1973; du Plessis & Duncan, 1989). The dormant corm consists of several internodes (du Plessis & Duncan, 1989), 3 or 4 nodes, each with an axillary bud in alternate positions, an apical bud (Hussey, 1977a; b; Ziv & Lilien-Kipnis, 1990; De Hertogh & Le Nard, 1993), a large scar at the base, indicating where it was attached to the mother corm, and root initials at the edge of this scar (Rees, 1992).

It is more or less globose to depressed globose in shape, although corms of different species vary considerably in size, which is often directly related to plant size (Goldblatt, 1996; Goldblatt & Manning, 1998).

The surface and internal tissue may be white, yellow or red to orange (McLean, 1941; Goldblatt, 1996). Corm pigmentation develops due to exposure to light and oxygen (Griesbach, 1972). There is a weak correlation between corm size and colour, larger corms often having orange flesh. This is perhaps related to tannin content and defence against predation (Goldblatt, 1996).

Gladiolus corms are almost always enclosed by protective layers called tunics, which serve to protect the corm from injury and dehydration (McLean, 1941; Goldblatt & Manning, 1998). These consist of 1 to 12 dead, fibrous layers (Wilfret, 1992) which overlap on the corm, facing the pointed tip (Greving, 1992) and can be hard and clawed or soft and papery, depending on the species (du Plessis & Duncan, 1989). They are derived from the bases of the previous year's cataphylls and lower leaves (Rix, 1983) and are usually characteristic of species groups (Goldblatt, 1996; Goldblatt & Manning, 1998).

Stolons and cormels: Short, thread-like, branched outgrowths called stolons develop between the mother and daughter corm on which grow clusters of small cormels (Ginzburg & Ziv, 1973; Steinitz & Lilien-Kipnis, 1989; Wilfret, 1992; Manrique, 1993; Ju & MingFang, 2004) or stem tubers, often in large numbers (Rees, 1992) and is also characteristic of species or species groups (Goldblatt, 1996; Goldblatt & Manning, 1998).

Roots: The primary root system consisting of 5 to 15 adventitious or assimilatory roots, develops from the base of the corm (Shillo & Halevy, 1976a; Robinson *et al.*, 1980; De Hertogh & Le Nard, 1993; Goldblatt, 1996). These originate from deep within the corm and are involved in the uptake of water and nutrients (Griesbach, 1972).

Corms also usually produce one or more thick, fleshy contractile roots which constitutes a secondary root system (Anderton & Park, 1989). These emerge from the base of the daughter corm and pull the newly formed corm deeper into the soil, ensuring a suitable level for plant growth (Halevy, 1986a; Goldblatt & Manning, 1998). The number of contractile roots that form are inversely related to the depth of the planted corm (Halevy, 1986b). Halevy (1986a) found that contractile root formation is regulated by corm size, temperature fluctuations and light, and that only small and medium sized corms produce contractile roots.

Sheath leaves (cataphylls): These are the first foliar organs produced by the shoot from the main bud of the corm (Goldblatt & Manning, 1998). This bud contains primordia of 9 to 11 sheath leaves, most of which are damaged during emergence, and only the inner most 3 to 4 grow 2 to 4 cm above the soil (Shillo & Halevy, 1976a).

These basal leaves are composed of bladeless sheaths (Halevy, 1986b) and protect the underground part of the shoot (Goldblatt, 1996). They grade from pale and membranous, to firm in texture and the above ground portions are sometimes pubescent and downy (Lewis *et al.*, 1972; Goldblatt & Manning, 1998). They are generally purplish to red in colour due to the presence of anthocyanins, but sometimes remain green (Pfeiffer, 1931; Goldblatt, 1996).

Foliage leaves: These are the 'true' leaves composed of basal sheath parts which gradually merge into the blade (Halevy, 1986b). They are the main organs of photosynthesis (Goldblatt & Manning, 1998) and vary in number and length according to the species (Buch, 1972; Bryan, 1989), but the genus generally produces 8 to 10 sword-shaped leaves (Armitage, 1993). *Gladiolus* leaf production can be regarded as pleisomorphic, that is, the first 4 to 5 long-bladed foliage leaves grow from compressed nodes below the ground level (basal leaves) and the remaining shorter-bladed ones are attached to the flowering spike above ground level (cauline leaves). The upper 2 to 3 leaves are less developed and resemble bracts (Shillo & Halevy, 1976a; Goldblatt, 1996).

The leaves are flattened, with parallel venation (McLean, 1941) and are sometimes coarse to touch (Bryan, 1989; 2002). Typically there is a thickening of the midrib and margins (Griesbach, 1972; Goldblatt, 1996).

The 'stem': This is actually the flowering spike although the corm also forms a part of the stem system. All *Gladiolus* species have an aerial flowering stem bearing the basal cataphylls, the lower and upper foliage leaves and the bract leaves. The stem may be simple or branched and stem thickness is directly related to plant size and diameter at the base of the spike (Goldblatt, 1996; Goldblatt & Manning, 1998).

Inflorescence: Gladioli flowers are arranged in a spike (Wilfret, 1992). They are sessile and each flower is subtended by a pair of green, firm, opposed bracts which protect the flower buds before opening (Goldblatt & Manning, 1998). The florets number up to 30 or more (Wilfret, 1992), however, this is variable even within populations and depends largely on the health and age of the plant (Goldblatt, 1996).

Florets vary in colour, shape, size and markings (Pfeiffer, 1931). They are zygomorphic, sometimes actinomorphic or star-shaped (Lewis *et al.*, 1972; Doutt, 1994). The perianth tube is funnel-shaped, narrow at the base and gradually widening towards the throat (du Plessis & Duncan, 1989). In many species the flowers open wide, while in the hybrids they are not as open, are fairly crowded on the stem and are usually one-sided (Bryan, 1989; 2002; Manrique, 1993). They frequently close at night (Manning *et al.*, 2002).

Flowers are bisexual and floral parts are divided into threes (Delpierre & du Plessis, 1973; Wilfret, 1992). Floral organs are initiated in the following order: 2 bracts, 3 stamens, 3 outer perianth lobes, 3 inner perianth lobes and finally the ovary (Shillo & Halevy, 1976a; Ziv & Lilien-Kipnis, 1990).

The inferior, trilocular ovary or capsule, contains between 50 and 100 ovules that mature within 30 days after fertilization (Pfeiffer, 1931; Wilfret, 1992). Normally for fertilization to take place, cross pollination must occur (Delpierre & du Plessis, 1973). In nature, various insects pollinate the flowers. To assist in pollination, the flowers of some species produce scent at certain times during the day to attract specific pollinators and most *Gladiolus* flowers have markings or nectar guides to which insects are attracted (Doutt, 1994; Manning, 2002). The fruit that forms ripens and eventually dehisces marking the release of the seeds (du Plessis & Duncan, 1989).

Seeds: *Gladiolus* seeds are usually 5 to 6 mm in size, light brown in colour and oval to round in shape. The seed surface is lightly reticulate and the outline of the epidermal cells is evident (Goldblatt & Manning, 1998).The seed body itself is globose but part of the seed coat extends into a broad flat, dry, membranous halo around the seed which acts as a 'wing'. This consists of large, loose cells containing airpockets which ensures lightness enabling them to be scattered easily by the wind (Lewis *et al.*, 1972; Delpierre & du Plessis, 1973; Goldblatt, 1996; Goldblatt *et al.*, 2001; Manning *et al.*, 2002). Seed number is dependant on capsule size and to some extent on seed size (Goldblatt & Manning, 1998), but generally 60 or more seeds are produced per capsule (Delpierre & du Plessis, 1973). The seeds are anatropous (Pfeiffer, 1931), thus the ovary turns through 180° so that the micropyle is folded over and lies near the base of the funiculus (Tootill, 1984).

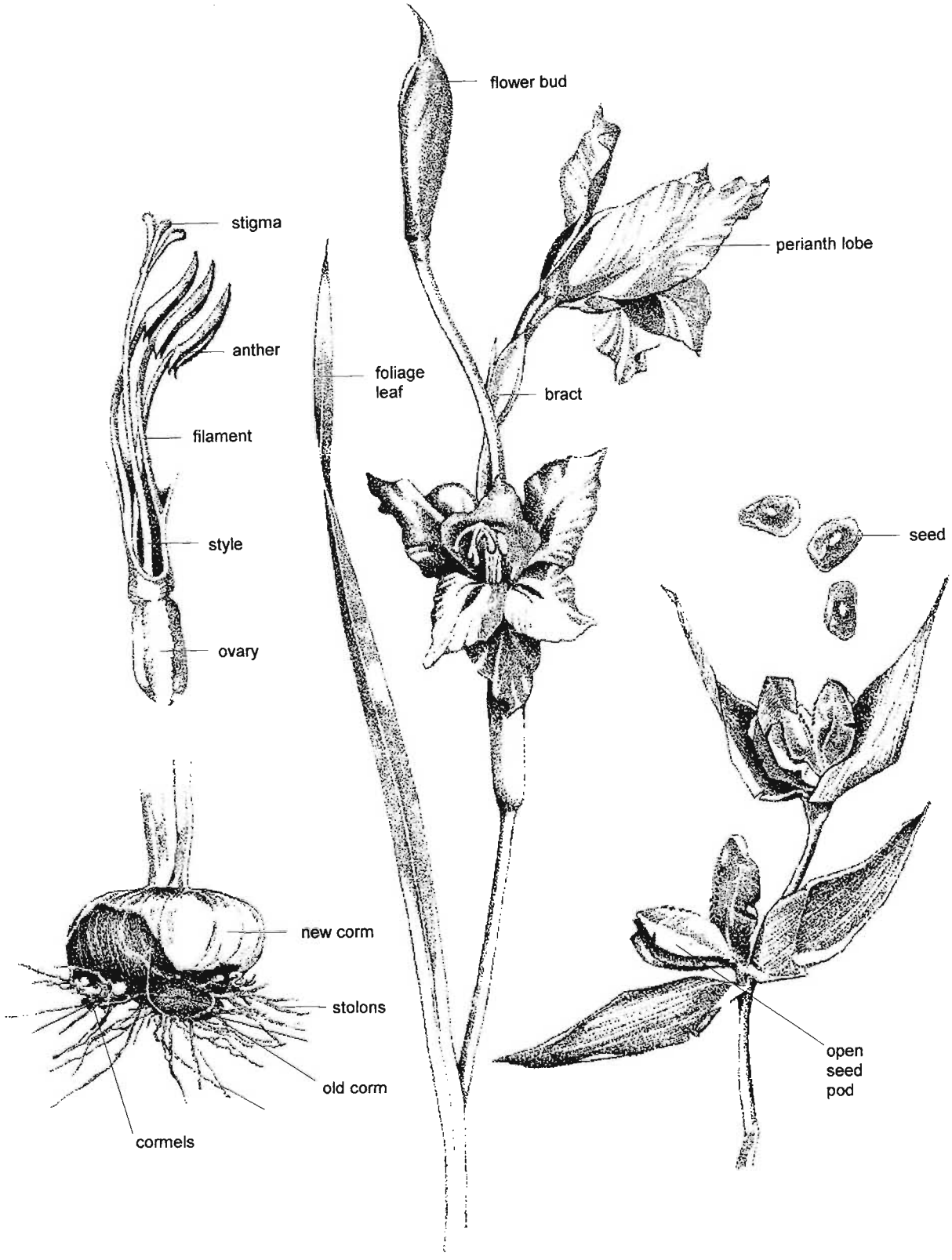


Figure 1.1 Schematic illustration of the morphology and anatomy of *Gladiolus* (adapted from Delpierre & du Plessis, 1973)

1.2.2 Growth and developmental cycle of summer flowering species

At the beginning of the growing season, after a period of cold storage during which corm dormancy is broken, the adventitious roots develop at the base of the corm (du Plessis & Duncan, 1989). Following water absorption, the apical regenerative bud emerges from the protective bud scales, 12 to 17 days after planting (Pfeiffer, 1931). The first foliage leaf emerges from the sheath approximately 20 days after planting (Buch, 1972). The foliage leaves emerge one at a time with subsequent leaves appearing from a split in the edge of the previous one (Delpierre & du Plessis, 1973). After planting, one or more of the lateral axillary buds may also sprout but this is dependant on cultivar and corm size (Buschman, 1998b; du Plessis & Duncan, 1989). Stolons form during the 4th to 6th leaf stage before spike emergence (Ju & MingFang, 2004).

Floral differentiation takes place only after the full number of leaves have been initiated (Shillo & Halevy, 1976a). It begins from the 2nd to 3rd visible leaf stage to the 6th to 7th visible leaf stage, 3 to 8 weeks after planting of mature corms (Buschman, 1998b; De Hertogh & Le Nard, 1993). During floral differentiation, the daughter corm begins to develop above the existing corm at the base of the stem (Delpierre & du Plessis, 1973; Salinger, 1985; Greving, 1992) as a result of cortical cell enlargement (Rees, 1992). This is formed by the inflation of the lower internodes of the stem which develop from the terminal bud (Halevy, 1986b) and contractile roots begin to develop at the base of this new corm (du Plessis & Duncan, 1989). During this time, the new corm becomes a strong sink and photosynthates from the mature leaves and mother corm are directed towards it (Robinson *et al.*, 1980).

The inflorescence stalk elongates rapidly once the 4th leaf is fully expanded (Shillo & Halevy, 1975; 1976a) and appears about 30 days before anthesis (Delpierre & du Plessis, 1973). Inflorescence formation is an autonomic process and floret development is acropetal. Flowers at different stages of development are found on the spike at any single time with fully developed florets at the base and primordia towards the top (Pfeiffer, 1931; Shillo & Halevy, 1976a). Waithaka *et al.* (2001) found that during the development of the inflorescence, there is a transfer of carbohydrates from the senescing lower florets to those developing acropetally. During inflorescence development, corm sink weakens and the inflorescence becomes the main sink until the end of flowering when the daughter corm assumes priority for assimilates yet again (Robinson *et al.*, 1980; 1983). Depending on the cultivar, anthesis occurs 60 to 90 days after planting (Armitage, 1993). Cormel growth occurs during a period

of about 5 weeks starting when flowering ends, although this is species dependant (Buch, 1972; Rees, 1992; Ju & MingFang, 2004).

After fertilization, the seeds develop and the capsule dehisces 4 to 6 weeks after anthesis (Delpierre & du Plessis, 1973). Seed formation may deplete corm reserves so that newly formed corms from seeded plants are smaller than corms from non-seeded corms (Pickell, 1972). Following seed dispersal, the above-ground parts begin to yellow, dry out and die. The dry flowering stem is shed and the corm enters a resting phase, the tiny subapical bud in the axil of a leaf base is then already formed and is ready to sprout at the beginning of the next favourable growing season (Goldblatt & Manning, 1998). If more than one bud on the old corm sprouted, then 2 or more daughter corms may also be produced, a process known as splitting (Halevy, 1986b). Roots normally senesce at this time (Salinger, 1985).

1.2.3 Factors affecting growth and development

1.2.3.1 Environmental effects

Light intensity: Gladioli need full sun to flower successfully (Rockwell, 1948; Barnhoorn, 1995; Hessayon, 1995). Insufficient light can result in a lanky, weak stem, too short spikes and flower desiccation due to a deficiency of assimilation products (Buschman, 1998b; Goldblatt, 1996). Plants at the 4th to 6th leaf stage (floral differentiation) are most sensitive to the prevailing light conditions (Shillo & Halevy, 1976b; Imanishi & Imae, 1990). A lower plant density means more light interception by the plants (Buschman, 1998b). Light is also important for the formation of contractile roots with higher light intensities for a longer period of time necessary for their formation (Halevy, 1986a; b).

Photoperiod: Shillo & Halevy (1976c) found *Gladiolus* to be a facultative short day plant and that the response to photoperiod is species dependant. However, it appears that flowers are initiated autonomously, although long days (LD) increase the flowering percentage, enhance floral quality, floret number per spike and spike length (Shillo & Halevy, 1981; JinQuan & QiXiang, 2003), but delays flower development and anthesis in some species. Short days (SD) directly promote corm growth and cormel formation, so that the photoperiodic effect can be envisaged as operating on the competition for assimilates between flower and corm (Shillo & Halevy, 1981; Rees, 1992; De Hertogh & Le Nard, 1993). SD also hasten anthesis, increase the incidence of flower blasting, decrease the number of florets per spike and the

height of the plants and reduce the number and size of the leaves (Shillo & Halevy, 1976c; McKay *et al.*, 1981). However, SD treatments have resulted in higher mineral contents in the spikes (JinQuan & QiXiang, 2003). The plants are more sensitive during the 1st to 5th leaf stage and the 4th to 6th leaf stage (Armitage, 1993).

Temperature: Temperature is also important and affects many growth processes (De Hertogh & Le Nard, 1993). Gladioli display optimum growth between 10 and 25 °C (Buschman, 1998b). Anything higher than 27 °C means a greater chance of flower desiccation and crooked stems (Buschman, 1998a). Shillo and Halevy (1976d) reported that gladioli are tolerant of high temperatures (up to 50 °C) as long as the air humidity and soil moisture are at an optimum. They also noted that plants are more sensitive to high temperatures from planting to the first leaf stage and that this damaging effect is indirect through its effect on plant water balance. Lewis *et al.* (1972) observed leaf elongation under hot conditions. Cool night temperatures, an average temperature below 10 °C and a long cold period, can cause an arrest in the growth and development of the plant (Buschman, 1998b; Imanishi & Imae, 1990; Armitage, 1993). Temperatures between 1 and 4 °C at any stage, increase the occurrence of flower blasting (Shillo & Halevy, 1976d). Gladioli do not tolerate temperatures below freezing point which causes cold damage and even perishing of the crop (Buschman, 1998b). Chilling damage has been observed at 2 developmental stages: immediately after planting and at the 7th leaf stage (Shillo & Halevy, 1976d). Halevy (1986a; b) found that contractile root formation requires a difference between day and night temperature.

Growth situation: Gladioli can be grown outdoors in open-field production, in a glass or plastic greenhouse or under shadecloth, however, when choosing the best site for planting the corms, the planting time and anticipated climatic conditions during the period of growth, must be considered (Maree, 2003). Protected cultivation has the advantage of being less dependant on unfavourable weather influences, but the reduction in light must be accounted for and the fact that crop and soil temperatures may be high during the summer months (Buschman, 1998b).

1.2.3.2 Growing medium

Any growing medium is suitable for cultivating gladioli as long as its structure and drainage are good (Rockwell, 1948; Hessayon, 1995; Buschman, 1998a). A poor structure will impair the flowering percentage and the quality of the gladioli (Buschman, 1998b). Compost, tree-bark, sand or gravel can be incorporated into a medium to improve drainage (Hancke & Verryn, 1989b). Many commercial growing mixes contain different ratios of perlite, vermiculite, bark, peat, sand, pumice, rockwool and other amendments (Hartmann *et al.*, 1997), depending on the growth requirements. The addition of pine bark to a growing medium improves aeration, water holding capacity, humidity, increases the amount of organic substances and percent of total nitrogen, produces excellent root growth and improves the growth and yield of *Gladiolus* plants (Grzeszkiewicz, 1978; Beardsell, 1999). Pine bark is not toxic to gladioli and composted bark is better as a soil conditioner because it has smaller bark particles which are easier to use in a soil mix (Grzeszkiewicz, 1978). The bark breaks down slowly and therefore maintains its structure for long periods in a container. It is also lightweight material and compares favourably with peat moss, mainly because it is more readily available worldwide and is less expensive (Beardsell, 1999). The best soils for outdoor field production are sandy loams and sands that allow for easy mechanical lifting without corm bruising and minimal losses of the cormels (De Hertogh & Le Nard, 1993; Bryan, 2002).

The growing medium or soil must be free of insects, nematodes and diseases (Buschman, 1998a), especially if it was used the year before. Therefore, it is recommended that it be fumigated or steam sterilized for the control thereof (Hancke & Verryn, 1989b). It is, however, more advisable to use a fresh medium for each new planting (Buschman, 1998b), as the best results are obtained with new or disinfected growing media or soils (De Hertogh & Le Nard, 1993).

Although growth medium and soil pH appears to play a minor role in the cultivation of some *Gladiolus* species (Delpierre & du Plessis, 1973), it is very important as it influences the availability and plant uptake of all essential plant nutrients (Ball, 1998). A high pH can lead to non-availability of some minor elements, for example, manganese and iron. A low pH can lead to toxicity (Parnes, 1990). The optimum pH for gladioli is between 6.5 and 7.0 (Hancke & Verryn, 1989b), whilst Greving (1992), Rees (1992) and Armitage (1993) reported that it is from 6.0 to 6.5. A pH lower than 5 results in a greater chance of fluorine damage. In this situation, the media or soil will need to be limed which should be done at least one month

before planting (Buschman, 1998b). If the pH is higher than 7, materials such as upgraded black peat, ammonium fertilizers (Buschman, 1998a) or sulphur-containing fertilizers can be used to decrease it (Follett *et al.*, 1981).

1.2.3.3 Nutrition

The nutrient requirements of gladioli in any growing situation are influenced by numerous factors such as media, pH, climatic conditions, corm size (small corms require 40 to 50 % more nutrients) and history of the previous nutritional status of the mother corm (Manrique, 1993; Hancke & Verryn, 1989b). Gladioli do not require heavy fertilization, as over fertilization could lead to harmful high salt concentrations (Hancke & Verryn, 1989b) and gladioli are salt sensitive plants. Gladioli corms contain sufficient mineral nutrients for good growth during the first few weeks and because the roots of newly planted corms are quite sensitive to high salt concentrations, a fertilizer application can best be postponed until some weeks after planting (Buschman, 1998b). Gladioli in sandy soils require more frequent fertilization than those in heavier soils (Armitage, 1993).

Proper nitrogen (N), phosphorus (P) and potassium (K) applications are essential for longevity of the cut spikes (Deswai & Patil, 1983; Amen & Kovanci, 1990). Fertilizer with an NPK formula can be used (Hancke & Verryn, 1989b). Generally, gladioli seem to require NPK applications of 90 to 135, 40 to 80, and 91 to 150 kg ha^{-1} respectively (Manrique, 1993). De Hertogh and Le Nard (1993) reported that the mineral requirements have been determined to be 122 kg ha^{-1} of N, 36 kg ha^{-1} of P_2O_5, 257 kg ha^{-1} of K_2O, 150 kg ha^{-1} of CaO and 34 kg ha^{-1} of MgO. The fertilizer can be applied as a liquid or in a granular form (Rix, 1983; Armitage, 1993). The phosphate fertilizer may be scattered before planting (Buschman, 1998b). During the first two-and-a-half months, a ratio of 3N to 2K can be applied and a ratio of 2N to 3K is then applied during the last phase. When corms are small, half of the fertilizer can be applied before planting, a quarter of the total fertilization before flowering and the last quarter after the flowers have been harvested (Hancke & Verryn, 1989b). Alternatively, 1 kg of 5-10-10 per 100 row feet can be applied before planting and as a side-dressing about 1 month after planting, as this results in sturdier stems. An additional side dressing may be used when the spikes are visible but is probably not needed in clay/loam soils in open-field production (Armitage, 1993).

Nitrogen influences the formation of daughter gladioli corms (Hancke & Verryn, 1989b). Too much N may increase the risk of infestation by *Fusarium* in gladioli. Dressing at a time when no N is taken up by the plant will result in losses, mostly by leaching of NO_3^- (Groen & Slangen, 1990). Lack of N appears to be a factor in causing blind plants (those failing to produce flowers) and can also seriously reduce the number of florets per spike and / or result in shorter spikes (Woltz, 1955; Wilfret, 1992). Plants deficient in N become light green in colour, beginning with the older leaves, as this element is an important component of the chlorophyll molecule (Ball, 1998). If injecting nitrogenous fertilizers into the irrigation water, the plants must be adequately watered thereafter to cause some leaching and prevent excess nitrate and soluble salt buildup (Ball, 1998).

Phosphorus is important for root system development, rapid growth and floral quality (Ball, 1998) and stimulates early flower formation (Hancke & Verryn, 1989b). Deficiency symptoms include slow root and top growth and in severe cases the foliage turns purple (Ball, 1998). Phosphate fertilizers containing fluoride, such as triphosphate, must be avoided as gladioli are damaged by fluoride (Buschman, 1998b). Woltz and Marousky (1975) found that regular superphosphate applications caused the greatest amounts of damage followed by triple superphosphate and then dicalcium phosphate. Visible damage is expressed primarily in characteristic patterns of leaf scorch (Woltz & Marousky, 1975). Therefore phosphate fertilizers poor in fluoride are preferred (Buschman, 1998b).

Potassium influences the size of the corms (Hancke & Verryn, 1989b). K applications during the growing period have a tremendous effect on flower emergence and significantly increase flowering and stem length (Aouichaoui & Tissaoui, 1989). Visible deficiency symptoms include marginal yellowing or chlorosis of the oldest leaves and spotting over the entire leaf (Ball, 1998). Woltz (1955) found that deficiencies also result in shorter spikes and appears to delay flowering. Quality and vase life of *Gladiolus* spikes are influenced by the source and level of K application in the field. The maximum number of flowers per spike have been obtained using K_2SO_4 both at low (4 g m^{-2}) and high (12 g m^{-2}) levels. Maximum flower diameter was obtained with a medium (8 g m^{-2}) application of K_2SO_4 (Roychowdhury & Roychowdhury, 1995).

Calcium (Ca) availability for plant uptake depends on growth medium pH and the levels of other cations present (Ball, 1998). Gladioli require a calcium (Ca_2O) concentration of 900 to 1680 kg ha^{-1} (Hancke & Verryn, 1989b). Ca deficiencies seem to lower the average floret

count, result in shorter spikes and reduce the mass of the spikes (Woltz, 1955). A low Ca content of the spikes may also cause physiological bud rot and stem topple (breaking over of the cut spike) (Wilfret, 1992; De Hertogh & Le Nard, 1993). To effectively change the available Ca level (and medium pH), lime must be thoroughly mixed into an adequately moist growth medium (Ball, 1998).

Magnesium (Mg) deficiencies can delay flowering (Woltz, 1955) and are exhibited as interveinal chlorosis of the older leaves (Wilfret, 1992; Ball, 1998). Adjusting the medium pH with dolomitic lime also increases the presence of available Mg (Ball, 1998).

Essential micronutrients include ***manganese*** (Mn), ***boron*** (B), ***iron*** (Fe), ***copper*** (Cu) and ***zinc*** (Zn). They may be applied to the media or sprayed on the foliage. B, Fe and Cu deficiencies are known (Salinger, 1985; De Hertogh & Le Nard, 1993). B deficient florets produce petals which fail to grow and expand normally, with the pistils extending past the florets (Woltz, 1955). It also results in a horizontal cracking of the leaves and a blunt curving of the leaf tip (Salinger, 1985). Effective Fe-containing fertilizers seem to be important for flowering, corm growth and cormel formation in gladioli, although corm and cormel growth and development are more sensitive to Fe deficiency than the flowers (Chen *et al.*, 1982/83). Cu deficiency is evident as an abnormal wilting of the leaves (Salinger, 1985). $ZnSO_4$ fertilizers have shown to increase *Gladiolus* spike length and the number of flowers per spike (Singh & Singh, 2004).

1.2.3.4 Watering

Gladioli are sensitive to water stress, showing a differential response between corm and flower growth (Salunkhe *et al.*, 1990; Manrique, 1993). Generous amounts of water must be provided on a regular basis to the root level from planting time and throughout cultivation, or they will do poorly and might not bloom at all (Rockwell, 1948; Barnhoorn, 1995; Buschman, 1998a). Robinson *et al.* (1983) found that a water deficit in gladioli, even to the slightest degree, decreased the assimilate mobilizing ability of the inflorescence, increased that of the corm and delayed translocation out of the leaves. A decrease in soil moisture certainly reduces flowering at most stages of development (Shillo & Halevy, 1976d).

Water requirements are high between planting and emergence (De Hertogh & Le Nard, 1993). The soil should be well moistened a few days before planting so that the corms are planted in a sufficiently moist but not overly wet soil (Buschman, 1998b) and are thus able to absorb water faster after planting (Buschman, 1998a). Watering during the first and second week after planting can then be reduced to a minimum or even omitted as this allows the soil to retain its structure so that the rooting of the corms can proceed undisturbed (Buschman, 1998b). Two growth stages have been identified when gladioli are more sensitive to water stress: from emergence to flower initiation, during which the contractile roots are also formed (Shillo & Halevy, 1976d; De Hertogh & Le Nard, 1993) and from spike emergence, through elongation to anthesis (Shillo & Halevy, 1976d; Manrique, 1993).

Halevy (1972) found that the flowers react differently to water stress according to their stage of development. Young flowers, up to about 2 days prior to the opening of the first floret, were more sensitive to water stress than older flowers and were the first to wilt with water being translocated to the young leaves.

The method of irrigating the plants is important. Many growers use overhead irrigation, however, this method can cause soil compaction from too large droplets and the free water on the plants can cause diseases. Therefore drip irrigation is preferable (Hancke & Verryn, 1989b). Some growers use trickle irrigation (Buschman, 1998a) and still others use controlled subirrigation (Ball, 1998).

Frequency of irrigation will depend on weather conditions and corm size. The quantity of water applied can be increased weekly up to and including time of flowering, after which it can be reduced just before the corms are lifted (Hancke & Verryn, 1989b).

1.2.3.5 Planting out

Only healthy corms must be planted and each corm must have a bud (Hancke & Verryn, 1989b). The corms should be disinfected before planting for 10 to 15 minutes depending on the disinfectant used (Buschman, 1998a). They must either be dusted or dipped in a fungicide (Greving, 1992). For open-field production, gladioli can be planted in ridges or in a bed. If the soil is heavy and if irrigation is to be used, planting in ridges is preferable as this will allow for easier lifting of the corms (De Hertogh & Le Nard, 1993; Buschman, 1998a). Beds are suitable for sandy soils (De Hertogh & Le Nard, 1993).

The planting depth is important: planting too shallow can cause wind damage and dessication and corms planted too deeply do not form contractile roots. Planting depth will depend on corm size and soil moisture content (Buschman, 1998a). As a rule, planting depth will be slightly shallower on heavy soils than on lighter soils (Buschman, 1998b). A final planting depth of 5 to 10 cm seems to be the most suitable (Buschman, 1998a) or a depth about 3 times the corm height (du Plessis & Duncan, 1989; Duncan, 1996). The corms must be planted with the pointed side facing upwards and the scar side down (Barnhoorn, 1995).

Spacing and plant density is also important as this affects the firmness of the plants and the flower quality. The size of the corms and the cultivar used are determining factors (Hancke & Verryn, 1989b) as well as available light, temperature and desired floret quality (Buschman, 1998a). The probability of disease increases with denser plantings. Two to four times the corm diameter is a good spacing rule, or about 15 cm apart (Barnhoorn, 1995). A wider spacing of 20 x 30 cm has produced significantly superior results in terms of growth, flowering and corm production (Pillewan *et al.*, 2004).

The use of support wire (Buschman, 1998b) or stakes (Rockwell, 1948; Greving, 1992; Barnhoorn, 1995; Bryan, 2002) during growth is recommended. Supporting the plants early on can lead to good growth all season as it will ensure tall, straight spikes. However, when staking is done, the flower spike must still be allowed some movement to prevent damage and breakage (Greving, 1992).

1.2.3.6 Pests and diseases

Gladioli are excellent hosts to a large number of pests and diseases (Littlejohn & van der Walt, 1992; Wilfret, 1992). In many areas, thrips (*Thrips simplex*) is the major insect pest of *Gladiolus* (Greving, 1992; Barnhoorn, 1995). They cause streaky stems from their sap sucking and affected leaves and flowers have small white spots and streaks, whilst corm surfaces develop rough, grey-brown patches (Delpierre & du Plessis, 1973; Rees, 1992). Aphids are a problem because of the damage they cause and because they are also vectors for non-persistent viruses (Adams *et al.*, 1975; Ball, 1998). They are usually found on developing foliage and flower buds (Duncan, 1996). Red spider mites may also attack during hot, dry weather (Barnhoorn, 1995) and cause localised yellowing of the plant. Other pests include a variety of caterpillars, beetles, cutworms and eelworms, whitefly, mites and mealybugs. These pests can all be combatted with a wide selection of pesticides (Delpierre

& du Plessis, 1973), reflective mulches (Smith *et al.*, 1972) and with careful and hygienic horticultural practices (Hartmann *et al.*, 1997).

Nematodes have been known to occur in soil surrounding *Gladiolus* roots. They injure the plants by direct feeding or by exposing the plants to attack by other pathogens (De Hertogh & Le Nard, 1993).

Dangerous air-borne fungi affecting gladioli include rusts (Uromyces) which develop as tiny yellow pustules on leaf surfaces (Ferreira, 1988; Bryan, 1989). The soil-borne fungus *Fusarium oxysporum* is considered to be a very serious threat to *Gladiolus* cultivation in most areas (Wilfret & Woltz, 1973; Remotti *et al.*, 1997; Mishra *et al.*, 2000; Pathania & Misra, 2003). The symptoms are basal corm-rot with nectrotic spots, deformed, blind plants and reduced flower production (Wilfret, 1992; Remotti & Loffler, 1996). *Botrytis gladiolorum* (*Sclerotinia draytonii*) is a rot that infects the corm first then spreads to the above ground portions causing them to go yellow, then brown. The spores produce light to dark brown lesions on foliage and flowers (Moore, 1979; Buschman, 1998b; Rees, 1992; Lorbeer *et al.*, 2004). Dry rot (*Stromatinia gladioli*) is another serious pathogen that causes the well-known yellowing of leaves, followed soon by browning and the rapid death of the plant. Small brown lesions on the corms develop into dry rot and shrivelling during storage (Buschman, 1998b). Protective spraying is advisable in order to avoid and adequately control these diseases. Soil fumigation and / or sterilization is also recommended, however, corms can only be planted 3 weeks later (Delpierre & du Plessis, 1973; Ball, 1998). Bio-control agents have been used with some success (Vaidya *et al.*, 2004). Alternatively, corms can be hot water treated (Hancke & Verryn, 1989a).

Gladioli worldwide suffer from bacterial scab (*Pseudomonas marginata*) which causes lesions on the corms, attacks leaf bases within the outer sheathing base, visible as reddish-brown spots, and finally causes the collapse of the plant with typical neck rot. Bacterial leaf blight is also a problem (Rees, 1992). It is referred to as 'fire' because of the fire-like symptoms present on the leaves of the spikes (Lorbeer *et al.*, 2004). Prevention can be achieved by dipping the corms in a fungicide or sterilizing the soil. Affected corms should be destroyed (Buschman, 1998a).

Viruses can also infect gladioli (Armitage, 1993; Asjes, 1997). Symptoms include colour breaking, flower malformation, leaf mosaics, mottling, dwarfing, plant and corm deformation or a lack of visible symptoms altogether (Stein, 1995; Mangal *et al.*, 2003). Unfortunately, little can be done against viral infection apart from controlling the vectors of these diseases and ensuring balanced fertilization to produce healthy and hence more resistant plants. Infected plants should be burned (Duncan, 1996). The use of virus-indexed corms is highly recommended (Stein *et al.*, 1988).

1.2.3.7 Forcing of gladioli corms

Forcing, by definition, is the flowering of a bulbous plant using other than naturally occurring conditions (De Hertogh *et al.*, 1983). The forcing ability of a geophyte, is its ability to produce a fully-grown flowering plant from a pre-cooled bulb within a reasonably short time (Sparnaaij *et al.*, 1985). The ultimate goal commercially is year round forcing, allowing a constant supply of high quality cut flowers to consumers. Hence, for some markets, the flowering process must be accelerated, whilst for others it must be retarded until the flowers are required (Waithaka, 1986; De Hertogh, 1997).

Many factors influence the growth, development and flowering of bulbs and are thus important considerations in the forcing process. These include temperature, moisture, nutrient levels, light (photoperiod, intensity and quality), planting medium composition, plant growth regulators and pests and diseases (De Hertogh, 1997; 1999). Bulbous plants continuously monitor their environment whether dormant or actively growing. Thus it is important that they are handled carefully and correctly at all times, even more so during the forcing process (De Hertogh, 1977; 1989). Each season of the year brings about a different response from the *Gladiolus* corms and this provides a guide for the manipulation of the corms during forcing (Table 1.1).

To integrate the knowledge of the bulb reproduction cycle and the influence of external factors, forcing systems are usually divided into 4 phases:

Marketing and consumer phase: Forcers must always start with this phase to allow subsequent management of the crop for the selected market (De Hertogh, 1989). The primary industry focus for forced flower bulbs must be to provide consumers with maximum satisfaction of the marketable product (De Hertogh, 1997).

Production phase: This encompasses all aspects involving the production of high quality, problem free corms suitable for forcing (De Hertogh, 1977; 1989; 1999) through propagation, harvesting, handling and storing of the corms prior to forcing (De Hertogh & Le Nard, 1993).

Programming phase: This is a complex phase requiring the control of many environmental factors (De Hertogh, 1997) (Table 1.1). The most crucial step in this phase is a low temperature treatment to root the corms and mobilize them to flower. In gladioli, low temperatures of 10 °C or less break dormancy more easily (De Hertogh & Le Nard, 1993). Waithaka (1986) found that *Gladiolus* corms require at least 8 weeks at 3 to 5 °C for dormancy release and Littlejohn and van der Walt (1992) note that the natural flowering time of gladioli can be shifted with a cold treatment at 5 to 8 °C for 8 to10 weeks after harvesting. Dormancy of gladioli corms can also be shortened with ethylene (Halevy *et al.*, 1970), by soaking corms in 20 ppm benzyladenine (BA) followed by 100 ppm gibberellic acid (GA) (Tsukamoto, 1979), with ethanol (Hosoki, 1983), with a 4-hour treatment of methyl disulfide (Hosoki & Kubara, 1989) or with 400 mg l^{-1} ethephon (Ram *et al.*, 2002). The standard forcing technique is to place the corms in controlled temperature rooting rooms thus manipulating the corms to obtain year-round control of the corm cycle (De Hertogh & Le Nard, 1993). The corms are placed in pots, flats or plastic trays in a suitable medium (De Hertogh, 1989; 1999) which most commonly consists of varying parts of soil, sand, peat, perlite, vermiculite, bark or other ammendments (De Hertogh, 1977). Gladioli prefer a deep, well, drained medium at a pH of 6.0 to 6.5 (De Hertogh, 1989).

Greenhouse phase: After the rooting and cooling requirements have been satisfied, the corms are transferred to the greenhouse where the corms are subjected to warm temperatures which promote organogenesis and sprouting and thus result in elongation of the flower stalk and anthesis (Groen & van de Rotten, 1980; De Hertogh & Le Nard, 1993). Regulation of the light intensity and photoperiod are essential (De Hertogh, 1989). Light intensity is very important in gladioli. Long days delay blooming but increase the flowering percentage and enhance flower quality (Shillo & Halevy, 1976b; 1976c). Low light in winter can lead to blindness and reduced flower quality (Imanishi & Imae, 1990). Proper watering and fertilizing practices and control of pests and diseases are essential for the production of high quality plants (De Hertogh, 1989). The greenhouse phase can be reduced by using gladioli varieties for which the number of days between planting and flowering is normally low (Groen & van de Rotten, 1980).

***Table* 1.1 Forcing of summer flowering *Gladiolus* corms showing seasons and important events (adapted from De Hertogh, 1977)**

Programming Phase			Greenhouse phase
Autumn	**Winter**	**Spring**	**Summer**
Harvesting of corms	Resting phase, induction of floral development under cool temperatures	Planting out, rooting and mobilization for flowering under mild temperatures	Flower stalk elongation and flowering under warm temperatures

1.2.3.8 Postharvest aspects

Handling of the spikes

Gladiolus spikes can be harvested 60 to 100 days after planting, depending on the cultivar and time of year (Wilfret, 1992). They should be cut early in the morning or evening for maximum freshness (Greving, 1992). Spikes must be cut when all the florets are still in the tight-bud stage with the lower 2 or 3 buds beginning to show colour (Hancke & Verryn, 1989a; De Hertogh & Le Nard, 1993; Ball, 1998; Bryan 2002) and semi-opened (Meir *et al.*, 1995), leaving 2 to 3 leaves on the remaining stem for corm development (Wilfret, 1992). The stems must be cut at an angle with a sharp knife as this does not compress the stem which prevents water uptake (Hancke & Verryn, 1989a). The stems should be placed in warm, fluoride-free (deionised) water (Armitage, 1993; De Hertogh & Le Nard, 1993) immediately after cutting to prevent air bubbles entering the vascular tissue and to keep the florets from wilting (Hancke & Verryn, 1989a; Greving, 1992). About 20 mm must be removed from the stems which have been left out of water for 10 minutes or more (Hancke & Verryn, 1989a).

Cut *Gladiolus* spikes should be kept cool and in a moist environment while sorting and before and after packing (De Hertogh & Le Nard, 1993). They should be packed in bunches of 10 between layers of paper or plastic to prevent damage and then placed in cartons to prevent

excessive moisture loss (Hancke & Verryn, 1989a). Spikes can also be placed in polyethylene bags which are removed after storage (Nowak & Rudnicki, 1990). They should be stored at 2 to 5 °C for 5 to 8 days at a humidity of 90 to 95 % (Buschman, 1998b; Nell, 1992) as the low temperature hardens the stems (Hancke & Verryn, 1989a). Temperatures below 1 °C usually result in chilling injury (Salunkhe *et al.*, 1990). Gladioli are very susceptible to grey mould during storage (Nowak & Rudnicki, 1990). They must also be kept upright to prevent curving or crooking of stems and the expression of negative geotropism (Wilfret, 1992), although this has been found to be reduced by packing the spikes with ice (Song *et al.*, 1992). The use of special boxes or other packaging that hold spikes vertically should be used for transporting these flowers. It must be ensured that there is appropriate air circulation in the cold storage room to maintain a uniform temperature and adequate gaseous exchange (Nowak and Rudnicki, 1990). After storage, stem-ends should be recut before placement in solution (Nowak & Rudnicki, 1990; Roychowdhury & Sarkar, 1995).

The typical life of a *Gladiolus* spike placed in water, is 4 to 6 days (Marousky, 1968; Mayak *et al.*, 1973). However, the use of preservatives does appear to prolong the vase life of the flowers , improve quality and prevent desiccation (Hancke & Verryn, 1989a; Salunkhe *et al.*, 1990). The length and weight of the spike as well as the number, size, shape and colour of the florets, are some of the criteria used for determining the quality of cut gladioli (Salunkhe *et al.*, 1990). As the florets fade, they must be removed to keep the gladioli looking their best (Greving, 1992). However, because the dead flowers are unattractive, senescence of the bottom florets does mark the end of the flower spike's commercial display life (Serek *et al.*, 1994).

Waithaka *et al.* (2001) found that the opening of *Gladiolus* florets requires the import of carbohydrate, most importantly fructose with substantially lower concentrations of glucose and sucrose. Increases in the life of *Gladiolus* flowers have been obtained by pulsing with sucrose or using vase preservatives containing sucrose (Marousky, 1971; 1972; Singh *et al.*, 2004). Anserwadekar and Patil (1986) observed that 6 % sucrose was a good solution for prolonging the vase life of *Gladiolus* spikes, whereas Mayak *et al.* (1973) and Bravdo *et al.* (1974) found that higher sugar concentrations (up to 50 %) improve the opening and size of the florets and increase flower spike longevity, the amount of solution absorbed decreasing with increasing solution concentration. Treatment with 0.1 M trehalose, a disaccharide consisting of 2 linked glucose units, has been found to prolong vase life and improve spike quality whereas inhibitors and other sugars had no effect (Otsubo & Iwaya-Inoue, 2000; Yamane *et al.*, 2005).

Pulsing immediately after harvest with 20 % sucrose in combination with silver nitrate ($AgNO_3$), a known biocide, for 20 hours at 21 °C, has also been successfully used (Kofranek & Halevy, 1976). Apelbaum and Katchansky (1977) found that the immersion of the cut stems for 24 to 72 hours in a solution containing sucrose and thiabendazole, facilitated opening, improved quality and prolonged the vase life of gladioli. Murali (1990) and Murali and Reddy (1993) found that sucrose, in combination with metal salts, increases gladioli vase life from 19 to 76 %, enhances the water uptake by the cut spikes and extends the time of a positive water balance within the cut flowers with a significant increase in flower fresh mass which was a maximum with sucrose and cobalt (Co). Some metal salts appear to overcome water stress through their effect as a germicide. Roychowdhury and Sarkar (1995) noted that nickel chloride ($NiCl_2$) at 500 ppm as a vase solution, gave the best preserved quality and increased the longevity of *Gladiolus* spikes when compared to sucrose at 2 % and 4 %, distilled water and other metal salts.

Marousky and Woltz (1971) found that the opening and size of the florets and longevity of the spikes are improved by holding them in a 600 ppm solution of 8-hydroxyquinoline citrate (8-HQC) and 4 % sucrose. Another solution used very successfully for gladioli is 50 ppm silver nitrate ($AgNO_3$), 300 ppm aluminium sulphate, 250 ppm 8-hydroxyquinoline sulphate (8-HQS) and 2 % sugar. Spikes are kept in this solution for 18 hours at 2 °C before the flowers are packed (Hancke & Verryn, 1989a). Iron sulphate ($FeSO_4$) sprays have also had a beneficial effect on vase life of gladioli spikes (Bala *et al.*, 2002).

A pulsing solution containing 20 % sucrose, 50 ppm $AgNO_3$, 300 ppm aluminium sulfate ($Al_2(SO_4)_2$) and 250 ppm (8-HQC), used for an 18 hour pulse, was better for *Gladiolus* than either Physan 20 (a quaternary ammonium compound) or silver thiosulphate (STS) plus sucrose. An 8 minute STS treatment gave a good response but anything above a 50 minute pulse was phytotoxic (Farhoomand *et al.*, 1980). Meir *et al.* (1995) observed that pulsing spikes with sucrose and STS prior to packing further improved flower quality and opening. The addition of STS to the pulsing solution doubled the levels of glucose and fructose in all florets, suggesting that STS may improve sucrose uptake and its subsequent hydrolysis. STS appears to have an antimicrobial effect on the cut spikes (Al-Humaid, 2004) and may also reduce the detrimental effects of carbohydrate depletion in flowers (Serek *et al.*, 1994). Barman *et al.* (2004) successfully used a pulsing solution of STS followed by holding in $AgNO_3$ to delay spike bending and improve floret opening and vase life in spikes of *Gladiolus* cv Eighth Wonder.

The involvement of ethylene in flower senescence of gladioli has been evidenced with treatments with ethylene synthesis inhibitors (Rudnick *et al.*, 1986) and the favourable effects of hypobaric storage (Staby *et al.*, 1982). 'Green Keeper', an ethylene absorber, was found to be effective in delaying *Gladiolus* flower wilting, thus prolonging their vase life and enhancing flower opening (Merodio & de la Plaza, 1989). Murali and Reddy (1993) found that a treatment with metal salts reduced ethylene evolution in *Gladiolus* spikes. In contrast, Serek *et al.* (1994) found that gladioli were not sensitive to ethylene found in the air surrounding the flowers, thereby suggesting that gladioli are ethylene-insensitive geophytes.

Carbohydrate analysis

In one form or another, carbohydrates make up the bulk of the organic components of the natural world (Smith, 1993). They are the primary energy-storing molecules in most living things and are formed from smaller molecules known as sugars. There are 3 kinds of carbohydrates, classified according to the number of sugar molecules they contain: monosaccharides (single sugars), disaccharides (2 monosaccharides joined together) and polysaccharides (several monosaccharides linked together in a long chain) (Raven *et al.*, 1992). In all plants, the major reserve carbohydrates are disaccharides and polysaccharides (of the monomers glucose and fructose) (Smith, 1972), the most important being sucrose, the form in which most sugar is transported in plants (Raven *et al.*, 1992; Proctor *et al.*, 1996).

The role that these sugars play in improving the vase life and quality of cut *Gladiolus* spikes, appears to be linked to their ability to maintain turgidity, thereby improving solute uptake, providing an energy source for respiration and supplementing sugars which are rapidly used by the florets (Marousky, 1968; Mayak *et al.*, 1973; Bravdo *et al.*, 1974; Kofranek & Halevy, 1976; van der Merwe *et al.*, 1986; Gowda & Gowda, 1990; Rekha *et al.*, 2001).

A large number of analytical techniques have been developed to measure the presence and concentration of sugars in plant material. Each procedure is detailed but most methods are based on the oxidation of the sugars with an alkaline solution of metal salts. The reduced metals are then determined by various means (Ting, 1956; Smith, 1972). Analytical methods for sugar determination include gas chromatography (Li & Schuhmann, 1981; Dashek, 1997), liquid chromatography (Palmer & Brandes, 1974; Brushwood, 2000), thin-layer chromatography (Vomhof & Tucker, 1965; Shanahan, 1991), enzymatic techniques (Johnson

et al., 1964; Gamble, 2001), titrational methods (Smith, 1981), and colorimetric and spectrometric methods which include the ferricyanide (Ting, 1954; Haslemore & Roughan, 1976), Somogyi (Somogyi, 1945; Furuholmen *et al.*, 1964), phenol sulphuric acid (Dubois *et al.*, 1956; Buysse & Merckx, 1993) and the anthrone (Yem & Willis, 1954; Jermyn, 1975) methods.

1.2.3.9 Harvesting and storage of gladioli corms

Gladioli corms can be left in the ground over winter, provided they are kept cool and dry, but they should preferably be lifted, cleaned and kept in storage during dormancy to prevent disease problems (du Plessis & Duncan, 1989; Greving, 1992). They should be removed after the foliage has declined, approximately 8 weeks after flowering. Any foliage still attached to the corms should be removed and the corms then placed in trays with no more than 4 layers of corms in each tray (Armitage, 1993). To help prevent storage problems, corms should be placed in a light, warm, well-ventilated place for several weeks for curing to remove excess corm moisture for them to husk as rapidly as possible. The old corm must be removed from the new corm as well as any loose husks, leaving the wrapper husks still intact (Greving, 1992). Curing should be done for 10 to 15 days at 22 to 25 °C (Armitage, 1993). The corms are often graded before storage by measuring the corm diameter (not the circumference) (Rees, 1992). The corms should then be placed in a dark, cool, dry, well-ventilated location (Greving, 1992) and stored for a minimum period of 6 weeks at 4 to 8 °C, with 70 to 75 % relative humidity, during which corm dormancy is broken (Hancke & Verryn, 1989a; Armitage 1993). The corms must not be stored with ethylene producing plants (Kamerbeek & de Munk, 1976).

1.3 PROPAGATION PRACTICES

1.3.1 Seed

Despite the capacity for gladioli to reproduce vegetatively, sexual reproduction and the creation of new plants by seed production and dispersal are essential for long-term survival through population variability, stability and the establishment of new colonies (Goldblatt & Manning, 1998).

The seeds of summer flowering gladiolis should naturally be planted out in spring, however, seeds of most bulbous plants are adapted to withstand a dry season so they tend to keep their viability for a few years without special treatment. They will, however, germinate better if sown fresh (Hessayon, 1995). Seeds should generally be placed about 2.5 cm below ground level. They should be slightly shaded and protected from excess drying, even in summer (Rix, 1983; Bryan, 2002).They normally take from 17 to 21 days to germinate (McLean, 1941; Pickell, 1972). The first leaves that emerge are thin and grass-like and should be kept green for as long as possible. (Rix, 1983). These young plants will take 2 to 3 years to flower (Hessayon, 1995).

South African gladioli species, all naturally develop a waxy, oily wing surrounding each seed (Lewis *et al.*, 1972; Goldblatt & Manning, 1998). This wing is thought to delay the germination of the freshly harvested seeds (Griesbach, 1972), however, this becomes useful during adverse germination conditions when a delay in germination gives the developing seedling a better chance of survival later on (Mayer & Poljakoff-Mayber, 1982; Venter & Venter, 1996; Baskin & Baskin, 1998). Carpenter *et al.* (1991) advises that the seeds be 'dewinged' before planting.

Carpenter *et al.* (1991) found that *Gladiolus grandiflora* seed germination was light-independent but temperature influenced the germination rate. Constant 20 °C promoted higher total germination (97 %), fewer days to 50 % of final germination, and a shorter span of days between 10 and 90 % germination than other constant temperatures. At 20 °C, 14 to 21 days are required to germinate 50 to 70 % of the seeds (Pickell, 1972).

Seed viability is an important aspect of seed quality and is a measure of how many seeds are alive and are able to develop into normal plants. It is usually expressed as a percentage

germination (Anon, 2005). There are numerous tests available for measuring seed viability, the most important being the standard germination, excised embryo (Hartmann *et al.*, 1997) and tetrazolium tests (Young & Young, 1986; McDonald & Copeland, 1989).

Seed vigour is defined by the Association of Official Seed Analysts (1983) as "those seed properties which determine the potential for rapid, uniform emergence and the development of normal seedlings under a wide range of field conditions". Seed dormancy may obscure seed vigour potential but should not be regarded as a component if vigour is unaffected in field sowings (Perry, 1987a). Vigour tests include seedling growth rate tests (incorporating the slant board (Smith *et al.*, 1973; Geneve, 2004) or rolled towel tests (Hartmann *et al.*, 1997), the cold test (McDonald, 1980; Fiala, 1987b), the accelerated aging test (Baskin, 1987; Jianhua & McDonald, 1996), the electrical conductivity test (McDonald, 1980; Matthews & Powell, 1987b), the controlled deterioration test (Matthews & Powell, 1987a), tetrazolium tests (Fiala, 1987a; Perry, 1987b) and the use of computer-aided analysis of digital images (Sako *et al.*, 2001; Geneve & Kester, 2001; Oakley *et al.*, 2004). There is currently no industry standard for flower seed vigour testing (Geneve, 2004).

The moisture content of seeds is also an important aspect of their longevity. It can be defined as the loss in weight when seeds are dried and is expressed as a percentage of the weight of the original sample (International Seed Testing Association, 1999). Seed moisture can be determined through the bite test, oven drying tests, using the Karl Fischer reagent or infrared balances (Young & Young, 1986).

1.3.2 Cormels

Cormels have the structure of corms but are much smaller and possess a hard tunic (De Hertogh & Le Nard, 1993). A single large *Gladiolus* plant can produce 100 or more cormels. The need for artificial means of propagation is therefore largely dictated by the natural rate of plant growth. Removal of the inflorescence as soon as it appears, doubles the yield of cormels (Rees, 1992). They vary in size and are usually graded accordingly (Manrique, 1993). Cormels should be planted in well-drained soil or growing media at a depth of 8 cm (Rees, 1992) and kept constantly moist because their roots are fairly shallow (Hancke & Verryn, 1989b). Their water requirements are high between planting and emergence because of their shallow planting depth and they have a long duration of sprouting (De Hertogh & Le Nard, 1993). Cormels give rise to a single shoot and they will form small corms the following year (Greving, 1992).

1.3.3 Micropropagation

The tissue culture of higher plants can be defined as the culture of plant parts on nutrient media under sterile conditions (Pierik, 1988). *In vitro* propagation may allow for virus elimination and the rapid increase of pathogen-free *Gladiolus* plants (Wilfret, 1971; Logan & Zettler, 1985; Ziv & Lilien-Kipnis, 1997; Mangal *et al.*, 2003; Dhane *et al.*, 2005). It is especially useful for producing bulbous plants because they are otherwise generally slow to multiply (Sutter, 1986). Other advantages include the culturing of new species for breeding programmes, germplasm preservation, the removal of pressure of a species from its natural habitat and its ultimate commercialization (Wilfret, 1971; Dickens *et al.*, 1986; Ball, 1998). However, the potential use of micropropagation *in vitro* for mass production of plants is currently limited by high costs due to intensive manual handling and low survival rate *ex vitro* (George & Sherrington, 1984; Ziv, 1992).

The following techniques have been described for the micropropagation of gladioli (Nicol, 1993): (i) meristem, shoot tip and single node cultures, involving axillary bud enlargement and proliferation (Hussey, 1976; Logan & Zettler, 1985; Dhane *et al.*, 2005), (ii) organ culture, involving the isolation of entire structures (Bajaj *et al.*, 1982/1983), (iii) direct organogenesis from corm and young inflorescence stalk explant surfaces (Ziv *et al.*, 1970; Sutter, 1986; Ziv & Lilien-Kipnis, 1997) and (iv) initiation of *Gladiolus* callus cultures from which (v) indirect regeneration of plantlets (Ziv *et al.*, 1970; Remotti & Loffler, 1995; DaeHoe *et al.*, 2003; Suzuki *et al.*, 2005; Kasumi *et al.*, 2005) or (vi) suspension cultures (Kamo *et al.*, 1995; Lipsky *et al.*, 1997) have been established.

1.3.3.1 Tissue culture media

The culture medium includes a basal medium of inorganic elements, usually a semi-solid support (agar or other commercial products such as Gelrite), an energy source (mainly sucrose), and vitamin supplements (Hartmann *et al.*, 1997). The required ingredients of the culture medium vary with plant species, plant age, organ age, organ type and the propagation stage of the culture (Pierik, 1987). Many different formulations have been developed over the years including Murashige and Skoog (MS) (1962), Nitsch and Nitsch (1969), Schenk and Hildebrandt (SH) (1972) and Gamborg *et al.* B5 (GB5) (1976), but the most popular and successful medium for *Gladiolus* culture appears to be MS medium.

Inorganic salts: These provide the explant with macro and microelements which are made up into concentrated stock solutions and stored in the refrigerator (Hartmann *et al.*, 1997).

Sucrose: This satisfies the carbohydrate requirement of most plants at a concentration of 2 to 3 % (de Bruyn & Ferreira, 1992; Collin & Edwards, 1998).

Vitamins: These are included in the media in very small quantities and generally function as important co-enzymes in reactions crucial to primary plant metabolism. The most essential vitamin is thiamine (Collin & Edwards, 1998).

Agar: This is added when a solid medium is required. It is advantageous in that it melts when heated, changes to a semi-solid gel at room temperature and is relatively inert. Its performance is affected by concentration and pH (Hartmann *et al.*, 1997). The literature indicates that 0.7 to 0.8 % agar is sufficient for medium solidification in *Gladiolus* culture. Ziv (1989) noted higher *Gladiolus* multiplication rates and reduced handling with liquid media culture which allows for a more cost effective and less labour intensive culture.

Growth hormones and regulators: Most cultures require auxin and cytokinin at particular ratios as these are the most important growth regulating substances for organ and tissue development and *in vitro* culture is often impossible without them (Murashige, 1974; Pierik, 1987; Hartmann *et al.*, 1997). The concentration used depends on the species, the cultivar and the desired morphological response (Pierik, 1988; Collin & Edwards, 1998).

In *Gladiolus* cultures, auxins are important for callus initiation (Ziv *et al.*, 1970; Wilfret, 1971), cell elongation and tissue swelling, formation of adventitious roots, inhibition of adventitious and axillary shoot formation and embryogenesis in suspension cultures (Pierik, 1987). The natural auxin, indole-3-acetic acid (IAA), can be used but the use of synthetic auxins, which are more active, such as 1-naphthalene-acetic-acid (NAA), 3-indolebutyric acid (IBA) and 2, 4-dichlorophenoxyacetic acid (2, 4-D), is more common (Hartmann *et al.*, 1997; Collin & Edwards, 1998).

Cytokinins promote cell division, induce adventitious shoot formation (Pierik, 1987), encourage axillary shoot growth but inhibit root initiation and prevent dormancy (Ginzburg & Ziv, 1973; Hussey, 1976; 1977a; b; Sutter, 1986). Compounds used in this regard include 6-benzyl-amino-purine (BAP), kinetin (K), N-isopentenylamino purine (2iP) and zeatin (Z) (Hartmann *et al.*, 1997; Collin & Edwards, 1998).

pH: The literature indicates that the pH of media for *Gladiolus* cultures should be between 5.6 and 5.9. This can be adjusted with HCl (Pierik, 1987), NaOH (Jager *et al.*, 1998) or KOH (Steinitz & Lilien-Kipnis, 1989) prior to autoclaving.

Autoclaving: The media should be autoclaved for 15 to 20 minutes at 120 to 121 °C (Steinitz & Lilien-Kipnis, 1989; Dantu & Bhojwani, 1995; Kamo *et al.*, 1995). The autoclaving process lowers the pH of the media by 0.3 to 0.5 units (Pierik, 1987).

1.3.3.2 Environmental factors

Light: The most important factors with regards to lighting are day length, irradiance and spectral composition (Thorpe, 1981; Pierik, 1987). Daylength is culture dependant but light is given continuously or in photoperiods of 8 to 16 hours (Thorpe, 1981; Hartmann *et al.*, 1997). High irradiances can be damaging to *in vitro* cultures (Pierik, 1987). Typical irradiance levels are between 40 and 80 µmol m^{-2} s^{-1} (Hartmann *et al.*, 1997). Cool, white fluorescent tubes are almost always used (Pierik, 1987).

Temperature: This is usually kept constant at 20 to 27 °C but is species dependant (Dodds & Roberts, 1985; Hartmann *et al.*, 1997). Lower temperatures can be chosen for special processes and often alternating temperatures may be required. The optimal temperature for *in vitro* culture is usually 3 to 4 °C higher than for *in vivo* growth, however, the growth room temperature can be maintained at optimal *in vivo* temperatures, because the temperature within the growth tubes is 3 to 4 °C higher than the growth room due to the internal warming effect of the irradiation (Thorpe, 1981; Pierik, 1987).

Humidity: The humidity in the sealed growth tubes is relatively high, evident by condensation inside the tubes and so growth chamber humidity only affects the loss of water from the tubes. A high humidity in the growth chamber can increase the rate of infection (Pierik, 1987).

Gaseous exchange: Good aeration is important for healthy growth and development (Pierik, 1987). The concentration of the ambient gases in growth chambers and incubators, such as oxygen (O_2) and carbon dioxide (CO_2), can be measured to ensure correct concentrations. Monitoring equipment is commercially available (George, 1996). All closures and caps used for tissue culture are permeable to gases to some degree (Hartmann *et al.*, 1997) which is important as their should be an exchange of respiratory gases. The O_2 supply to the plantlets

can be promoted with the use of metal caps, non cotton wool plugs, apolar inoculation and the use of liquid media. CO_2 will be produced by the plantlets within the culture tubes (Pierik, 1987).

1.3.3.3 Stages of micropropagation

Hartmann *et al.* (1997) recognise 4 stages of micropropagation: stage 1 - establishment, stage 2 - multiplication, stage 3 - root formation and stage 4 - acclimatization. Debergh and Maene (1981) and George and Sherrington (1984), include another stage, donor plant establishment or stage 0.

Stage 0 - Donor plant preparation

This involves the production and preparation of healthy and vigorous plants grown under hygienic conditions. Plants are cultivated in a controlled greenhouse environment, although this is not always possible, in sterile potting media with irrigation applied directly to the soil not via overhead irrigation. Pests and diseases are controlled with insecticides, fungicides and often antibiotics (Debergh & Maene, 1981; George & Sherrington, 1984).

Stage I - Establishment and stabilization of explants in culture

The objectives of stage I are to successfully place explants into aseptic cultures while avoiding contamination and to provide an *in vitro* environment that promotes a morphological response, that is, shoot tip enlargement or callus formation (Murashige, 1974; Hartmann *et al.*, 1997).

Explants: An explant is a small, excised segment of plant tissue or organ taken from a plant used to initiate a culture (Tootill, 1984; Pierik, 1987). The success of manipulation of stage I is determined by the choice of explant (Murashige, 1977). The explant must contain dividing, living cells and the mother plant must be actively growing and not about to enter a period of dormancy (Collin & Edwards, 1998). The choice of explant depends on the plant source, the age of the organ, the size of the explant, the season and climatic conditions and the overall quality of the source plant (Murashige, 1977).

Gladioli have been successfully cultured using a wide variety of explants (Table 1.2). From an original 20 corms, up to 1000 plants can be produced in one year (Sutter, 1986). The best results have been achieved from axillary buds as this material has provided the highest rate of propagation (Dickens *et al.*, 1986; Ziv, 1989; de Bruyn & Ferreira, 1992). For the purposes of large scale propagation, it is assumed that proliferation by precocious axillary shoot formation is the most reliable way of ensuring genetically uniform propagules (Hussey, 1977a; b).

Young, elongating *Gladiolus* inflorescence stems have proved to be the most reactive tissue from growing tissue explants (Ziv *et al.*, 1970; Hussey, 1975). However, the response of stem explants varies considerably with cultivar, many giving few or no plantlets (Hussey, 1977a). Some cultivars do not respond consistently to the induction of adventitious shoots on inverted stem segments, therefore the direct culture of axillary buds is preferred (Hussey, 1977a). The *Gladiolus* cormel, because of its size, is very convenient for experimental handling in micropropagation (Ginzburg, 1973). Plant regeneration has also been obtained via somatic embryogenesis for four cultivars of *Gladiolus* (Stefaniak, 1994). Callus and cell suspension cultures have generally proved unsuitable for *in vitro* propagation because they tend to accumulate chromosomal abnormalities and lose their capacity to regenerate whole plants (Hussey, 1976). However, they may be used where it can be shown that they will remain genetically stable for long periods (Hussey, 1977a; b; Sen & Sen, 1995).

***Table* 1.2 Explants used for the micropropagation of *Gladiolus* (adapted from Nicol, 1993)**

Organ	Reference
corm, cormel segments	Hussey, 1975; Bajaj *et al.*, 1982/1983; Dickens *et al.*, 1986; Sutter, 1986; Kamo *et al.*, 1995; Remotti & Loffler, 1995; Remotti *et al.*, 1997; Babu & Chawla, 2000; Nhut *et al.*, 2004; Dhane *et al.*, 2005
axillary buds, apical buds	Simonsen & Hildebrandt, 1971; Wilfret, 1971; Hussey, 1977a; b; Ziv, 1979; Bajaj *et al.*, 1982/1983; Lilien-Kipnis & Kochba, 1987; 1989; Ziv, 1989; Steinitz *et al.*, 1991; de Bruyn & Ferreira, 1992; Dantu & Bhojwani,1995; Sen & Sen, 1995; Lipsky *et al.*, 1997; Beura *et al.*, 2003
basal plates	Nhut *et al.*, 2004
shoot tips	Logan & Zettler, 1985; Babu & Chawla, 2000; DaeHoe *et al.*, 2003; Nhut *et al.*, 2004
inflorescence stalks	Ziv *et al.*, 1970; Hussey, 1975; Hussey, 1976; Hussey, 1977a; Bajaj *et al.*, 1982/1983; Ziv & Lilien-Kipnis, 1997
seedling parts	Jager *et al.*, 1998
root sections	Kasumi *et al.*, 2005
stolon tips	Ginzburg & Ziv, 1973
meristems	Bertaccini & Marani, 1986; Lilien-Kipnis *et al.*, 1992; Mangal *et al.*, 2003
perianth	Bajaj *et al.*, 1982/1983
bracts, leaves, young anthers, rachis	Bajaj *et al.*, 1982/1983; Gupta & Datta, 2003/2004

Sterilization: Pierik (1987) identifies four sources of infection: (i) plant (internal and external), (ii) nutrient medium, (iii) air and (iv) inaccurate work. Disinfestation involves the use of chemicals that are toxic to microorganisms but not toxic to plant material (Hartmann *et al.*, 1997). Surface sterilization is essential for the removal of microorganisms which cause contamination and ultimately plant loss (George & Sherrington, 1984). Internal pathogens are not easily destroyed and often remain suppressed until the explant is subcultured (Hartmann *et al.*, 1997). The use of antibiotics in the culture medium (Haldeman *et al.*, 1987; George, 1996) and / or meristem tip culture (Bertaccini & Marani, 1986; Lilien-Kipnis *et al.*, 1992) has helped in this regard, although no known antibiotic is effective against all microorganisms that cause contamination (Dodds & Roberts, 1985).

Underground perennating organs are usually heavily contaminated, both externally and internally (George, 1996; Ziv & Lilien-Kipnis, 1997) and are difficult to free from contamination (Hussey, 1975). However, lifting the newly formed corms soon after flowering while the surrounding leaf bases are still firm and green, will enhance the aseptic conditions (Hussey, 1977b).

Mature *Gladiolus* corms should be gently wiped or washed to remove any soil particles (Hussey, 1977b; Collin & Edwards, 1998). The outer dead leaf bases must be removed, the corms washed thoroughly under running tap water for 24 hours (Logan & Zettler, 1985; Dickens *et al.*, 1986; Ziv, 1989), immersed in 70 % (Steinitz & Lilien-Kipnis, 1989; Beura *et al.*, 2003) or 100 % ethanol (Dickens *et al.*, 1986) and then rinsed 3 times in sterile distilled water (Ziv, 1989). Corms are often treated with a fungicide such as Benomyl® (methyl-1-butyl carbomyl-2-benzimidazole carbamate) (de Bruyn & Ferreira, 1992) or Zineb WP (Logan & Zettler, 1985).

There is a wide variety of other sterilants that can be used for this process (Hartmann *et al.*, 1997). ***Sodium hypochlorite*** (NaOCl), or commercial bleach, has proved to be very effective in *Gladiolus* culture. For example, corms have been successfully cleaned when treated for 30 minutes in 1 % NaOCl (Hussey, 1975). ***Calcium hypochlorite*** ($CaClO_4$) should be used for more sensitive tissues (Pierik, 1987). A 9 % solution applied for 20 minutes was used by Ziv (1989) to surface sterilize corms. ***Mercuric chloride*** ($HgCl_2$) is very effective but is a toxic substance and should be used with care (Pierik, 1987). Dickens *et al.* (1986) found that soaking corms in 0.1 % $HgCl_2$ for 10 minutes was successful.

Corms can then be cut into smaller explants each with an axillary bud or the apical bud attached to a small piece of corm tissue (Ziv, 1989). These may be further sterilized with 0.1 % $HgCl_2$ for 7 to 10 minutes (Sen & Sen, 1995), 5 to 9 % $CaClO_4$ for 10 minutes (Ziv, 1979; Bertaccini & Marani, 1986) or 6 to 8 minutes in freshly prepared chlorine water (Bajaj *et al.*, 1982/1983) followed by repeated washings in sterile distilled water (Ziv, 1989; Sen & Sen, 1995).

Cormels are treated in much the same way as the corms: they are dehusked and any soil particles and husk residues removed by washing them in tap water (Remotti & Loffler, 1995). They are sterilized by dipping for 10 seconds to 2 minutes in 70 to 80 % ethanol (Sutter, 1986; Remotti & Loffler, 1995) followed by immersion for 10 to 15 minutes in a 0.5 to 1.2 % NaOCl solution (Simonsen & Hildebrandt, 1971; de Bruyn & Ferreira, 1992). They are then rinsed in several changes of sterile distilled water (Simonsen & Hildebrandt, 1971; Sutter, 1986; Remotti & Loffler, 1995).

A few drops of Tween 20 or 80 is usually added to the sterilizing solutions at concentrations of 0.08 to 0.12 %. It allows for better surface contact between the plant tissue and the sterilant solution (Sutter, 1986; Pierik, 1987; Remotti & Loffler, 1995; Jager *et al.*, 1998).

Callus culture: The advantage of callus culture is the ability to speed up multiplication rates and it allows for the development of new hybrids via somaclonal variation (George & Sherrington, 1984; Pierik, 1987). Corm, cormel (Hussey, 1975; Kamo *et al.*, 1990; 1995; Remotti & Loffler, 1995; Remotti *et al.*, 1997) and young inflorescence stalk segments (Ziv *et al.*, 1970; Bajaj *et al.*, 1982/1983; Kamo et *al.*, 1990) have been the preferred explant sources from gladioli for the initiation and production of callus. Growth regulators play an important role in callus formation (Hartmann *et al.*, 1997). Auxins have been found to be essential for this process in *Gladiolus* culture (Ziv *et al.*, 1970; Bajaj *et al.*, 1982/1983). The higher the auxin concentration, the more callus is produced or the greater the chance of it developing (Hussey, 1975; Bajaj *et al.*, 1982/1983; Sutter, 1986). NAA and 2, 4-D appear to be the better auxin source for callus induction (Wilfret, 1971; Kamo *et al.*, 1990; Stefaniak, 1994) with a minimum concentration of 5.0 mg l^{-1} necessary for callus initiation (Wilfret, 1971). Callus initiation is improved when cytokinins are included in the medium (Simonsen & Hildebrandt, 1971). Ziv *et al.* (1970) found the best callus was produced at 5.0 ppm NAA in combination with 0.5 ppm K.

Stage II - Multiplication

The aim of stage II is to maintain the microcultures in a stabilized state and to multiply the shoots to the number required for rooting (Hartmann *et al.*, 1997).

Medium: The basic medium of this stage is similar to that of stage I (Ziv, 1979; Hartmann *et al.*, 1997). *Gladiolus* explants are split and subcultured onto fresh medium every 21 to 28 days (Steinitz & Lilien-Kipnis, 1989; Sen & Sen, 1995; Jager *et al.*, 1998).

Growth regulators: The addition of BAP to the tissue culture medium enhances the initiation and multiplication of shoots (Dantu & Bhojwani, 1995; Hartmann *et al.*, 1997). The slower the natural propagation rate, the higher the BAP concentration required (Hussey, 1977a). BAP also promotes branching and appears to prevent apical dominance (Hussey, 1976). Bertaccini and Marani (1986) observed successful shoot growth at an optimum concentration of 0.12 mg l^{-1} BAP, whereas Sen and Sen (1995) found 1.0 mg l^{-1} BAP to be a suitable concentration for shoot development.

Although shoot growth is successful without the presence of an auxin, there appears to be a marked interaction between cytokinin and auxin in the proliferation medium (Bajaj *et al.*, 1982/1983). Adventitious shoot proliferation has been obtained with low levels of NAA with varying concentrations of BAP and K (Hussey, 1975; Bajaj *et al.*, 1982/1983; Lilien-Kipnis & Kochba, 1987). When the ratio of cytokinin to auxin is high, as many as 10 buds per explant

can develop (Ziv & Lilien-Kipnis, 1997). Jager *et al.* (1998) successfully multiplied *Gladiolus* shoots on an MS medium with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP and Ziv (1979) found 2.0 mg l^{-1} K and 0.1 mg l^{-1} NAA was adequate to induce axillary bud outgrowth. However, Sutter (1986) found that shoots were thicker and more numerous with BA than with NAA, and Hussey (1977b) found that the addition of NAA inhibited shoot growth and enhanced basal swelling of the bud explant with the ultimate formation of callus, especially at concentrations nearer to that of BAP. He concluded that NAA should be omitted from media with BAP.

Hussey (1975) and Sen and Sen (1995) observed the formation of plantlets on MS medium without growth regulators added to it. *Gladiolus* bud clusters and corms left on medium without hormones form thin, grass-like shoots, and roots also develop (Sutter, 1986).

Environmental conditions: Environmental factors usually remain the same throughout stage I and stage II (Hartmann *et al.*, 1997). The literature indicates that *Gladiolus* cultures should be maintained in growth chambers between 23 to 25 °C ± 2 °C for a 16 hour light / 8 hour dark photoperiod. Cultures are sometimes placed at temperatures of 25 °C day / 20 °C night temperature and / or under continuous light (Steinitz & Lilien-Kipnis, 1989). Dantu and Bhojwani (1995) found the elongation of shoots was better in the light than the dark. The type of lighting and intensity used varies between authors: cool fluorescent tubes providing light at 32.8 $\mu mol^{-2} s^{-1}$ (Jager *et al.*, 1998) to 100 $\mu mol^{-2} s^{-1}$ (Steinitz & Lilien-Kipnis, 1989) have been used. The use of warm, white fluorescent tubes is also noted at 1000 to 8000 lux (Hussey, 1976; Hussey, 1977b; Bertaccini & Marani, 1986) as well as incandescent bulbs (Simonsen & Hildebrandt, 1971).

Stage III - Root formation

During this phase, single microplants must now be subcultured or moved to a medium or suitable environment for root induction as well as the preparation for transplantation into the new environment (Hartmann *et al.*, 1997), making this a labour intensive stage (Debergh & Maene, 1981). It is also beneficial for the plants to produce cormlets during this time (Ziv *et al.*, 1970; Ziv, 1989). Plantlets not subcultured onto fresh medium eventually become dormant with the formation of small corms (Hussey, 1977b). Shoots formed during stage II do not usually have roots although at times they do spontaneously root on the multiplication media. De Bruyn and Ferreira (1992) found that *G. dalenii,* readily formed a corm on the shoot induction media and no additional transfer was therefore necessary.

Media: Agar, inorganic salt and sucrose concentrations are usually decreased for the rooting stage (Ziv, 1979; Logan & Zettler, 1985; Hartmann *et al.*, 1997) although corm formation and production is increased at higher (4 to 10 %) than normal sucrose levels with the optimum level being 6 % (Dantu & Bhojwani, 1995). Cormlets initiated on media containing 3 % sucrose become dormant after 5 to 6 weeks in culture (Dantu & Bhojwani, 1995). Logan and Zettler (1985) found that roots on a substrate containing vermiculite were better in appearance and multi-branched with numerous root hairs and had better transplant success than those in a medium with 0.8 % agar.

Growth regulators: Transferring micropropagated shoots to a medium with low concentrations or a lack of cytokinin induces root formation (Hussey, 1977a; Lilien-Kipnis & Kochba, 1987; de Bruyn & Ferreira, 1992). Rooting is also greatly affected by the auxin content (Bajaj *et al.*, 1982/1983), although high auxin concentrations inhibit root and leaf elongation (Lilien-Kipnis & Kochba, 1987). Logan and Zettler (1985) noted that roots induced in the presence of an auxin, were thicker than those produced on a basal MS medium and the number of roots produced was directly related to the auxin concentration. The maximum number of roots were initiated with an optimum concentration of 1.0 mg l^{-1} NAA, however, the roots remained unbranched and there was slight callusing. Ziv (1989) found that a rooting medium lacking K and with 0.5 mg l^{-1} NAA produced several roots. Rooting has also been obtained using a hormone free medium (Jager *et al.*, 1998). Bertaccini and Marani (1986) achieved successful rooting using a half strength MS medium.

Plantlets show a strong tendency to become dormant and to form a resting corm in cultures without cytokinin (Hussey, 1976; Hussey, 1977a; Steinitz & Lilien-Kipnis, 1989; de Bruyn & Ferreira, 1992). However, cytokinins have been found to suppress corm formation and reduce corm size and fresh mass, especially with the use of BAP (Steinitz & Lilien-Kipnis, 1989; Dantu & Bhojwani, 1995). Auxin and cytokinin interactions also control corm formation and the continued proliferation thereof (Dickens *et al.*, 1986; Sutter, 1986; Ziv, 1989). This is enhanced when 0.5 mg l^{-1} NAA is used in combination with 0.5 to 5.0 mg l^{-1} K and inhibited by 5.0 mg l^{-1} NAA with low levels of K (Ginzburg & Ziv, 1973). Jager *et al.* (1998) found that shoots readily formed corms when using 2 mg l^{-1} BA.

Activated charcoal (AC): Activated charcoal has been used successfully by various authors for the *in vitro* rooting of a number of plant species. Lilien-Kipnis and Kochba (1987) found that *in vitro* rooting of gladioli is improved with the presence of AC which also enhances the yield of corms and cormels although high concentrations of AC can inhibit the frequency of corm formation and corm size (Dantu & Bhojwani, 1995). Root number and length increases in medium supplemented with AC and cultures produce long, fine, white roots with more secondary branches than if cultured in auxin alone (Logan & Zettler, 1985). Better root development has been observed under high light intensities when 0.3 % AC was added to the pretransplanting medium (Ziv, 1979; Bertaccini & Marani, 1986) or used in combination with NAA (Ziv, 1979).

The benefit of AC might be attributed to the absorption of inhibitory substances formed by the explants themselves or by the medium, the slow absorption of auxins thereby improving the rooting environment and removing any detrimental effects on root growth, darkening the culture medium (Lilien-Kipnis & Kochba, 1987; George, 1996) or stabilizing the medium pH (Pierik, 1987).

Growth retardants: Steinitz and Lilien-Kipnis (1989) found that paclobutrazol, an inhibitor of GA biosynthesis, had a two-fold effect on corm development: it reduced the initiation period and once initiation had taken place, it stimulated corm enlargement, but only at concentrations greater than 1.0 mg g^{-1}. De Bruyn and Ferreira (1992) also noted that paclobutrazol promotes corm development and improves fresh mass. These results correspond to work done by Ginzburg and Ziv (1973), who observed that GA inhibits corm development and promotes leaf blade and stolon elongation at low cytokinin levels. They suggest that GA is involved in the regulation of assimilate flow by determining the main sink in the plant system. Steinitz *et al.* (1991) found that paclobutrazol at 10 ml l^{-1} shifts the assimilate allocation towards the growing corm.

Abscisic acid (ABA) also has a retarding effect on *in vitro* cultured plantlets. The addition of ABA appears to inhibit cormel growth and leaf elongation either alone or in combination with NAA and K (Ginzburg & Ziv, 1973).

Environmental conditions: Improved root growth has been observed under high light intensities (Ziv, 1979; George & Sherrington, 1984; Hartmann *et al.*, 1997). Dantu and Bhojwani (1995) note that root formation was very poor in the dark and Logan and Zettler (1985) doubled the light intensities of stage III. The optimal temperature for root development seems to be 17 °C as higher temperatures between 25 and 27 °C inhibit growth (Lilien-Kipnis & Kotchba, 1987). The frequency of corm formation and corm growth is better in the light (Logan & Zettler, 1985) and increases at a temperature of 15 °C (de Bruyn & Ferreira, 1992).

Stage IV - Acclimatization

This stage is one in which the microplant undergoes a change from a heterotrophic environment (sugar-requiring) to an autotrophic (free-living) one and becomes acclimatized to the outdoor environment (Hartmann *et al.*, 1997). Ziv (1979) and Dantu and Bhojwani (1995) note how difficult this final stage is. *In vitro* cultured plants are very sensitive (Ziv *et*

al., 1970) and differ in structure from those grown *in vivo*, hence need time to acclimatize to their new environment (Pierik, 1987).

In *Gladiolus* culture, the nutrient medium must first be gently removed from the roots (Pierik, 1987). The plant is then rinsed under running tap water to remove excess agar (Bajaj *et al.*, 1982/1983; Nicol, 1993). Logan and Zettler (1985) immersed the plants in a fungicide before planting in the new medium. Plantlets have been successfully hardened off in compost (Hussey, 1977b), sand : soil (2:1) (Jager *et al.*, 1998), a sand and peat mix (Ziv, 1979; Dickens *et al.*, 1986), a mixture of sand, soil and peat (Bertaccini & Marani, 1986), a peat : vermiculite mixture (1:1) (Ziv, 1989) and a peat, perlite and vermiculite mix (Logan & Zettler, 1985). Simonsen and Hildebrandt (1971) found that sterile sphagnum was superior to perlite or vermiculite.

In vitro plants have a poorly developed cuticle and an inadequate root system. On transfer, there is a rapid loss of water and a great risk of plant loss hence the hardening off environment must be highly humid (Collin & Edwards, 1998). High humidity is maintained by placing the plants in a mist house or covering them with plastic (Simonsen & Hildebrandt, 1971; Lilien-Kipnis & Kochba, 1987; Nicol, 1993; Jager *et al.*, 1998). Root growth and elongation is induced at 17 °C as high temperature inhibits this development (Lilien-Kipnis & Kochba, 1987) and rate of plantlet survival after transfer to a growing mix is temperature dependant (Hussey, 1977b). Pots can then be transferred to the greenhouse under 'natural' daylength conditions at 25 ± 1 °C (Ziv, 1989) which will be season dependant and may need to be provided by artificial means.

Ziv (1979) pointed out the advantage of avoiding a dormant cormel stage by transferring actively growing plantlets to soil. Simonsen and Hildebrandt (1971), Steinitz and Lilien-Kipnis (1989), Nicol (1993) and Jager *et al.* (1998), all had success when hardening off *in vitro* *Gladiolus* plantlets after transferring them from agar to soil in the greenhouse. However, once in the new growing environment, *Gladiolus* plantlets do show a strong tendency to die back and become dormant often leaving a small resting cormlet or bulbil (Hussey, 1977b; Dickens *et al.*, 1986; Dantu & Bhojwani, 1995), although this does appear to be cultivar dependant (Bertaccini & Marani, 1986). This dieback cannot be prevented by maintaining plantlets under high humidity (Dickens *et al.*, 1986). Cormels formed *in vitro* do not differ in their structure from those grown *in vivo* (Ginzburg & Ziv, 1973). Therefore attempts are made to produce *in vitro* corms from shoots developing from axillary buds. These small corms can then be

conveniently stored and easily established under non-aseptical conditions (Ziv, 1979; 1989; Steinitz *et al.*, 1991; de Bruyn & Ferreira, 1992). A cold period of 2 to 5 °C for 3 to 8 weeks prior to planting is necessary to break dormancy, promote sprouting and obtain vigorous growth of shoots (Ginzburg, 1973; Hussey, 1977b; Sutter, 1986; Dantu & Bhojwani, 1995).

1.4 *GLADIOLUS SCABRIDUS*

1.4.1 History

Gladiolus scabridus was first recorded in 1944 in the Wakkerstroom District of Mpumalanga by General Jan C. Smuts. It was, however, temporarily assigned to the high Drakensberg species *G. microcarpus* by Lewis *et al.* (1972) along with other specimens from the area. The species remained unnamed until 1976 when plants were collected in Itala Nature Reserve in northern KwaZulu-Natal. In 1978, a type collection was made and it was formally described in 1982 by A.A. Obermeyer as *G. microcarpus* subsp. *italaensis*, on examining the plants herself at Itala (Goldblatt & Manning, 1998). It was then later described as *Gladiolus scabridus*.

1.4.2 Diagnosis and relationships

G. scabridus or Itala Gladiolus (Pooley, 1998), gets its name from the descriptive term, scabrid, referring to the short, rough pubescence present on the upper stem, bracts and spike axis of the species. It is a member of the eastern southern African complex series *Scabridus*, a group of species with fairly large, pink flowers and white nectar guides outlined or streaked with red to purple (Goldblatt & Manning, 1998). It is closely allied to *G. microcarpus* and Pooley (1998) notes that *G. scabridus* and *G. microcarpus* subsp. *italaensis* are one in the same. However, *G. microcarpus* differs in having smooth bracts and stems. The perianth also differs in that the lower lateral tepals of *G. microcarpus* are shorter than the lower median and upper lateral tepals, a feature which unmistakably distinguishes it from *G. scabridus*. The stems and leaves of *G. microcarpus* are inclined or drooping whereas those of *G. scabridus* are erect (Goldblatt & Manning, 1998). The term *microcarpus* means 'bearing small fruit' (Pooley, 1998) so this may indicate a difference in seed size between the two species.

1.4.3 Morphology and anatomy

The plants grow up to 1 m tall (Scott-Shaw, 1999; Bryan, 2002). The ***corm*** is 30 to 35 mm in diameter and more or less globose in shape covered in firm, papery tunic layers which soon decay into fibrous fragments. The ***cataphylls*** are pale and membranous, the uppermost one reaching 5 to 8 cm above the soil. They are pale green or dull purple in colour and glabrous or with a short, fine pubescence (Goldblatt & Manning, 1998).

Seven to nine sword-shaped ***foliage leaves*** develop, forming a loose fan (Scott-Shaw, 1999). The blades are minutely scabrid-papillose and 10 to 24 mm wide, the midrib and a pair of secondary veins moderately thickened and raised. There are 2 or 3 upper cauline leaves with blades similar to the basal leaves, the uppermost leaves often forming an entire sheath, the margins of the sheaths open to the base (Goldblatt & Manning, 1998).

The ***stem*** is erect, scabrid, simple or with one, rarely two branches and 3 mm in diameter below the spike. The ***spike*** is erect and straight with a minutely scabrid axis. It produces 10 to16 flowers, the branches 2 to 8 flowered. The ***bracts*** are dull green to dull greenish purple in colour and minutely scabrid, often more or less dry above, the apices twisted outward. The outer bract is usually 25 to 35 mm in length and two and a half internodes long, the inner bract about three-quarters as long to slightly longer than the outer, forked apically for 1 to 2 mm (Goldblatt & Manning, 1998).

The ***flowers*** are large and bright pink to mauve in colour and appear to be unscented (Scott-Shaw, 1999; Bryan, 2002), although this may change at different times of the day (Manning, 2002). The lower three tepals each have a narrow, white longitudinal zone with a narrow reddish streak. The ***perianth tube*** is obliquely funnel-shaped and 35 to 40 mm long. The ***tepals*** are unequal, lanceolate, the larger upper three, more or less equal and 40 to 45 mm long. The lower three tepals are joined to the upper laterals for 3 to 4 mm and to each other for 4 mm, are straight and tilted toward the ground and 35 to 40 mm in length. The ***filaments*** are 15 to 16 mm long and exserted 8mm from the tube. The pale yellow ***anthers*** are 10 to 13 mm long and produce white pollen. The ***ovary*** is narrowly obovoid and 7 mm long. The ***style*** branch is 5 to 6 mm long and it arches over the stamens, dividing at or shortly beyond the anther apices. The three-lobed ***capsules*** are oblong, retuse and 16 to 22 mm long. They produce ***seeds*** that are broadly ovate, 6 to 8 mm by 4 to 5 mm in size, more or less evenly

winged or the wing is not developed on one side and rich brown in colour. The ***chromosome number*** 2n = 30 (Goldblatt & Manning, 1998).

1.4.4 Distribution and habitat

The distribution of *G. scabridus* is not yet well documented, however, the species appears to be restricted to the mountains of northern KwaZulu-Natal and southern Swaziland (Figure 1.2) (Goldblatt & Manning, 1998; Bryan, 2002) in the middle to upper catchment of the Pongola and Bivane Rivers, particularly in Itala Nature Reserve where it is protected (Scott-Shaw, 1999). It also occurs in parts of southern Mpumalanga immediately adjacent to KwaZulu-Natal, on the opposite bank of the Pongola River (Goldblatt & Manning, 1998).

According to Goldblatt and Manning (1998) the species grows at elevations of 1000 to 2000 m. Pooley (1998) states that it grows at lower altitudes, at about 250 m, whereas Scott-Shaw (1999) notes that it occurs between 800 to 1000 m. However, it appears to be restricted to well drained, rocky habitats and is especially common in quartzite outcrops often hanging over cliff edges (Goldblatt & Manning, 1998; Scott-Shaw, 1999) where the corms become wedged between crevices making them almost impossible to dig up, thus secured from predation by baboons and porcupines (Goldblatt & Manning, 1998). According to Scott-Shaw (1999) it grows in a savanna and open woodland habitat in bioclimatic regions P4 (Highland to Submontane) and P6 (Upland Moist) (Phillips, 1969), bioresource groups C8 (Moist Highland Sourveld) and C11 (Moist Transitional Tall Grassland) (Camp, 1997) with veld and vegetation types A8 (Northern-eastern Mountain Sourveld), A64 (Northern Tall Grassveld) (Acocks, 1988) and L43 (North-eastern Mountain Grassland) (Low & Rebelo, 1996).

The flowers appear in December to late January at lower altitudes and sometimes into late February in cooler, wetter sites (Goldblatt & Manning, 1998; Bryan, 2002). It is assumed that the long, tubular flowers of *G. scabridus*, with their linear, white and dark red streaked nectar guides, are adapted for pollination by long-tonged flies, but the pollination biology of the species still needs to be studied further (Goldblatt & Manning, 1998).

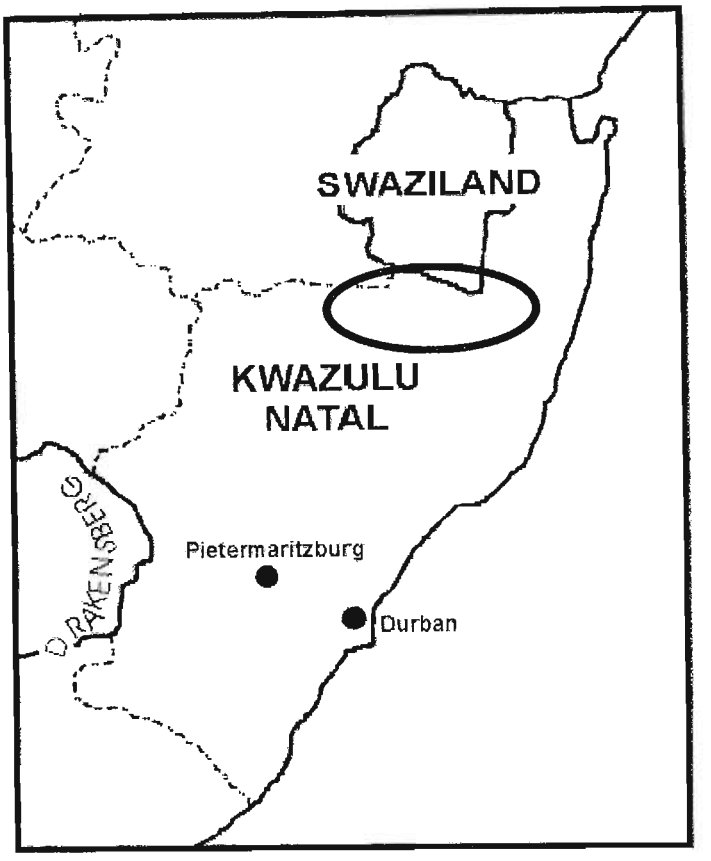


Figure 1.2 The distribution of *G. scabridus* in northern KwaZulu-Natal

1.4.5 Conservation status

The species is very rare with a narrow distribution and low abundance; the colonies seldom exceed 200 individuals (Scott-Shaw, 1999). Its habitat is severely fragmented and its area of occupancy is declining (B. Church, pers. commun., 2003). It has a vulnerable conservation status (faces a high risk of extinction in the wild in the medium-term future); it has a protected legal status and is classed as medium priority in its urgency for conservation action (Scott-Shaw, 1999).

1.5 CONSERVATION BIOLOGY

Although southern Africa is a species rich area, it has the highest known concentration of threatened plants in the world (Hilton-Taylor, 1996). In KwaZulu-Natal alone, approximately 16 % of the flora is endemic, however, 11 % is rare and threatened. Thirty of the 125 members of the Iridaceae family in KwaZulu-Natal are threatened (Scott-Shaw, 1999).

There are a number of reasons for this growing endangered status (Pooley, 1998). South Africa has one of the fastest growing populations in the world which, parallelled with rapidly increasing urbanisation, is bringing about major changes in plant habitats. This, together with the direct destructive forces of man himself, has caused widespread alterations in the sizes of populations of wild species (Hall, 1978). KwaZulu-Natal is undergoing an extensive rate of land transformation and habitat modification which is far greater than any other country or province in southern Africa (Scott-Shaw, 1999).

An important agent of transformation, where the soil is turned and the natural plant cover removed, often to such an extent that native species have been totally lost from the system, is crop and tree farming, such as the conversion of grasslands to timber plantations (Armstrong *et al.*, 1998; Pooley, 1998; Scott-Shaw, 1999). The permanent and rapidly increasing settlement of man since the mid 1800s, has led to increased domestic stock populations and unsustainable levels of subsistence farming. This has resulted in a rapid breakdown of the fragile natural resources (Winter & Botha, 1994; Scott-Shaw, 1999). Modern farming methods and fire have an impact on dispersal, phenology and pollination biology (Stirton, 1980). Grazing is also an area of concern. However, it is less of a threat to bulbous plants than most other forms of vegetation. Many bulbous plants are poisonous and survive by being unpalatable to sheep and goats. Some do not have any adaptions but can only survive by being out of reach on cliffs, among rocks or inside spiny bushes (Rix, 1983).

Another threat to plant diversity is the uncontrolled harvesting of wild plants (Delpierre & du Plessis, 1973; Scott-Shaw, 1999). Bulbous plants are particularly vulnerable because they are so easy to collect and keep alive during the transfer from the wild to the garden (Rix, 1983). Alien plants also modify habitats to such an extent that native species are lost from the system. At higher altitudes the uncontrolled spread of wattle is transforming riparian habitats (Delpierre & du Plessis, 1973).

The commercialisation of threatened plants is just one solution to reduce the chances of species extinction (Winter & Botha, 1994). There is much scope for the selection and development of cultivars both from the wild state and from garden collections of genera that could be profitably investigated for use as cut flowers, ornamentals or as pot plants. Petaloid monocotyledons in South Africa offer great opportunities for both pure and applied research, however, the great majority have yet to be investigated for their horticultural potential (Stirton, 1980).

Threatened plants which can be released into the horticultural trade fall into 2 categories: firstly, those in demand, including traditional medicinal plants collected from the wild, ornamental species desired by collectors and those with commercial potential for cut flower production and nursery trade. The second group includes plants with horticultural potential which are still relatively unknown but have the potential for commercialisation, however, require research and promotion to create a commercial demand before they can be released (Winter & Botha, 1994).

Before any material is released, all horticultural requirements should be investigated and cultivation problems solved, documented and distributed to the horticultural trade. Possible objectives for cultivation and commercialisation to be considered are (i) bulking up of material involving the development of successful propagation techniques, (ii) field testing of new species to determine their tolerance to cultural conditions, (iii) distribution to botanic gardens and other interested groups resulting in *ex situ* conservation and the maintenance of genetic viability, (iv) store of germplasm and (v) the production and supplying of material for reintroduction into the wild which involves a good understanding of its ecology (Winter & Botha, 1994).

Few indigenous plants, particularly those that are naturally rare, have been studied in any detail (Smith *et al.*, 1995) and there appears to be little information available about ongoing research in South Africa. Information facilitates conservation, not only on which species are threatened, but what the threats to their survival are and where the plants still occur so that their localities may be protected (Synge, 1980; Smith *et al.*, 1996). Information is also important to plant breeders as it offers guidance in the selection and collection of breeding material (Stirton, 1980). Breeding methodology is largely determined by the ploidy differences between species (Littlejohn & van der Walt, 1992). Maintaining genetic diversity is relatively easy in South Africa as we have access to a pollen bank which is operated nationwide to

provide growers with pollen when required (Winter & Botha, 1994). When breeding with gladioli, disease resistance (mainly *Uromyces*), fragrance, new colours and shapes or the use of smaller varieties is considered (Jansen van Vuuren *et al.*, 1993).

For a while there were few concerted attempts to breed South African plants for commercial exploitation on a wide scale but this is gradually changing. One example of the result of this growing interest is the Indigenous Bulb Grower's Association of South Africa (established in 1961) which plays an important role in encouraging the cultivation of indigenous bulbous plants and from which much valuable material can be obtained (Stirton, 1980; Littlejohn & van der Walt, 1992).

Goldblatt and Manning (1998) emphasise the importance of sexual reproduction and seed dispersal in conserving geophytic plants such as gladioli. Their conservation does not simply require that they be left undisturbed in their habitat as this will certainly lead to their ultimate loss. Seed production depends on successful pollination therefore conservation also implies conservation of pollinators, and the maintenance of the environment to the extent that pollinators are available and predator populations are limited.

The National Botanical Institute (NBI), formed after the amalgamation of the Botanical Research Institute and the National Botanical Gardens, currently consists of eight national botanical gardens, five herbaria and five research groups (Winter & Botha, 1994). Together, with various other nature conservation organisations such as The Provincial Departments of Nature Conservation and The National Parks Board, they carry the responsibility of conserving the flora of southern Africa (Hall, 1978).

The NBI promotes conservation through cultivation by making threatened or endangered plants available to the horticultural trade. They have access to seed and other resources and the expertise to grow these plants. Plant material is collected by the NBI, which becomes their property, and is used as stock material for propagating purposes. The horticultural industry has the finances, outlets and other means to multiply and distribute these plants (Winter & Botha, 1994). Many endangered bulbous plants often prove easy to cultivate and propagate (Koopowitz, 1999). Hence gardeners, for example, can play an important role in the conservation of species that are at risk in their natural habitat that otherwise might be lost forever (Doutt, 1997). Making material available to private collectors as well, where much of the germplasm of rare varieties and species is maintained, can aid the survival of South Africa's genetic treasures (Koopowitz, 1999).

CHAPTER 2

ECOLOGICAL STUDIES OF *GLADIOLUS SCABRIDUS*

2.1 BIVANE DAM

2.1.1 Introduction

Paris Dam, now called Bivane Dam, is the largest private dam in South Africa (E. Granger, pers. commun., 2003) (Plate 2.1 A). It is situated in a precipitous gorge of the Bivane River, a major tributary of the Pongola River (27°31'32" S; 31°03'18" E) and is owned and was built by Impala Irrigation Board, now the Impala Water User Association, at a cost of approximately R 200 million (Department of Water Affairs and Forestry, 2001). The Association took the initiative to build Bivane Dam for reasons of water shortage in the region; at one stage the Pongola River stopped flowing because of forestry in the area. One third of the cost of the dam was met by the Department of Water Affairs, the remainder by a levy on commercial farmers by Impala Water User Association (Hemmen, 1998). They started building the dam on 2 April 1997 and it was completed in 2000 (McKenzie, 2001a). Bivane dam is 180 m wide with a 60 m long spillway (Plate 2.1 B) and is able to store 118 million cubic metres of water (Linscott, 1998; Hemmen, 1998).

The implementation of the Bivane Dam water supply scheme had four major economic spin-offs: it would provide a large water reserve for one of southern Africa's most productive areas of irrigated sugar cane farming, it would introduce significant numbers of small farmers to highly lucrative irrigated sugar cane growing for the first time, it would bring potable water to more than a quarter of a million people in rural settlements and, as it is set in one of the most picturesque parts of the province, it would establish a significant niche of tourism development (Linscott, 1998).

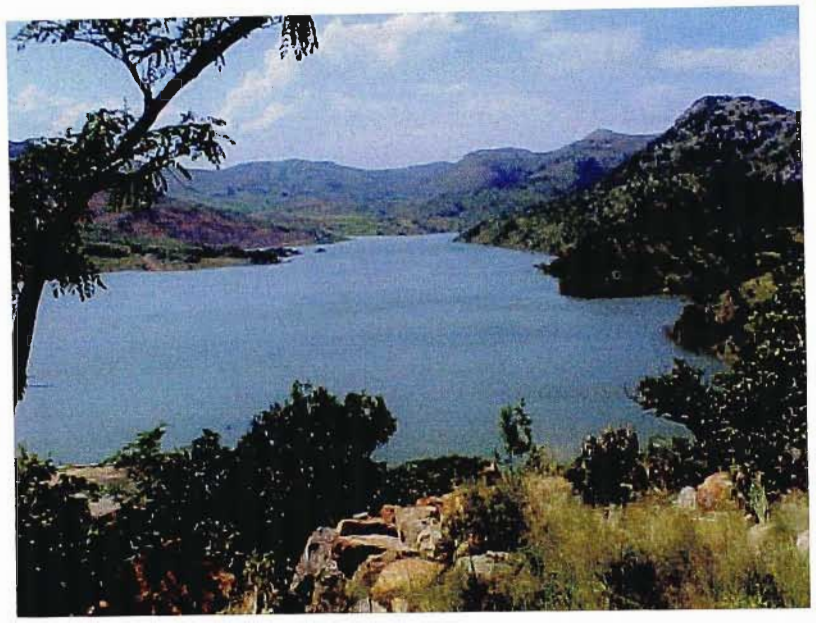
The dominant feature of the Bivane area is rolling hills with exposed quartzite ridges, wooded drainage lines and steep cliffs and gorges and of course the open water of the dam. The area is home to many rare and localised species of birds (McKenzie, 2001a). The area is also well known for its plant diversity, which is unrivalled in Zululand (northern KZN). It is home to one of the plant hotspots in the province and many botanists visit this area annually to

enjoy the number of endemic or rare plants. These include the Mountain waterwood (*Syzygium guineense* subsp. *legatii*), Broad-leaved beech (*Faurea speciosa*), Wild teak (*Pterocarpus angolensis*), Live-long (*Lannea discolor*) and Itala gladiolus (*G. scabridus*) which are virtually confined to this area of the province. The wild flower displays in spring are quite spectacular (McKenzie, 2001b).

The planning of the dam raised two concerns: one was that it would flood the land of communities along the river banks and the other that it would cause environmental damage, destroying these rare plant species (Hemmen, 1998). The University of KwaZulu-Natal's Pietermaritzburg-based Institute of Natural Resources was engaged, at a cost of about R 4 million, to monitor the environmental impact of the project and to translocate rare species that were otherwise threatened with destruction (Linscott, 1998). *G. scabridus* formed a part of this translocation project and plants have been sent to the dam from KZN Wildlife as part of a replanting project to aid the survival of the species in its natural environment.

One of the areas where *G. scabridus* grows naturally at Bivane Dam, is on a small hillside overlooking the dam and is easily accessible by road and foot. It was therefore decided to conduct a field study on the species at this site in an attempt to learn more about its ecology and to improve understanding of its growth in the wild. It is hoped that the information obtained on the species will aid the difficult task that conservationists and researchers face in working towards improving its conservation status.

A



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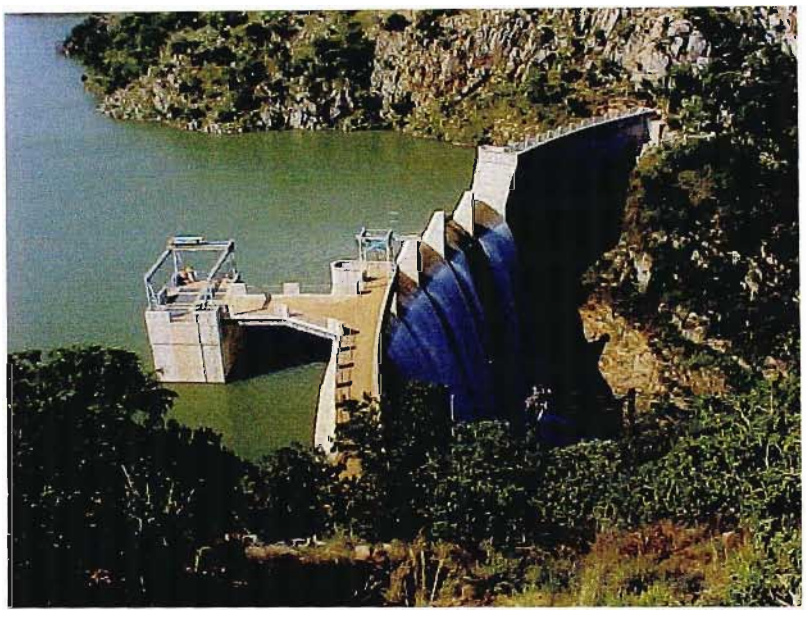


Plate 2.1 Bivane Dam is set amongst hillsides and rocky gorges with a lovely view of (A) the open water and (B) the spillway.

2.1.2 Materials and Methods

The field trip was undertaken over 3 days during January 2001. A Global Positioning System (GPS) was used to determine altitude and reference points. Soil samples were taken at the *G. scabridus* sites using an auger. Soil samples were also collected from two areas at the dam where the species did not occur: a flat, slightly rocky grassland (Plate 2.2 A) and a sloping, unrocky hillside (Plate 2.2 B). All samples were sent to the Soil Science Department at Cedara Agricultural College for mineral analysis. Nitrogen analysis was carried out by Allerton Provincial Veterinary Laboratory. *G. scabridus* leaf samples were also collected at the sites. The most recently matured leaves from flowering plants were sampled and these were sent for mineral analysis. Soil data was statistically analysed using Genstat 5 (5th Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station) and was treated as a completely randomized design.

General features that were also noted at each *G. scabridus* site were plant population size, plant condition, flowering stage and number of leaves, as well as the other plant species growing in the area as an indication of the habitat type that *G. scabridus* prefers and is limited to.

2.1.3 Results and Discussion

2.1.3.1 General ecological findings

The GPS indicated that the species occurred between 27°31'28"S to 27°31'40"S and 31°03'12"E to 31°03'19"E at an altitude of 820 to 842 m ASL at the study area at Bivane Dam. Within this area, 61 *G. scabridus* sites were found. A site was classified as a colony of 1 to 200 plants growing in close proximity to one another and 5 m or more away from the next site / colony. *G. scabridus* colonies seldom exceed two hundred individuals (Scott-Shaw, 1999). Colonies at Bivane Dam varied from 1 to 108 in number.

G. scabridus plants were found to be at various stages of development. There were many non-flowering, young plants growing in the colonies, although at times it was not clear whether these were individual plants growing from their own cormels or shoots from axillary buds of the corms of the larger plants. Some of the more mature plants were still in the tight

bud stage and many were in flower (Plate 2.3 A). However, the majority were almost finished flowering and / or starting to set seed (Plate 2.3 B). Other plants had already set seed and some had even been damaged, with flowers, seeds and leaves that had been chewed and eaten presumably by insects or animals. The fact that these plants can be decimated in such a way, needs to be carefully considered when looking at the survival strategies of the species. Reduction in seed set and dispersal as well as the above ground vegetative organs, will mean less chance of continued plant survival through seed dispersal as well as inhibiting the development of the daughter corms and cormels underground due to a depletion of the above ground reserves. However, an animal may well play a part in seed dispersal but this will depend on where the seeds get deposited by the animal as to whether germination and subsequent growth will take place.

The number of leaves on young seedling plants varied from 2 to 6, whereas those on the flowering plants were 7 to 11 in number, an indication that leaf number play a roles in determining when the plant will flower, as observed in the nutritional studies (Chapter 4). The number of flowering branches on the spikes varied from 1 to 4. Although most florets had 6 petals, this did vary from 4 to 6 in number and the number of petals that were striped varied from 2 to 6. Open floret width was between 20.35 to 54.63 mm and floret length from 24.90 to 58.27 mm. Many of the spikes had ants feeding off the extra-floral nectar produced by the florets (Plate 2.3 C) (Chapter 5).

2.1.3.2 Habitat and soil type

Most *G. scabridus* plants were growing in full sun, indicating their adaption to hot, dry, harsh conditions. However, some colonies were found in the dappled shade of nearby trees and shrubs. The species can thus withstand lower light conditions, although these plants were more spindly in growth habit than those in full sun.

The rocky quartzite outcrops that have been noted to be associated with populations of *G. scabridus* (Goldblatt & Manning, 1998; Scott-Shaw, 1999), were particularly evident in the study area at Bivane Dam (Plate 2.4). A number of plants were found growing alongside the rocks but most were wedged tightly between them. Such a growth habit means less chance of illegal collecting and harvesting of the species as they are virtually impossible to dig up when wedged so tightly between the rocks. These unreachable crevices may act as moisture

or nutrient pockets for the corms or may provide suitable temperature regimes for adequate corm growth as the rocks give off or retain heat at the various times of the year.

Due to the fact that the area where *G. scabridus* grows is extremely rocky, it proved very difficult to take soil samples. The soil type was deduced to be either Mispah, Glenrosa or Mayo (Soil Classification Working Group, 1991).

Many wild species of gladioli are restricted to unusual habitats and seem to favour a particular soil type, many of these soils having a limited distribution in southern Africa. Species in the summer rainfall regions favour either course-grained soils derived from quartzites, or clay soils derived from shales and dolerite (Goldblatt & Manning, 1998). *G. scabridus* appears to fall into the first category and prefer course-grained soils from quartzite mother rock.

Bivane Dam is surrounded by a succession of resistant siliceous quartzite ridges of the Mozaan Group, Pongola Supergroup. Quartzite beds also underlie the dam site (Hemmen, 1998). Quartzite is thus a prominent feature of the dam area and thus very characteristic of the habitat where *G. scabridus* is to be found. This is an important consideration when attempts are made to locate other populations of the species in northern KwaZulu-Natal as prolific quartzite sites seems to be an indicator of the presence of *G. scabridus* colonies. Quartzite is a metamorphic rock formed from sandstone and is composed mainly or entirely of quartz, a silicon oxide (SiO_2) compound. Quartzite is a common and widely distributed rock found primarily amongst ancient rocks (Raven *et al.*, 1992). This indicates that *G. scabridus* prefers well weathered, older soils containing quartz compounds and related minerals. Perhaps these plants have adapted to soils high in silicon (Si) and are thus restricted to sites where such an element is present.

Silica minerals generally have low adsorption capacities because they have negligible charge, and thus have low cation exchange capacities (CEC). Hence these minerals have a diluting effect on the overall CEC of most soils which contributes to low levels of cations in the soil (Monger & Kelly, 2002) as indicated by Table 2.1, which shows the results of the soil analysis from *G. scabridus* sites as well as the other two areas at Bivane Dam where the species was not growing. This low CEC will mean an acidic soil which *G. scabridus* plants have been shown to thrive in (Chapter 4).

Generally there were no significant differences between most of the soil constituents of the three sites (Table 2.1). However, soils taken from *G. scabridus* sites showed significantly higher levels of phosphorus (P) (7 mg l^{-1}) ($p=0.001$) and zinc (Zn) (2.2 mg l^{-1}) ($p=0.002$) than the other two sites. This is important considering the three sites were within a 1 km radius of each other and have such vast differences in soil type and habitat, perhaps indicating an important reason why the plants are just restricted to the rocky, quartzite sites. Phosphorus (P) and Zn may thus be important for and hence play a significant role in the growth of the species in the wild. Previous nutrient studies did not focus on P, although as with all elements it is an important for plant growth and development. However, previous results have indicated that Zn may play an important role in corm and cormel development.

Although there was no significant difference between the levels of potassium (K) ($p=0.348$), there was a tendency for soil from *G. scabridus* sites to be lower in K. Soil from the hillside had 170 mg l^{-1} K, the flat area 82 mg l^{-1} and *G. scabridus* sites 46 mg l^{-1}. Hence the species appears to have adapted to soils lower in K. Studies have shown that this species thrives on nutrient levels much lower than the norms for hybrids (Chapter 4).

There was also a significant difference between the levels of organic carbon found at the three sites ($p=0.016$). The *G. scabridus* site showed the highest organic carbon level of 3.8 %. This is surprising because the soil from *G. scabridus* sites was courser and sandier in texture and derived from older, more weathered material. Soil from the other two areas was darker in colour indicating the presence of more organic matter and it was much softer in texture.

There was a significant difference between the pH from each site ($p=0.002$), although all three soil types were acidic. Soil where *G. scabridus* grows has a mean pH value of 4.52, the steep area slightly higher at pH 5.16 and the flat site, lower with pH 3.93. Although the soil of the areas where the species did not occur was acidic, perhaps the lack of quartzite material on the steep slope meant an unsuitable soil type for *G. scabridus* growth and perhaps the soil pH of the flatter site was too acidic.

***Table* 2.1 Results of the soil analysis for sites where *G. scabridus* occurred and two areas where it did not occur at Bivane Dam**

Analysis	Site 1 [x]	Site 2 [y]	Site 3 [z]
Nitrogen (%)	0.054 a	0.161 a	0.109 a
Phosphorus (mg l^{-1})	2 b	1 b	7 a
Potassium (mg l^{-1})	82 a	170 a	46 a
Calcium (mg l^{-1})	113 b	2249 a	627 b
Magnesium (mg l^{-1})	46 c	721 a	136 b
Zinc (mg l^{-1})	0.8 b	0.7 b	2.2 a
Manganese (mg l^{-1})	40 a	7 b	17 b
Organic carbon (%)	1.5 b	2.8 b	3.8 a
pH (KCl)	3.93 c	5.16 a	4.52 b
Total cations (cmol l^{-1})	2.08 b	17.64 a	4.81 b
Acid saturation (%)	44 a	0 b	13 b
Clay (%)	20 b	33 a	17 b
Density (g ml^{-1})	1.21 a	1.02 b	1.20 a
Exchangeable acidity (cmol l^{-1})	0.93 a	0.05 a	0.44 a

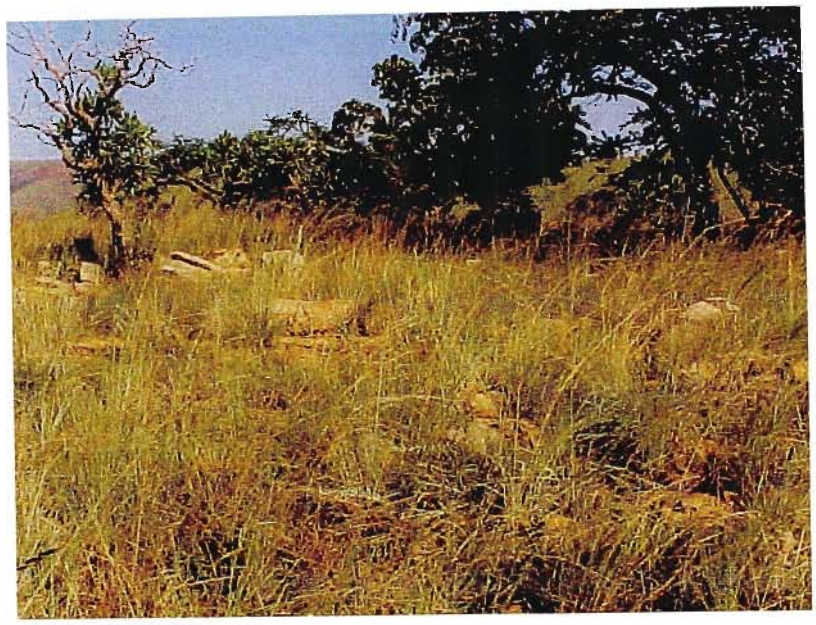
[x] flat, slightly rocky site without *G. scabridus*

[y] sloping, unrocky hillside

[z] *G. scabridus* sites

Letters a to c compare means in each row.

A



B

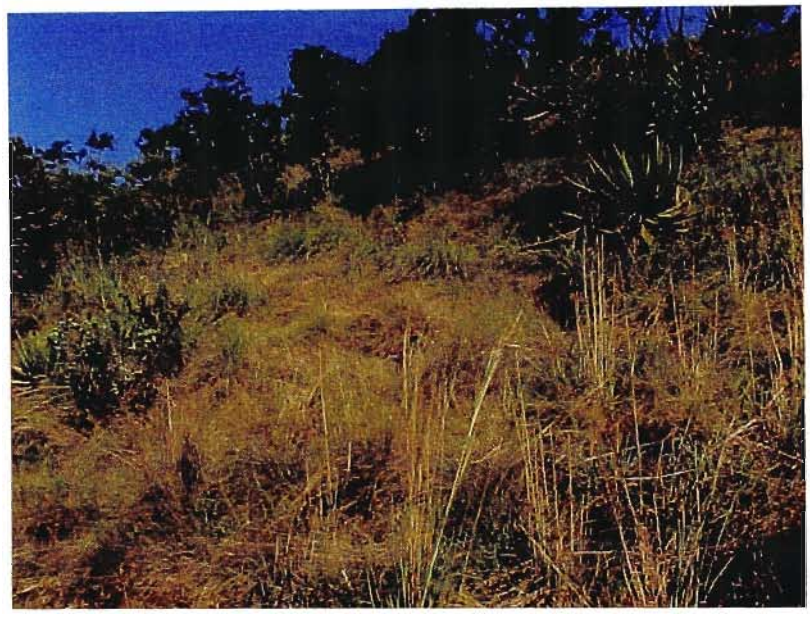


Plate 2.2 (A) A flat, slightly rocky grassland and (B) an unrocky hillside at Bivane Dam where soil samples were taken as a comparison to where *G. scabridus* is found

A

B

C

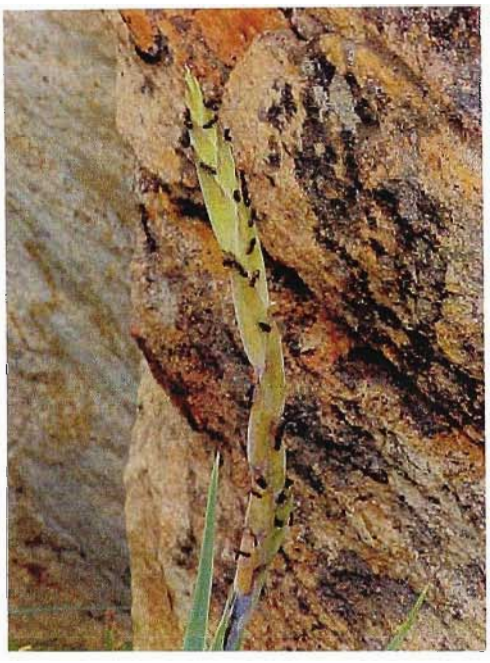
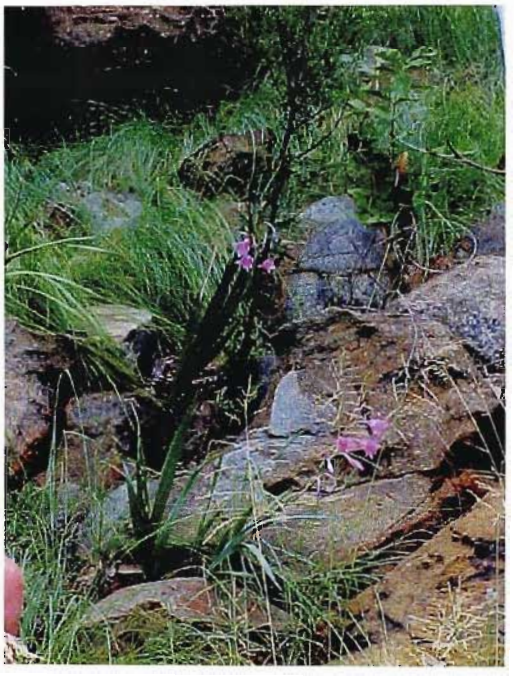


Plate 2.3 ***G. scabridus*** **floral spikes at Bivane Dam (A) in flower, (B) seeding and (C) with ants feeding off the extra-floral nectar**

A

B



C

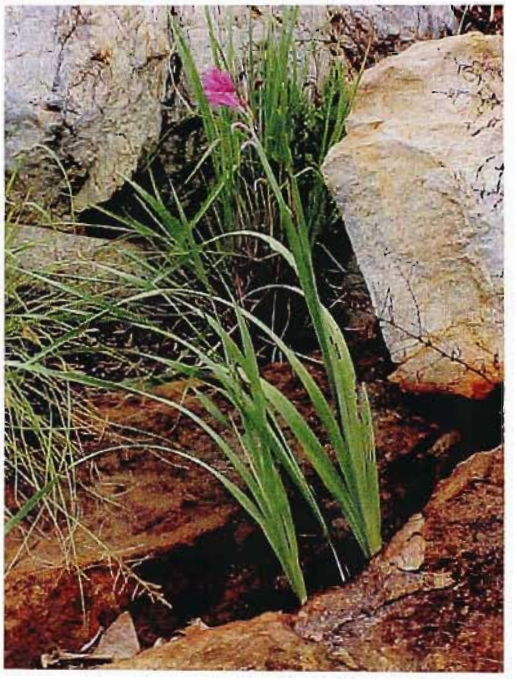


Plate 2.4 (A, B and C) *G. scabridus* plants at Bivane Dam closely associated with quartzite outcrops

2.1.3.3 Leaf analysis

Table 2.2 shows the mineral analysis results for *G. scabridus* leaf samples from Bivane Dam. A comparison with Appendix 1, the leaf norms for gladioli, indicates that the levels of N, P and K are all below the low level for gladioli leaves, supposedly insufficient to allow for optimum plant performance. However, the *G. scabridus* plants that were observed at Bivane Dam, appeared to be thriving in this environment with no deficiency symptoms evident on the leaves. These gladioli norms thus do not apply to the wild gladioli and the lower nutrient levels in the leaves cannot be considered deficient in these plants. The plants would have adjusted their growth over time to adapt to the low levels of these nutrients and hence thrive in soils with low nutrients and have a low nutrient requirement. Previous studies have shown that the addition of nutrients is beneficial to growth. However, the plants do not demand them and have adapted their growth accordingly. This will, however, mean that growth in the wild will be slower compared to commercial greenhouse conditions and corms will take longer to develop and produce flowering spikes.

Although leaf mineral data is not necessarily linked to the soil data, both indicated that there were lower levels of N and K in the soil and that which was taken up. P was, however, higher in *G. scabridus* soils yet low in the leaves but it may not necessarily be taken up that quickly by the plants hence this lower level. Even though *G. scabridus* plants have adapted to soils higher in P, they may not utilize much of the available P in the soil or perhaps it is not mobile in the quartzite parent material.

According to Appendix 1, *G. scabridus* leaf Ca and Zn levels are in the sufficient range and Mg and Mn levels are high. This may imply that these elements are in good supply in the soil or may just mean that these plants do not use large quantities of these elements for growth but may be needed for daughter corm formation. The plants appear to have adapted their growth to soils low in Cu as well, as this level was in the low range for hybrid gladioli.

Perhaps further studies in the wild will help develop a better understanding of the nutrient status of the species in the wild. As with the growth studies, this should include leaf sampling at different times in the growing season to show the nutrient status of wild plants at the crucial stages of plant development.

***Table* 2.2 Mineral analysis of *G. scabridus* leaf samples collected at Bivane Dam**

Element	Concentration
Nitrogen (%)	1.74
Phosphorus (%)	0.11
Potassium (%)	0.9
Calcium (%)	1.34
Magnesium (%)	0.32
Sodium (%)	0.04
Manganese (ppm)	277
Zinc (ppm)	20
Copper (ppm)	5

2.1.3.4 Companion plant species

There were a variety of plant species associated with *G. scabridus*. Appendix 2 shows the grass, tree, shrub and flower species that occurred in the area along with their habitat characteristics.

During summer, the species will flower at the same time as many other plants in their communities and are thus exposed to high levels of competition for space, light, pollinators (Goldblatt & Manning, 1998) and nutrients. Thus competition with other plant species may have played a role in contributing towards the vulnerable status of *G. scabridus* reducing their distribution. Perhaps certain pollinators, like the long-tongued proboscid flies that pollinate *G. scabridus* flowers (Goldblatt & Manning, 1999) are limited to the areas where the species grows or vice versa.

There are many similarities with habitat preference of the plant species listed in Appendix 2 which should give some indication as to the soil type and habitat preferred by *G. scabridus* or that which it is limited to. Most of the species seem to prefer well-drained, shallow, sandy or stony soils in disturbed or undisturbed sites. Many are common on rocky hillsides at low to medium altitudes as preferred by *G. scabridus*.

2.1.3.5 Overview

Although various reasons for the low distribution of *G. scabridus* have been speculated on as a result of the field study at Bivane Dam, no particular factor can be assumed to be the reason for its limited distribution. It was originally thought that nutrition could play a vital role in this regard but the plants appear to have low nutrient requirement, and therefore this is not a limitation to their growth.

Even though they were in close proximity to each other, the three sites, where the soils samples were taken from, showed distinct visual habitat differences (Plates 2.2 A, B and 2.4 A, B and C). The species is definitely only found where quartzite outcrops occur but the question remains as to why? Microclimate differences may occur between these sites but this unlikely to cause major differences.

An important consideration is that they may have adapted their growth to these rocky places to be free from predation. The rocks would prevent certain predators from getting to the plants due to the difficulty in accessing them and the steepness of some of the crevices. By growing between the rocks they are also free from predation by baboons and other such animals (Goldblatt & Manning, 1998). It would definitely be worth investigating this effect on *G. scabridus* distribution through further studies of the species in its natural environment.

2.2 ITALA NATURE RESERVE

2.2.1 Introduction

Itala Nature Reserve is owned by KwaZulu-Natal Wildlife and is located in northern KwaZulu-Natal, 60 km east of Vryheid, immediately north of Louwsberg (27°24'-27'26" S, 31°9'-31'27" E) (Anon, 1991). Proclaimed in 1972, this 29,653 ha nature reserve lies in part of the old Pongola Game Reserve, the very first game reserve ever proclaimed in Africa (O' Hagan, 1996). The northern boundary of the reserve lies on the south bank of the Pongola River and several tributaries flow through the area and the mountain range, of which the *Ngoje* mountain is part, forms the southern boundary (McKenzie, 2001b; Anon, 1991).

Altitude ranges from 1348 m above sea level (ASL) in the south west to 350 m ASL in the north east. The scenic terrain consists mostly of grassed hilltops and rugged mountainsides (Plate 2.5 A) dotted with numerous aloes, steep valleys and open woodland savannah or bushveld. Rivers cut sharply into the mountain forming steep rocky embankments, however, ridge tops are generally flatter and have deeper, less rocky soils. Itala is home to diverse geological features which is dominated by shales, quartzite and granite (Pooley & Player, 1995).

The summers are generally warm to hot with an average annual rainfall of 680 mm, although the higher areas receive up to 1200 mm. Winter days are generally mild, but nights can become cold. Mean monthly temperatures range from between 4.3 °C in winter and 32 °C in summer (Cooper, 1981).

The great variety of habitats provide suitable environments for many species of birds, mammals and plants. Some 910 plant species have been identified to date at Itala. The diversity of plant life is due to the many soil types resulting from erosion of the rock formations (Pooley & Player, 1995). One of KZN Wildlife's major objectives for Itala Nature Reserve is to conserve the optimum number of appropriate indigenous (to the Reserve) species and their habitats, maintain breeding populations and protect the specificity of these gene pools (Anon, 1991).

The first recorded collection of *G. scabridus* at Itala Nature Reserve was in 1976 (Goldblatt & Manning, 1998). Known populations of the species appear to be mainly located in the reserve, hence its common name Itala Gladiolus, and it has a protected status in the reserve (Scott-Shaw, 1999). Replanting of the species has been undertaken: KwaZulu-Natal Wildlife sent about 60 plants to Ntshondwe camp in the Reserve to be used for a public awareness campaign (B. Church, pers. commun., 2003).

Locations of *G. scabridus* at Itala are in very remote areas and hence are difficult to access. There are, however, areas at Itala with similar quartzite rocky outcrops where one would normally find the species (C.R. Scott-Shaw, pers. commun., 2001). These occur along the Bergvliet Loop Road in the Reserve (Figure 2.1). A field trip to Itala Nature Reserve was undertaken but due to the difficulty in accessing areas where the species grows, this more easily accessible area was visited for observational purposes.

Figure 2.1 A map of Itala Nature Reserve showing the Bergvliet Loop Road

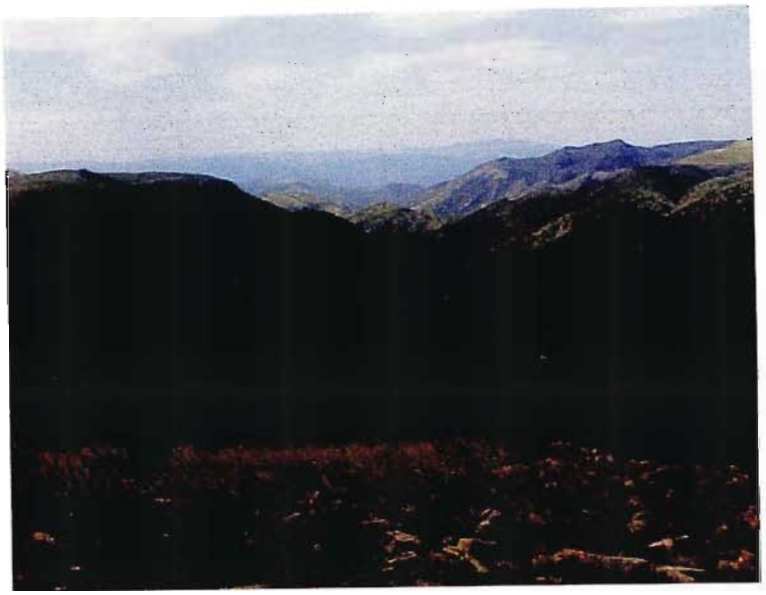
2.2.2 Materials and Methods

The rocky areas along the Bergvliet Loop Road were easily found but were some distance from the road. Due to the access restrictions of the Reserve, these hillsides could not be reached by foot and could only be viewed from the road. However, it was possible to take photographs of this area.

2.2.3 Results and Discussion

The Bergvliet Loop hillsides were similar in appearance to the *G. scabridus* sites at Bivane Dam with an abundance of quartzite outcrops as noted in Plate 2.5 B. Unfortunately the distance from the road did now allow for definite plant species identification or soil sampling. There is definitely a need for future research to be undertaken at Itala with regards to the habitat preference of the species and looking at these hillsides as well as accessing the remote *G. scabridus* areas. Appendix 3 shows the sites of recorded populations of *G. scabridus* in the reserve to date.

A



B

Plate 2.5 (A) Itala Nature Reserve is characterised by scenic terrain, grassed hilltops and rugged mountain tops. (B) The quartzite hillside along the Bergvliet Loop Road at Itala.

2.3 CONCLUSIONS

G. scabridus appears to be restricted to rocky areas, predominant features being quartzite outcrops with shallow soil that is low in nutrients, fairly high in organic carbon and possibly high in silicon. Elements like Zn and P may also be important. However, it is more likely that its limited distribution to these areas is more of an adaption to predation by animals as this is an important way of ensuring the survival of the species. These factors will all need to be considered when replanting the species in the wild.

It is hoped that visitors to Bivane Dam will not attempt to remove *G. scabridus* plants from their natural environment. Their growth habitat does mean that they should also be protected from illegal harvesting by man, another important process to aiding their survival in the wild.

G. scabridus plant population size varies and they appear to reproduce through seed set and dispersal, however, this appears to be limited to a certain extent by predation of the flowering stalks and seeds. Nevertheless, animals could play a role in seed dispersal if the seeds are deposited in the correct habitat for the subsequent growth of the plants.

Further studies in the wild, especially at Itala Nature Reserve, are vital to learning more about the growth habit of *G. scabridus* in the wild and especially as to why its distribution is threatened. We can only speculate at this point as to the reason behind this, although certain factors like nutritional restrictions appear to be ruled out. It is hoped that this study will lead the way for further studies on this species.

CHAPTER 3

PROPAGATION OF *GLADIOLUS SCABRIDUS*

3.1 SEED STUDIES

3.1.1 Introduction

Most agricultural crops, vegetables, garden and floricultural plants are reproduced by seed (Hartmann *et al.*, 1997). The main advantage of propagating bulbous plants from seed, is that the seedlings are initially healthy and not debilitated or disfigured by viral diseases which are more commonly transmitted by vegetative propagation (Rix, 1983). Optimum seed germination of gladioli is also important to breeders for the production of new species (Carpenter *et al.*, 1991). Plants grown from seed do, however, take longer to reach maturity (Rix, 1983).

A relatively recent area for seed propagation has developed through an interest in restoring natural areas and wildlife habitats through the reproduction of the natural range of species occurring in these environments (Hartmann *et al.*, 1997). Seed production provides an efficient and economic method of propagating such plants that are under threat in their natural environment. The National Botanical Gardens, as well as various seed companies, promote conservation through cultivation by making seeds of endangered species available for purchase and propagation. Thus it is important to know the optimum conditions for the storage and germination of these seeds.

G. scabridus flower spikes produce prolific numbers of seeds (Delpierre & du Plessis, 1973; Goldblatt & Manning, 1998). This provides an important source of material for the production of large numbers of plants of this vulnerable species. Hence, a study was conducted to ascertain the optimum conditions for *G. scabridus* seed germination. Once this was ascertained further studies were undertaken to determine the vigour, viability and moisture content of fresh and stored *G. scabridus* seeds. Vigour tests are essential for determining the storability of endangered species. They also provide commercial growers with the means to independently assess seed vigour, to justify buying seeds and to have the confidence in the performance of their crops (Karlovich, 1998). Breeders rely on the long-term storage of

seed to develop breeding lines (Carpenter *et al.*, 1991).

G. scabridus germination tests will aid in the commercial production of the species and determine how successful seed propagation would be in creating a large source of plant material for further propagation and for the bulking up of stock plants for replanting in the wild.

3.1.2 Materials and Methods

3.1.2.1 Germination

Seeds of *G. scabridus* (Plate 3.1) were collected from the KwaZulu-Natal Wildlife Biodiversity Research gardens, Queen Elizabeth Park, Pietermaritzburg. They were stored in a dry, dark place at room temperature (22 °C) for 6 months before the germination trial commenced.

The seeds were prepared by either leaving or removing the winged appendage and dusting with Captab® (Carpenter *et al.*, 1991). They were placed in 9 cm petri dishes on a double layer of Whatman No. 1 filter paper moistened with 5 ml distilled water. Ten seeds were placed in each petri dish with a resulting 100 seeds per treatment. Treatments of winged and dewinged seeds were subjected to either constant light or dark conditions. Petri dishes to be placed in constant darkness were wrapped in aluminium foil. All petri dishes were placed into plastic bags with a few drops of distilled water to maintain moisture. The bags were sealed and placed into four germination chambers set at 15, 20, 25 and 30 °C respectively and 60 % relative humidity, all with constant light provided by Osram fluorescent grow lamps (6.08 $\mu mol\ m^{-2}\ s^{-1}$). Daily germination counts were made of the seeds based on visible radicle protrusion through the testa. Seeds germinated in constant darkness were checked under a green safe light.

Data was analysed using the Genstat ANOVA programme (5th Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

3.1.2.2 Viability and vigour

Two pieces of germination paper (55.6 x 30.7 cm) were placed together. A pencil line was drawn across the centre of the top layer and they were then moistened with distilled water. Fifty dewinged *G. scabridus* seeds were placed evenly apart along the pencil line. The test consisted of seeds from 4 treatments: 2 year old and 1 year old seed which had been stored in a dry, dark cupboard at room temperature (22 °C) and fresh seed from field plants and plants grown in the greenhouse. Four replications per treatment were used. A third piece of germination paper was moistened as before and placed over the seeds. The paper was rolled together to form a tube of about 4 cm in diameter (Hartmann *et al.*, 1997). An elastic band was tied around the paper and the rolled paper was placed upright in a 2 L glass beaker with 40 ml distilled water. Each beaker was sealed in a plastic bag. Beakers were placed in a germination chamber set at 20 °C with constant lighting (6.15 µmol $m^{-2} s^{-1}$).

Seeds were monitored on a daily basis; the parameters noted were number of days to start of germination, 50 % germination and 100 % germination, number of seeds that had germinated and the length of the shoot and root of each seedling. The test was terminated when the seedlings of highest vigour were about 10 cm long (roots and shoots).

The test was repeated, however, the seeds were lightly dusted with Captab® (Carpenter *et al.*, 1991) before placing on the germination paper and 5 drops of Sporekill® was added to the 40 ml of distilled water in the 2 L beakers.

Data was analysed using the Genstat ANOVA programme (8th Edition, 8.1 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

3.1.2.3 Moisture content

One hundred dewinged *G. scabridus* seeds were weighed and then placed onto a piece of aluminium foil. Two year old, 1 year old and fresh seed was used with 3 reps per treatment. Seeds were placed into a drying oven set at 130 °C for an hour (Young & Young, 1986; International Seed Testing Association, 1999) after which they were reweighed and their moisture loss determined.

Data was analysed using the Genstat ANOVA programme (8th Edition, 8.1 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

Plate 3.1 Freshly harvested winged *G. scabridus* seeds prior to germination studies

3.1.3 Results and Discussion

3.1.3.1 Germination

The non-dormant seeds of many plant species, germinate equally well in light or darkness (Baskin & Baskin, 1988). This appears to be true for *G. scabridus* as the presence or absence of light had no significant effect on *G. scabridus* seed germination ($p=0.689$) at any of the temperatures (results not shown), indicating that the species does not require light for germination. Carpenter *et al.* (1991) found that *G. grandiflora* seed germination was light-independent. Light is, however, needed for healthy seedling growth once germination has taken place (Raven *et al.*, 1992). Radicles that emerged in the dark from *G. scabridus* seeds, were brown, long and spindly compared to those grown in the light which were green, more vigorous and healthy in appearance (Plate 3.2).

One of the most crucial factors in germinating seeds is a suitable temperature (Pickell, 1972; Mayer & Poljakoff-Mayber, 1982). *G. scabridus* is a summer flowering species (Pooley, 1998; Scott-Shaw, 1999), therefore the seeds are adapted to germinate in spring when temperatures are cooler (du Plessis & Duncan, 1989). Barton (1939) noted that gladioli seeds germinate better during cooler weather at temperatures of about 20 °C and that higher temperatures tend to reduce the germination percentage.

There was a significant interaction between the temperatures at which the *G. scabridus* seeds were germinated and the presence or absence of the wings on the seeds for all three measured variables: days to start of germination ($p<0.001$), days to final percentage germination ($p<0.001$) and final germination percentage ($p=0.032$).

There were no significant differences between seeds germinated at 15 and 20 °C (Table 3.1). The lower temperatures caused a decrease in days to start of germination and days to final percentage germination while increasing the final germination percentage of both winged and dewinged seeds. However, there appeared to be a tendency for better germination at 20 °C. Radicles and shoots that emerged from seeds at this temperature were healthier and grew more rapidly after emergence than those at 15 °C.

Germination at 25 and 30 °C was significantly poorer than at 15 and 20 °C. Both winged and dewinged seeds at these higher temperatures, took longer to commence germination, displayed longer germination periods and lower final germination percentages (Table 3.1). Those radicles that did emerge from dewinged seeds at 30 °C, developed brown tips and senesced shortly afterwards, an indication that this temperature was too high for successful germination and growth. No winged seeds germinated at 30 °C (Table 3.1). The winged appendage surrounding *G. scabridus* seeds thus appeared to hinder or delay germination which was observed by Griesbach (1972) in gladioli seeds, although Pickell (1972) found a delay of only a day or two. The wing could play a role in stressful environments, such as conditions experienced at 30 °C, by inducing temporary dormancy through the presence of inhibitory compounds (Venter & Venter, 1996; Baskin & Baskin, 1998) or a prevention of imbibition or gaseous exchange (Bewley & Black, 1985). Postponement of germination due to a temperature requirement or some other factor, will give the developing seedling a better chance of survival until conditions once again become favourable (Mayer & Poljakoff-Mayber, 1982; Murray, 1984; Venter & Venter, 1996). This is ideal for a vulnerable species, like *G. scabridus*, as this allows plants in the wild to be 'protected' from adverse conditions thus preserving the plant material.

***Table* 3.1 Germination of winged and dewinged *G. scabridus* seeds at 15, 20, 25 and 30 °C**

Temperature (°C)	Winged (w) or dewinged (de)	Days to start of germination [x]	Days to final % germination [y]	Final % germination [z]
15	w	11.9 cd	24.1 b	89 a
	de	11.3 cd	21.5 bc	98 a
20	w	8.8 d	20.5 bc	93 a
	de	9.8 cd	22.8 bc	94 a
25	w	18.2 a	46.0 a	71 b
	de	16.8 ab	40.8 a	71 b
30	w	-	-	-
	de	13.6 bc	17.7 c	18 c

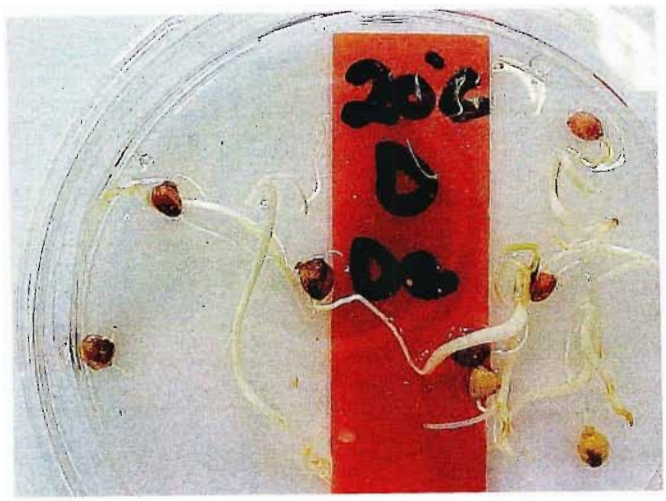
[x] LSD (5%) = 4.431

[y] LSD (5%) = 6.075

[z] LSD (5%) = 9.460

Letters a to e compare means in each column; n = 10 x 10 seeds

A



B

Plate 3.2 Dewinged *G. scabridus* seeds 4 days after germination at 20 °C in (A) the dark and (B) the light (6.08 µmol m^{-2} s^{-1})

3.1.3.2 Viability and vigour

The initial germination test for *G. scabridus* seed viability and vigour showed non-significant results between all the treatments as no seeds germinated. This was due to a severe fungal infestation which prevented the seeds from germinating. Hence the use of Captab® and Sporekill® during the second test in an attempt to reduce this infection as fungicides have been previously shown to reduce the presence of micro-organisms (Hartmann *et al.*, 1997; Cantliffe, 1998). During the second test, seeds that had been stored for a year or more yet again showed a high rate of fungal attack, however, there was far less fungal development on fresh seed.

None of the seeds that had been stored for 1 year germinated during the second test (Table 3.2). These seeds were slightly swollen in appearance at the termination of the test showing that water had been absorbed for germination (imbibition) but the fungal attack was severe and it is assumed that this inhibited any possible germination (Plate 3.3 A). Young and Young (1986) note that if seeds become covered with fungal or bacterial colonies soon after they are placed in an environment for germination, it is often an indication of (1) non-viable seeds, (2) seeds with low vigour, or (3) a grossly inadequate germination environment. The seeds were germinated under the ideal conditions determined during the previous germination test so germination environment was not the hindering factor. Thus it must be concluded that the fungus was an indication of a lack of vigour and viability amongst the older seed which was also evidenced by a lack of germination.

Gladiolus seed has been previously found to have irregular germination and a rapid loss of viability during storage (Carpenter *et al.*, 1991). Only 1.5 % of the seeds that had been stored for 2 years, germinated (Plate 3.3 B). There were no significant differences between seeds stored for 1 year or 2 years when comparing all the measured parameters (Table 3.2). As with 1 year old seed, the seeds swelled and those that did not germinate were covered with fungus, preventing healthy seed development.

The germination results for fresh seed were significantly different to seed that had been stored when comparing days to start of germination ($p=0.011$), days to 50 % germination ($p=0.007$) and days to final % germination ($p=0.002$). There were no significant differences between fresh seed from the greenhouse and fresh seed from the field when comparing these parameters (Table 3.2). Fresh seed took on average longer to start germinating and

longer to reach 50 % and final % germination because more seeds germinated under these treatments.

Fresh seed from greenhouse plants showed the significantly highest viability of 90 % ($p<0.001$) (Plate 3.3 C) which was significantly higher than all the treatments including fresh seed from field plants (46.5 %) (Plate 3.3 D). Thus fresh *G. scabridus* seed is significantly more viable than seed that has been stored for a year or more. Lewis *et al.* (1972) note that the seeds of many late-flowering wild species, such as *G. maculatus* 'Sweet', can only be germinated after 6 months of storage. However, the results show that *G. scabridus* requires no initial storage period and can be successfully germinated directly after harvest. Greenhouse seed showed little fungal infection and seed from the field slightly more so, however, this was certainly not as severe as stored seed. Thus *G. scabridus* seed that develops in a protected environment is less prone to attack from internal micro-organisms than seed from an open field or garden.

Plant viability during this germination test was directly proportional to plant vigour as noted by Cantliffe (1998). The results show that the more viable the seeds, the more vigour they had and the more healthy they were. This is evidenced by the seedling length results (Table 3.2). Fresh seed from the greenhouse was the most viable and these seedlings also had the significantly highest shoot ($p<0.001$), root ($p<0.001$) and total seedling length ($p<0.001$) of 4.016, 5.117 and 9.13 cm respectively on completion of the germination test. Seedling vigour was significantly lower in seed from the field, however, as with viability this was significantly better than seed that had been stored. Both viability and vigour do decline with time (Cantliffe, 1998). One year old seed showed no vigour due to a lack of germination and this was not significantly different to 2 year old seed which also had a very low vigour. Thus seedling length results show that fresh seed has more vigour than stored seed. Yet again the fungal attack is probable evidence of a lack of seed vigour.

***Table* 3.2 Germination and growth of fresh and stored *G. scabridus* seeds in moist, rolled paper at 20 °C with constant lighting (6.15 µmol m^{-2} s^{-1})**

Seed source	Days to start of germination [t]	Days to 50 % germination [u]	Days to final % germination [v]	Final % germination [w]	Shoot length (cm) [x]	Root length (cm) [y]	Total length (cm) [z]
2 year old seed	6.2 b	-	7.5 b	1.5 c	0.024 c	0.033 c	0.06 c
1 year old seed	-	-	-	-	-	-	-
Fresh seed from the green-house	14.5 a	19.8 a	24.0 a	90.0 a	4.016 a	5.117 a	9.13 a
Fresh seed from the field	18.5 a	11.8 a	26.5 a	46.5 b	2.044 b	2.494 b	4.54 b

[t] LSD (5%) = 10.12 [u] LSD (5%) = 11.02
[v] LSD (5%) = 14.50 [w] LSD (5%) = 34.50
[x] LSD (5%) = 0.3330 [y] LSD (5%) = 0.3884 [z] LSD (5%) = 0.710
Letters a to c compare means in each column; n = 4 x 50 seeds

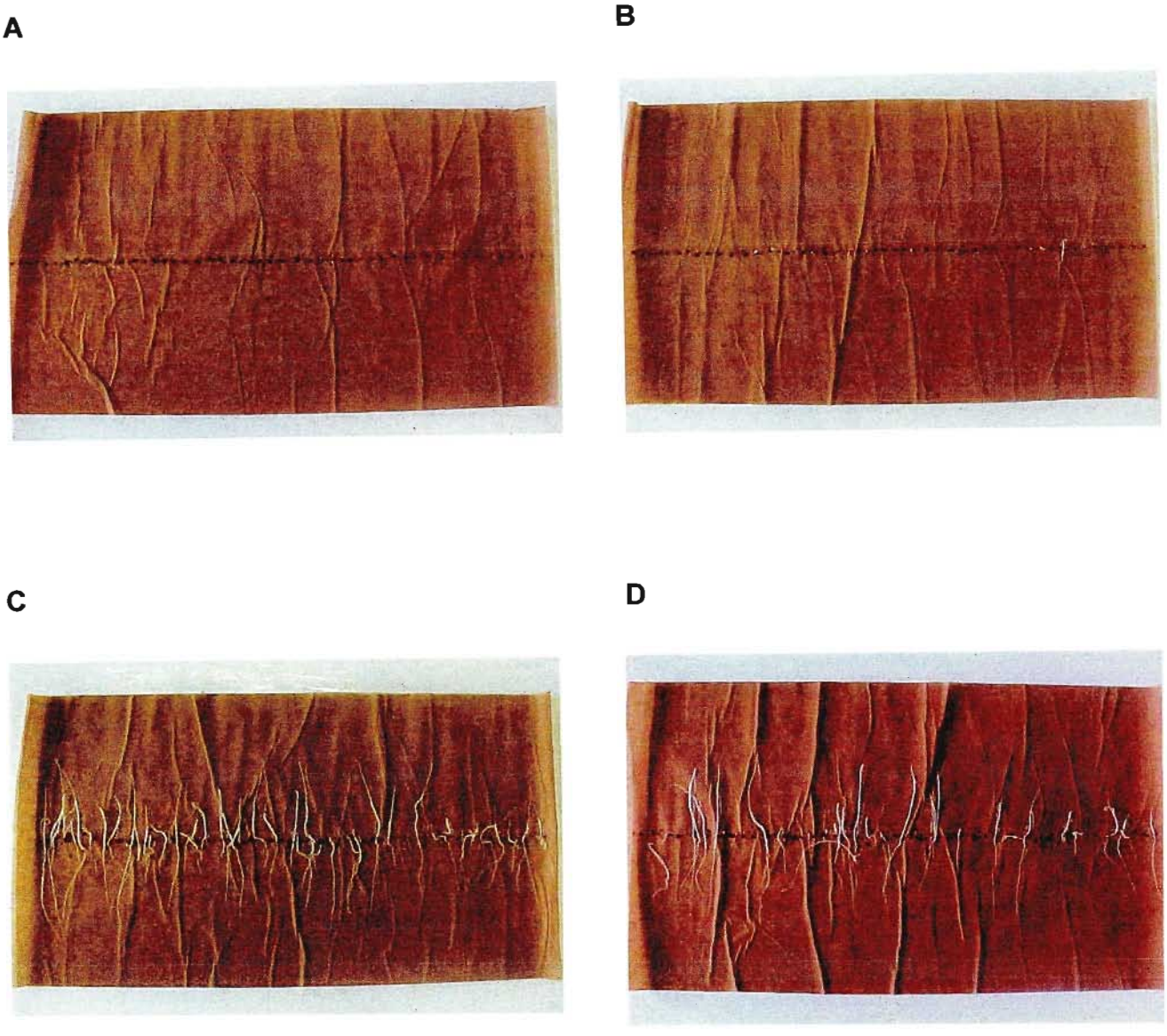


Plate 3.3 Germination of *G. scabridus* seed between moist paper at 20 °C and constant lighting (6.15 µmol m^{-2} s^{-1}) after storage for (A) 1 year and (B) 2 years, and fresh *G. scabridus* seed from (C) greenhouse and (D) field plants

3.1.3.3 Moisture content

The moisture content of seeds is one of the most important factors influencing their retention of viability and general appearance (U.S. Department of Agriculture, 1952). Visually, fresh *G. scabridus* seed appears larger, less shrivelled and lighter brown in colour compared to older seed. Fresh *G. scabridus* seed showed a significantly higher initial ($p=0.030$) and final ($p=0.034$) seed weight compared to seed that had been stored. There were no significant differences between stored seed for 1 year or 2 years. As seed ages, it loses moisture (Young & Young, 1986). Moisture loss results showed that fresh *G. scabridus* seed had the greatest moisture loss after drying ($p=0.021$) of 10.29 %, confirming that fresh *G. scabridus* seed contains a higher moisture content than older, stored seed. This result was significantly different to the moisture loss of 1 year old seed (8.95 %) and 2 year old seed (8.89 %).

Young and Young (1986) and Hartmann *et al.* (1997) note that a seed moisture content of about 10 % in oily seeds, such as those of *Gladiolus* (Griesbach, 1972), results in the active growth of storage fungi which destroy the seed embryo. This is a plausible explanation for the development of fungi in *G. scabridus* seeds. Seed preservation of endangered species is extremely important for long term survival and thus it is crucial to develop methods for the successful storage of *G. scabridus* seed to prevent fungal development during germination. Previously authored methods which aid in preventing fungal attack include drying the seeds as quickly as possible to a moisture content above 10 % and then maintaining them at this moisture content throughout storage, pouring boiling water over the seeds, rinsing the seeds with a 0.01 % solution of hydrogen peroxide (Young & Young, 1986), carefully monitoring the relative humidity through controlled atmosphere storage (Bass, 1979; Carpenter *et al.*, 1991), careful sanitation practises (Hartmann *et al.*, 1997) and storing the seed at low temperatures (Bass, 1979; Hartmann *et al.*, 1997). *Gladiolus* seed is tolerant of sub-freezing temperatures (Carpenter *et al.*, 1991) so this is certainly an option worth exploring.

***Table* 3.3 Moisture content of fresh and stored *G. scabridus* seed**

Seed source	Mean moisture content (%) [z]
2 year old seed	8.89 b
1 year old seed	8.95 b
Fresh seed	10.29 a

[z] LSD (5%) = 1.259
Letters a to b compare means in the column;
n = 3 x 100 seeds

3.1.4 Conclusions

The germination of *G. scabridus* seeds has proved to be a viable source of material for the successful propagation of large numbers of plants. These seeds germinate readily given the right conditions. To create the ideal germination conditions for these seeds, the winged appendage around the seeds should be removed and the seeds placed at 20 °C under constant light which allows for rapid and healthy radicle development. These temperature and light conditions appear to be linked to their natural conditions in the wild which should be used as an indicator of germination conditions both in this species and others like it. The presence of the oily wing appears to be very important as it protects the seeds during unfavourable conditions by preventing germination thus promoting the preservation of this threatened species in the wild and under controlled germination.

The seeds lose viability and vigour over time during storage. Fresh seed has a higher moisture content and is more viable, and hence should be the seed of choice. However, the storage of seed, particularly an endangered species like *G. scabridus*, is important and methods need to be developed to eliminate the fungal infection that reduces the viability and vigour of stored *G. scabridus* seed.

These initial studies provide scope for further studies to enhance germination of the species. This could involve the use of alternating light and dark conditions, scarification of the seeds and seed germination enhancers such as gibberellic acid. Any procedures that improve germination will mean that higher numbers of plants can be grown in a short space of time. This will aid in the building up of stock plants of this precious source of germplasm for further research and the bulking up of plants for returning to the wild to improve the conservation status of the species.

3.2 MICROPROPAGATION

3.2.1 Introduction

There is considerable commercial interest in gladioli, however, the relatively slow propagation rate of this crop by vegetative means, hinders its production. Micropropagation is an important method of speeding up the multiplication rate of the species. Therefore, it is highly desirable to develop methods accordingly (Bajaj *et al.*, 1982/1983). *In vitro* propagation thus provides an ideal method for the commercial multiplication and continuous distribution of large numbers of plants from limited sources (Wockok, 1981; Nicol, 1993; Armstrong, 2000) which would aid in the conservation or preservation of endangered species. This would be advantageous with a vulnerable species like *G. scabridus*.

Micropropagation has also evolved as a powerful and important tool for breeders and propagators alike, with breeders integrating aspects of tissue culture to speed up new product introductions and the expansion of product lines (van der Linde, 1992; Ball, 1998). The gene pool currently used for the production of hybrid gladioli is relatively small, so the tissue culturing of new or unknown species like *G. scabridus*, could greatly speed up these breeding programmes (Wilfret, 1971; Dickens *et al.*, 1986).

There is currently, however, a lack of commercially acceptable pathogen-free hybrid *Gladiolus* stock. *In vitro* culture provides an immediate and inexpensive way of producing disease and virus free material (Logan & Zettler, 1985; Pierik, 1988; Remotti *et al.*, 1997).

In vitro work on gladioli began in the early 1970's and knowledge and techniques have been developed and increased ever since. However, to date, very little investigation into the tissue culturing of wild species has been conducted. Due to the enormous benefits of such a propagation method and the potential that *G. scabridus* provides in this regards, it was decided to investigate and develop techniques for the micropropagation of this species.

3.2.2 Materials and Methods

Corms and cormels of *G. scabridus* were obtained from the KwaZulu-Natal Wildlife Biodiversity Research Gardens, Queen Elizabeth Park, Pietermaritzburg. They were dried in a cool place for 2 months and then stored for 6 weeks at 4 °C and 65 to 70 % relative humidity (Bertaccini & Marani, 1986), after which they were kept in the dark for a further 2 weeks at 24 °C and 65 to 70 % relative humidity. The material was then removed and prepared for tissue culture.

3.2.2.1 Stage 1 - shoot initiation

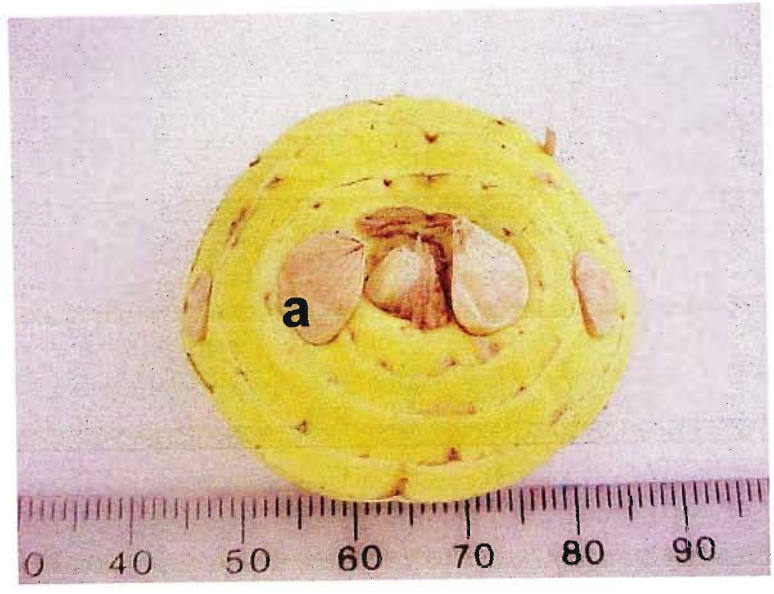
Explant disinfection and preparation

Corms: The dead, outer leaf bases surrounding each mature corm were removed (Plate 3.4 A) and the corms were washed thoroughly under running tap water. They were cut into smaller 20 x 20 mm pieces, each piece containing a visible axillary bud. These were treated in a 1 % (w/v) Benomyl® solution for 45 minutes, washed briefly in sterile ultra-pure water and further disinfected in 80 % (v/v) ethanol for 1 minute. This was followed by disinfection in 2 % (w/v) sodium hypochlorite (NaOCl) and a few drops of Tween 20 for 30 minutes. The material was then rinsed three times in sterile ultra-pure water for 10, 15 and 30 minutes respectively.

Each bud had its outer protective covering gently removed and was excised with a small wedge of corm material (8 x 8 mm) still attached (Ziv, 1979). These were disinfected in 1 % (w/v) NaOCl for 10 minutes followed by a rinse three times in sterile ultra-pure water.

Cormels: Each cormel had its outer husk removed (Plate 3.4 B) and was then gently washed in distilled water and a few drops of Tween 20. They were dipped for 20 seconds in 80 % (v/v) ethanol after which they were immersed in 0.5 % (w/v) NaOCl and Tween 20 for 15 minutes. This was followed by rinsing three times in sterile ultra-pure water.

A



B

Plate 3.4 (A) A mature *G. scabridus* corm showing axillary buds (a) and (B) *G. scabridus* cormels both used for *in vitro* propagation

Medium preparation

A modified Murashige and Skoog (MS) (1962) medium was used. The medium comprised seven stock solutions made up of analytical grade chemicals dissolved in ultra-pure water (Table 3.4). These were stored at 10°C with light sensitive compounds kept in the dark. The medium was supplemented with 0.01 % (w/v) myoinositol, 3 % (w/v) sucrose and growth regulators at various concentrations. The auxin, 1-naphthalene-acetic-acid (NAA) (Sigma), was dissolved in 2 ml of 0.2 M sodium hydroxide (NaOH) and the cytokinin, 6-benzyl-amino-purine (BAP) (Sigma), was dissolved in 0.2 M hydrochloric acid (HCl) and made up to volume with ultra-pure water. Medium pH was adjusted to 5.8 ± 0.1 with 0.2 M potassium hydroxide (KOH) and then solidified with 0.8 % agar (Associated Chemical Enterprises C.C.) which was dissolved by heating the medium in a microwave oven for 6 minutes.

Aliquots of 10 ml dissolved medium were dispensed into 20 x 100 mm glass test tubes which were then capped with aluminium tops (Cap-O-Test). The test tubes were autoclaved at 121 °C for 20 minutes.

Growth regulators

The limited availability of plant material made it necessary to limit the number of explants as well as the hormone concentrations tested. Corm and cormel explants were exposed to identical conditions and were cultured on MS medium with different combinations of NAA and BAP as follows: 0 mg l^{-1} NAA and 0 mg l^{-1} BAP, 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP, 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, and 5.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP.

Bench preparation

All culture processes were performed on a laminar flow blench which had been sprayed and sterilized with 80 % (v/v) ethanol before use. Further sterilization was achieved using an ultra violet (UV) light for 12 hours before culture. All transfer instruments and glassware were sterilized in the autoclave at 121 °C for 20 minutes.

***Table* 3.4 MS medium (adapted from Murashige & Skoog, 1962)**

Compound	Concentration (mg l^{-1})	Mass / 100 ml stock (g)	Stock solution
NH_4NO_3	1650	16.5	i
KNO_3	1900	19	
$CaCl_2.2H_2O$	440	4.4	ii
$MgSO_4.7H_2O$	370	3.7	iii
$MnSO_4.4H_2O$	22.3	0.223	
$ZnSO_4.7H_2O$	8.6	0.086	
$CuSO_4.5H_2O$	0.025	0.00025	
NaFeEDTA	74.5	0.745	iv
KH_2PO_4	170	1.7	v
H_3BO_3	6.2	0.062	
$Na_2MO_4.2H_2O$	0.25	0.0025	
KI	0.83	0.0083	vi
$CoCl_2.6H_2O$	0.025	0.00025	
Glycine	2	0.02	vii
Nicotinic Acid	0.5	0.005	
Pyridoxine HCl	0.5	0.005	
Thiamine HCl	0.1	0.001	

Culture process

All working surfaces and the culturer's hands and arms were regularly swabbed with 80 % (v/v) ethanol during culturing. All instruments were kept in a beaker with 95 % (v/v) ethanol and together with test tube openings, were flamed before and after transfer. The excised buds were placed onto the solidified MS medium in each test tube. Cormels were cut in half transversely and the cut surfaces placed on the medium (Sutter, 1986). Vials were recapped and sealed with Parafilm to prevent contamination. Ten bud and cormel halve explants were cultured per treatment and this process was repeated once a week over 5 culture dates.

From the second culture date, explant disinfection was further achieved using a fungicide and antibiotic combination (Haldeman *et al.*, 1987). The fungicide, Benomyl®, was added to the medium prior to autoclaving at a concentration of 0.2 % (w/v). The antibiotic, rifampicin (Sigma), was dissolved in dimethylsulphoxide (DMSO) and diluted by one-half with ultra-pure water resulting in a 5 mg ml^{-1} solution. Once the explants had been placed on the medium surfaces, 50 µl rifampicin was dispensed into each test tube through a syringe fitted with a 0.2 µm pore filter (National Scientific Company).

Culture conditions

Cultures were kept in a growth chamber at 25 ± 2 °C. Light was provided for 16 hours a day by Philips TLD 58 watt cool white fluorescent tubes (119.37 µmol m^{-2} s^{-1}).

Statistical analysis

The experiment was set up as a split-plot design and analysed using the Genstat statistical analysis program (5^{th} Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

3.2.2.2 Stage II - shoot proliferation

Proliferating shoot propagules were split and subcultured twice every 8 weeks onto fresh MS medium with the same treatments and culture conditions as used in stage I. The same statistical analysis was conducted as for stage I.

3.2.2.3 Stage III - rooting

Medium preparation

A modified MS medium was used and supplemented with 0.01 % (w/v) myoinositol, 1.5 % (w/v) sucrose and 0.2 % (w/v) Benomyl®. NAA was added at concentrations of 0, 0.5 and 1.0 mg l^{-1} with 0.3 % (w/v) activated charcoal (AC) being added to half the number of flasks. The medium was solidified with 0.8 % agar.

Glass Erlenmeyer flasks (Schott) of 500 and 1000 ml volumes were used and 130 and 250 ml aliqouts of medium were dispensed into each flask respectively and sealed with cotton wool plugs covered with aluminium foil. The flasks were then autoclaved for 20 minutes at 121 °C.

Culture process

Proliferating shoot propagules were removed from the test tubes, divided and placed on solidified rooting media in the Erlenmeyer flasks under sterile conditions on the laminar flow bench.

Culture conditions

As for stage I and stage II.

Statistical analysis

Shoot, root and cormlet data was statistically analysed as previously described. During this stage, a factor of 'previous treatment' was introduced into the treatment structure. This refers to the treatments used during stage I and II and was included to determine whether there were any residual effects of growth regulators used during these stages on the performance of explants during stage III.

3.2.2.4 Stage IV - hardening off

Electron microscopy

To investigate whether stomata had developed, a number of explants were removed from the flasks a few days prior to the commencement of stage IV. The shoots were cut into 5 mm^2 sections, placed on brass stubs and viewed under the Environmental Scanning Electron Microscope (ESEM) at magnifications of 500 to 3500 times and spot size of 4.0.

Medium preparation

The hardening off medium was comprised of vermiculite : perlite : coir (coconut hair) : river sand in the ratio 1:1:1:0.5 (v/v/v/v). This was autoclaved for 20 minutes at 121 °C in autoclave bags and allowed to cool overnight under a UV light on the laminar flow bench.

Transplanting process

Small, 10 cm-diameter, plastic pots were filled with hardening off medium. Rooted plantlets and plantlets with or without cormlets (cormels formed *in vitro*) were removed from the agar medium, washed with sterilized ultra-pure water to remove excess agar (Niemann, 2001), and planted in the medium. Each pot was watered with 30 ml sterile distilled water and sprayed with 2 % (w/v) Benomyl®. Pots were sealed in plastic bags, supported with wire, to retain high humidity and were placed in a modified growth chamber at 24 ± 2 °C with a 14 hour photoperiod provided by Osram flourescent lamps (17.3 µmol m^{-2} s^{-1}). Light intensity was later increased to 27.8 µmol m^{-2} s^{-1}.

At 3, 9, and 15 days after potting, plants were fertilized with 10 ml Chemicult® (1.0 mg l^{-1}) which was injected with a syringe into the media around the plantlets. Plantlets were watered again at 6, 12 and 18 days after potting. After 7 days, small holes were made in the plastic to allow for air circulation and to promote hardening off. A flat dish containing sterilized ultra-pure water was placed in the growth chamber to maintain a high humidity. Further holes were punched in the plastic bags after 12 days and larger holes after 16 days. Plantlets that survived were transferred in their pots to a well-lit room and placed near a light window. After 7 days plantlets were then transferred to a plastic mist tunnel (128 µmol m^{-2} s^{-1}). Those plantlets that became dormant in the media were allowed to dry out whilst still in the media.

The cormlets were then removed and stored at 4 °C and 65 to 70 % relative humidity for 8 weeks (Sutter, 1986; Dantu & Bhojwani, 1995) and planted out again in the same media outside under shade cloth.

Statistical analysis

Data was analysed using Genstat. Results for stage IV were analysed using the treatments from stage III as a factor to see whether they had any effect on this final stage.

3.2.3 Results and Discussion

3.2.3.1 Stage I - shoot initiation

Antibiotics have not been widely used in plant tissue culture as the possibility exists that they alter the growth and development of these tissues *in vitro* and may induce mutations (Dodds & Roberts, 1985; Pierik, 1988). There was 100 % contamination in the first *G. scabridus* culture. However, the use of the Benomyl® and rifampicin combination in successive cultures, proved to be successful in reducing infection. Although some latent contamination was observed, it occurred at a rate of 8 % or less in both axillary bud (Table 3.5) and cormel halve (Table 3.6) explants. Underground, bulbous organs are heavily contaminated with internal pathogens that are difficult to eliminate (Hussey, 1975; George, 1996) which often results in latent endogenous contamination as was observed in the *G. scabridus* cultures. Unfortunately, any permanent effects from the use of rifampicin will only be evident once the plantlets have been established for a few years and begun flowering. The use of meristem culture has been suggested as an alternative to using antibiotics (Pierik, 1988) although this is not a guaranteed method.

Axillary bud and cormel halve explants showed visible signs of shoot development after 5 and 7 days in culture respectively. Results shown in Tables 3.5 and 3.6 are prior to the first subculture onto fresh medium marking the beginning of stage II.

Axillary buds

The most successful results for *in vitro* shoot production are achieved using axillary bud explants (Dickens *et al.*, 1986; de Bruyn & Ferreira, 1992). This has become an important propagation method because it is simple, the rate of propagation is relatively fast, genetic stability is usually preserved and growth of the resulting plant is good (Pierik, 1988).

Shoots: *G. scabridus* axillary bud explants showed prolific shoot production by the end of stage I (Plate 3.5). Shoot production is usually initiated in the presence of BAP in the tissue culture medium (Dantu & Bhojwani, 1995; Hartmann *et al.*, 1997). The presence or absence of growth regulators had a significant effect on *G. scabridus* shoot growth ($p<0.001$) with the control cultures producing significantly more shoots (3.85) compared to the other treatments (Table 3.5). This implies that growth regulators are not critical for *in vitro G. scabridus* shoot production during stage I. This is similar to the results of Hussey (1975) and Sen and Sen (1995) who found that *Gladiolus* plantlets were formed without growth factors added to the medium. However, Ginzburg and Ziv (1973) observed that there was no growth of *Gladiolus* explants cultured on basal MS medium.

In the *G. scabridus* cultures, there was no significant difference in the number of shoots produced at each level of BAP (0.5 and 1.0 mg l^{-1}) within each NAA treatment, although more shoots were produced with the highest level of 1.0 mg l^{-1} BAP and the lowest level of 0.5 mg l^{-1} NAA (Table 3.5). Although lower in number than the control, these were slightly larger in size than other shoots with slight swelling of the shoot bases. Sutter (1986), Sen and Sen (1995), Lipsky *et al.* (1997) and Dinkleman and van Staden (1988), found that 1.0 mg l^{-1} benzyladenine (BA) favoured *Gladiolus* axillary bud regeneration but at the expense of elongation. Hussey (1976) noted that BAP promotes branching *in vitro*, however, it was found that BAP levels over 0.12 mg l^{-1} produced distorted shoots. *G. scabridus* shoots in the BAP cultures were all healthy, green and medium to large in size.

An increase in NAA from 0.5 to 5.0 mg l^{-1}, resulted in a significant decrease in the number of shoots at the various levels of BAP (Table 3.5). This is confirmed by Hussey (1975) who found that more shoots were induced at lower levels of NAA in *Gladiolus* cultures. Therefore higher levels of NAA had an inhibitory effect on shoot production in *G. scabridus* axillary bud cultures during stage I.

***Table* 3.5 Growth response of excised *G. scabridus* axillary buds cultured *in vitro* for 8 weeks**

Growth regulator	% aseptic cultures [x]	% uncontaminated explants		Average number of shoots [y]
		dead or browned	with shoots	
0 mg l^{-1} NAA [z] 0 mg l^{-1} BAP	94	10.63	89.37	3.85 a
0.5 mg l^{-1} NAA 0.5 mg l^{-1} BAP	92	8.69	91.31	2.76 b
0.5 mg l^{-1} NAA 1.0 mg l^{-1} BAP	94	8.51	91.49	3.02 b
5.0 mg l^{-1} NAA 0.5 mg l^{-1} BAP	94	6.38	93.62	2.04 c
5.0 mg l^{-1} NAA 1.0 mg l^{-1} BAP	92	10.86	89.14	1.94 c

[x] with the use of rifampicin and Benomyl®

[y] LSD (5%) = 0.397; Letters a to c compare means in the column; n = 5 x 10

[z] control treatment

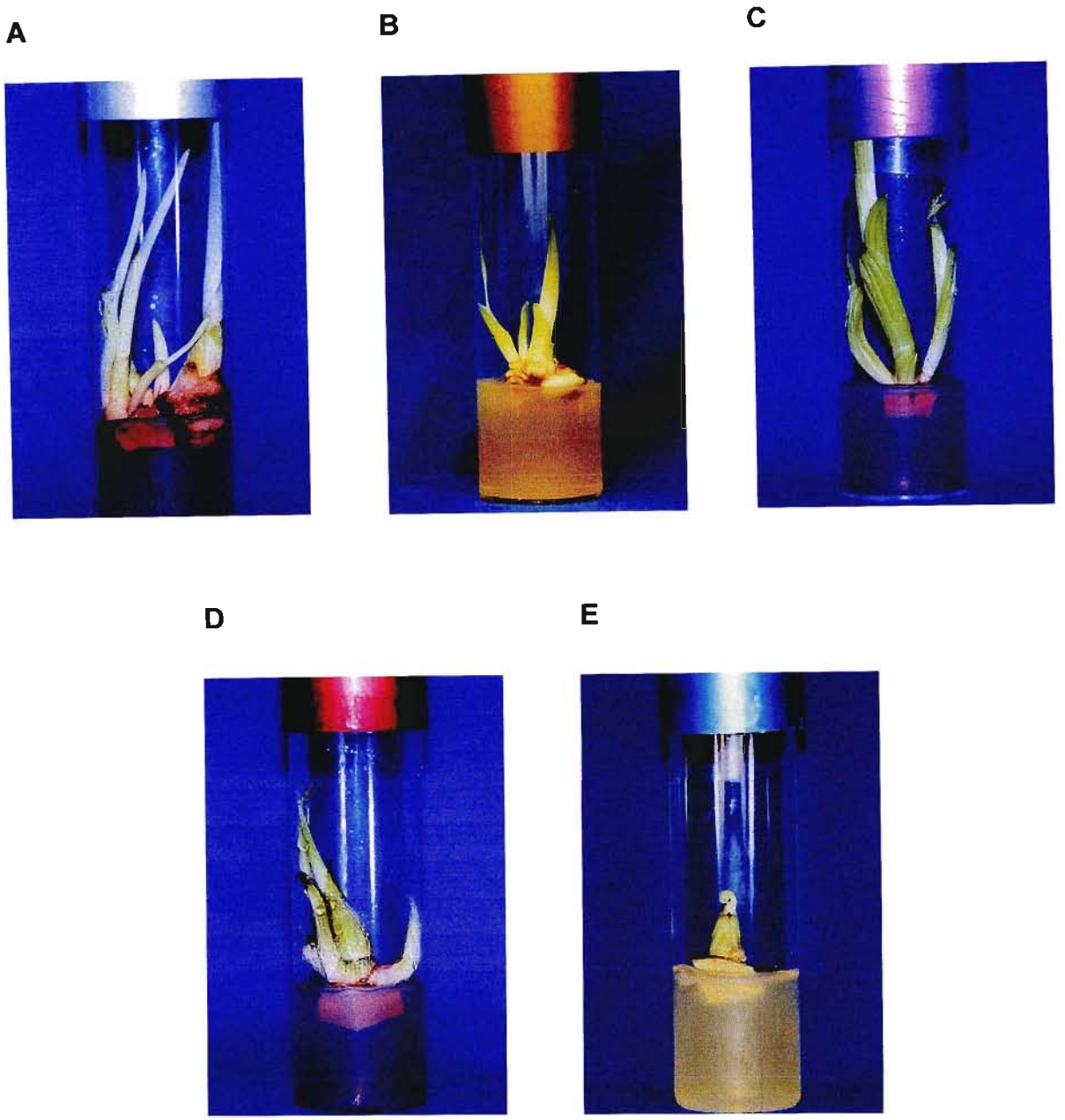


Plate 3.5 **Growth response of excised axillary buds from *G. scabridus* corms cultured *in vitro* on MS medium for 8 weeks with (A) 0 mg l^{-1} NAA and 0 mg l^{-1} BAP, (B) 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, (C) 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP, (D) 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP and (E) 5.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP**

Cormel halves

Cormel halve explants displayed both shoot and root development by the end of stage I (Plate 3.6). Cormel halves were placed with their cut surfaces on the media, therefore they either had their morphological bases facing towards or away from the media. Those explants with their bases facing downwards, developed shoots and roots, whereas those with their bases facing away from the media, produced mainly roots which were white and hairy in appearance (Plate 3.6 B).

Shoots: The treatments had a significant effect on both shoot number ($p<0.001$) and root number ($p=0.002$) (Table 3.6). Nicol (1993) found that in the absence of growth regulators, *Gladiolus* explants did not multiply but leaves elongated. However, control *G. scabridus* explants produced the highest number of shoots with an increase in NAA resulting in a significant decrease in shoot number, as with axillary bud explants (Table 3.6). Shoots with 0.5 mg l^{-1} NAA at both levels of BAP, were also taller and wider in appearance with some evidence of basal swelling, an indication of the start of cormlet formation. This level of NAA appears to be suitable for healthy and prolific *in vitro* shoot production of *G. scabridus* cormel halves. At both levels of NAA (0.5 and 5.0 mg l^{-1}), an increase in BAP from 0.5 to 1.0 mg l^{-1} resulted in a non-significant increase in shoot number. There was evidence of *G. scabridus* explant-splitting in the treatment containing 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP.

Roots: Auxin promotes root production in *Gladiolus* explants grown *in vitro* (Ziv, 1979; Bajaj *et al.*, 1982/1983). Nicol (1993) found that 1.0 mg l^{-1} NAA was the optimal concentration for rooting *G. tristis* var. *concolor*. A significantly higher number of *G. scabridus* roots were induced with 5.0 mg l^{-1} NAA at both levels of BAP (Table 3.6). Although greater in number, the roots at this treatment tended to be thicker and shorter in appearance than at 0.5 mg l^{-1} NAA. Root length and lateral branching decreases with an increase in NAA concentration (Nicol, 1993). The number of *G. scabridus* roots was not significantly different between the control and 0.5 mg l^{-1} NAA.

***Table* 3.6 Growth response of *G. scabridus* cormel halves cultured *in vitro* for 8 weeks**

Growth regulator	% aseptic cultures [w]	% uncontaminated explants			Average number of	
		dead or browned	with shoots	with roots	shoots [x]	roots [y]
0 mg l^{-1} NAA [z] 0 mg l^{-1} BAP	92	8.66	87.11	91.33	3.04 a	4.05 b
0.5 mg l^{-1} NAA 0.5 mg l^{-1} BAP	96	6.25	89.55	93.77	2.39 bc	4.10 b
0.5 mg l^{-1} NAA 1.0 mg l^{-1} BAP	94	8.51	87.1	89.32	2.53 b	4.35 b
5.0 mg l^{-1} NAA 0.5 mg l^{-1} BAP	92	10.94	89.77	87.55	1.93 c	5.45 a
5.0 mg l^{-1} NAA 1.0 mg l^{-1} BAP	94	4.26	91.32	95.77	2.00 c	5.22 a

[w] with the use of rifampicin and Benomyl®

[x] LSD (5%) = 0.462

[y] LSD (5%) = 0.758

[z] control treatment

Letters a to c compare means in each column; n = 5 x 10

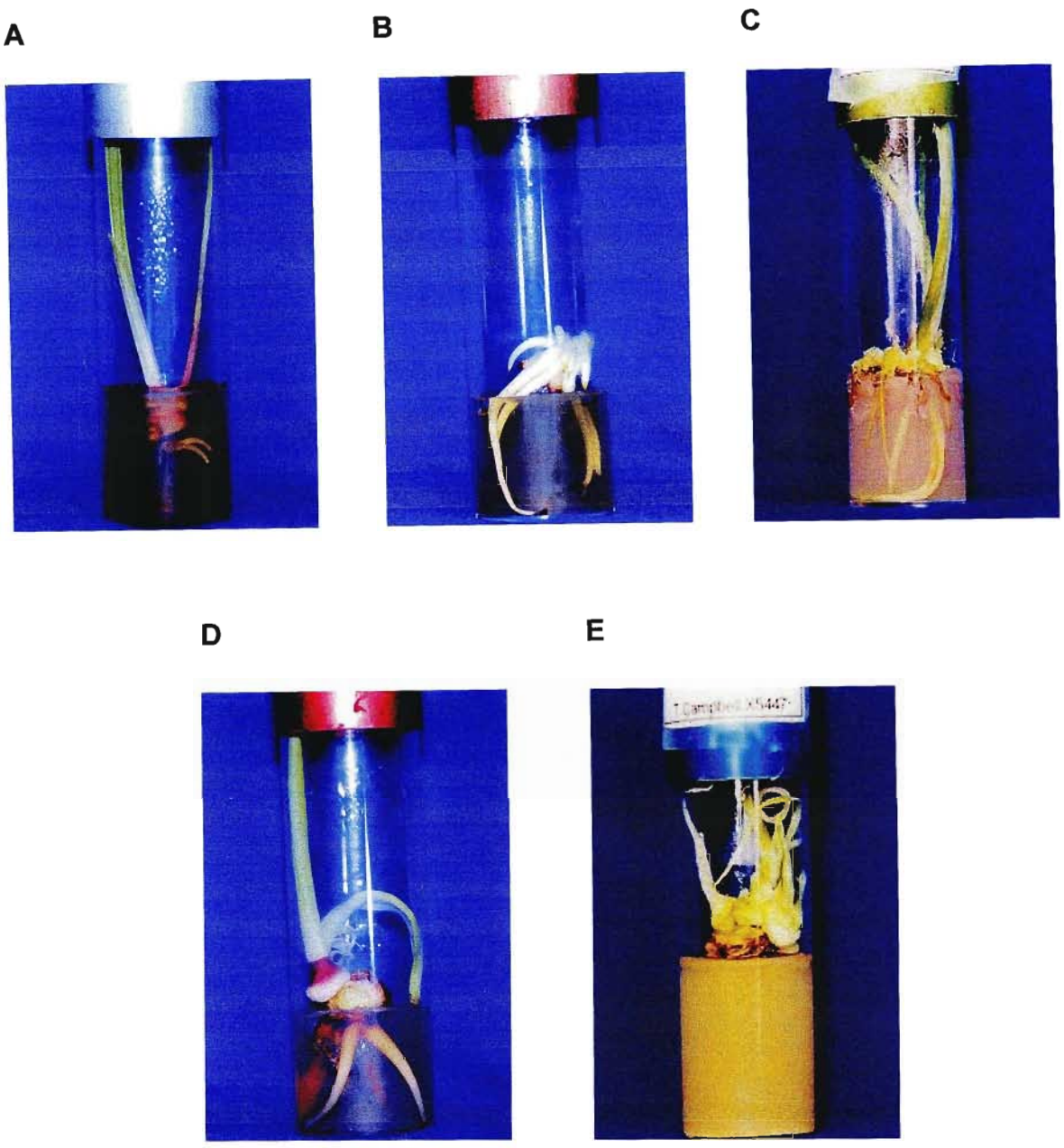


Plate 3.6 ***In vitro*** **growth response of** ***G. scabridus*** **cormel halves after 8 weeks in culture with their morphological bases facing towards or away from the MS media with (A) 0 mg l^{-1} NAA and 0 mg l^{-1} BAP, (B) 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, (C) 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP, (D) 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP and (E) 5.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP**

3.2.3.2 Stage II - shoot proliferation

Axillary buds

Shoots: The absence or presence of growth regulators had a highly significant effect on the number of shoots produced by axillary bud explants ($p<0.001$). The control, once again, showed a significantly higher shoot number (3.25) than the other treatments (Table 3.7). Hussey (1977b) noted that plantlets transferred to a basal medium, showed limited growth and eventually became dormant unless BAP was added to the medium. However, *G. scabridus* control cultures remained prolific throughout stage II. This was also observed by Sen and Sen (1995), Lipsky *et al.* (1997) and Remotti *et al.* (1997) who transferred regenerated plantlets from stage I to hormone free media for axillary bud proliferation and elongation and further shoot development during stage II. *G. scabridus* control explants were, however, taller and thinner in appearance than the other treatments (Plate 3.7). Sutter (1986) found that shoots formed on medium lacking hormones or containing NAA, were morphologically distinct from those formed on BA which were thicker in appearance.

BAP promotes leaf growth, the number of shoots increasing with increasing BAP concentration (Hussey, 1977b). However, in the *G. scabridus* trial, the increase in BAP from 0.5 to 1.0 mg l^{-1} at each level of NAA, did not have a significant effect on shoot number. De Bruyn and Ferreira (1992), however, observed significant differences in the average number of shoots formed per *G. tristis* explant due to different BA concentrations.

There was a significant difference in shoot number between each level of NAA (0.5 and 5.0 mg l^{-1}) with an increase in NAA causing a decrease in shoot number, indicating that higher NAA has an inhibitory effect on *G. scabridus* shoot proliferation. This was observed by Bajaj *et al.* (1982/83) who found that a lower NAA level of 0.1 mg l^{-1} resulted in better shoot production than 10 mg l^{-1} NAA. *G. scabridus* shoots with 0.5 mg l^{-1} NAA were taller and healthy in appearance whereas those at 5.0 mg l^{-1} NAA, were short, thick and pale in colour (Plate 3.7). The lower level of 0.5 mg l^{-1} is therefore the favoured level of NAA to use during stage II for *G. scabridus* culture as it resulted in the greater numbers of shoots that were better in appearance.

A few *G. scabridus* axillary bud explants at 5.0 mg l^{-1} NAA, displayed slight callusing along their periphery. Ziv *et al.* (1970) and Wilfret (1971) confirmed that high levels of NAA are necessary to produce callus from *Gladiolus* explants. However, the focus of the *G. scabridus* cultures was not on callus production and it did not affect the later stages of growth, so this was not pursued any further. *In vitro* techniques that enhance the natural mechanisms of axillary and adventitious shoot formation are better for long-term multiplication and genetic stability (Hussey, 1976; 1977a; b).

Roots: Axillary bud explants displayed root development on the shoot proliferating media during stage II. The effect of treatment on root number was highly significant ($p<0.001$). Overall root development of *G. scabridus* axillary bud explants was poor and appears to be inhibited by the presence of growth regulators during stage II as significantly more roots were produced in the control treatment (1.30) compared to all the other treatments (Table 3.7). Roots formed in the presence of NAA were thicker than those in the basal medium. There was no significant difference in root number between treatments with growth regulators, although there was a tendency for more roots to develop at 5.0 mg l^{-1} NAA within each of the BAP levels. BAP can inhibit root growth and development (Hussey, 1977b) but in the *G. scabridus* cultures, NAA may have a stronger role to play in initiating root formation. This is confirmed by Dickens *et al.* (1986) who found that 5.0 mg l^{-1} NAA with or without cytokinin stimulated *Gladiolus* root formation.

Cormlets: *G. scabridus* axillary bud explants also showed cormlet development by the end of stage II and this varied significantly between the treatments ($p=0.001$). The lowest number of cormlets were initiated by the control (0.41). Significantly better cormlet development occurred with 5.0 mg l^{-1} NAA than with 0.5 mg l^{-1} NAA (Table 3.7), showing that NAA is important for cormlet production in *G. scabridus* cultures. However, Dickens *et al.* (1986) found that NAA by itself (5.0 mg l^{-1}) or with kinetin at 0.5 mg l^{-1} had an inhibitory effect on corm proliferation.

Dantu and Bhojwani (1995) observed that the fresh mass and size of cormlets is reduced in the presence of BAP. De Bruyn and Ferreira (1992) indicated that the omission of BA from the culture media can result in significantly more corms being produced. However, in the work being reported on, BAP did not have a significant effect on *G. scabridus* cormlet production at each NAA level.

Table 3.7 **Growth response of excised *G. scabridus* axillary buds during stage II of *in vitro* propagation**

Growth regulator	% aseptic cultures	% explants dead or browned	Average number of		
			shoots [w]	roots [x]	cormlets [y]
0 mg l^{-1} NAA [z] 0 mg l^{-1} BAP	96.86	3.97	3.25 a	1.30 a	0.41 c
0.5 mg l^{-1} NAA 0.5 mg l^{-1} BAP	95.14	1.33	2.94 b	0.37 b	0.56 bc
0.5 mg l^{-1} NAA 1.0 mg l^{-1} BAP	94.28	4.32	2.93 b	0.36 b	0.45 c
5.0 mg l^{-1} NAA 0.5 mg l^{-1} BAP	96.32	3.94	2.00 c	0.46 b	0.73 ab
5.0 mg l^{-1} NAA 1.0 mg l^{-1} BAP	95.7	1.82	2.20 c	0.54 b	0.91 a

[w] LSD (5%) = 0.2612

[x] LSD (5%) = 0.2730

[y] LSD (5%) = 0.2326

[z] control treatment

Letters a to c compare means in each column

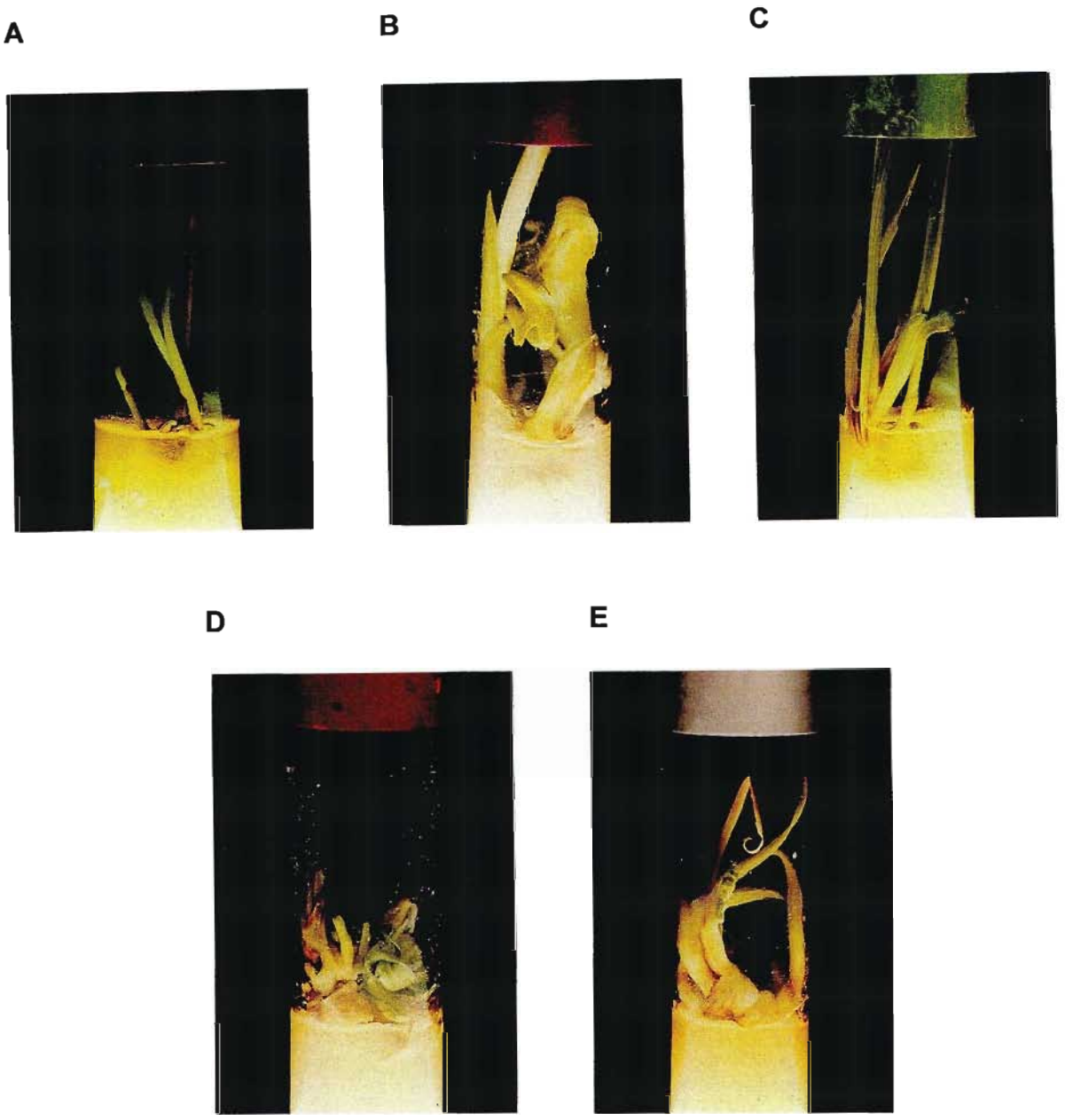


Plate 3.7 Growth response of excised axillary buds from *G. scabridus* corms at the end of stage II cultured *in vitro* on MS medium with (A) 0 mg l^{-1} NAA and 0 mg l^{-1} BAP, (B) 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, (C) 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP, (D) 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP and (E) 5.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP

Cormel halves

Shoots: Shoot number was not significantly affected by the presence or absence of NAA and BAP ($p=0.170$). There was, however, a tendency for the higher level of 5.0 mg l^{-1} NAA to result in fewer shoots (Table 3.8). As with the axillary bud cultures, the control shoots were thinner and taller than the other treatments. This was observed by Sutter (1986) who found that corms left on a medium without growth regulators develop thin, grass-like shoots. A difference in shoot appearance between the levels of NAA was not as prominent as in the axillary bud explants but shoots with 0.5 mg l^{-1} NAA did look healthier and stronger (Plate 3.8).

Roots: Significantly more roots ($p<0.001$) were produced with 5.0 mg l^{-1} NAA than 0.5 mg l^{-1} NAA or the control (Table 3.8). Lilien-Kipnis and Kochba (1987) report that root number is directly proportional to auxin concentration which appears to hold true for *G. scabridus* cultures. However, although not significantly different, *G. scabridus* control explants produced more roots than 0.5 mg l^{-1} NAA.

Within each level of NAA there was no significant difference in root number between each level of BAP. However, at both levels of NAA, there was a tendency for better root growth with 0.5 mg l^{-1} BAP than 1.0 mg l^{-1} BAP, yet again indicating that BAP appears to suppress root growth. This is confirmed by Hussey (1977a) who observed that shoots transferred to media with low concentrations or no cytokinin, spontaneously form roots.

Cormlets: There were no significant differences between the cormlet numbers at the various treatments ($p=0.662$) (Table 3.8). However, fewer cormlets were produced with the control as observed by Steinitz and Lilien-Kipnis (1989) who found that fewer *Gladiolus* cormlets develop in medium without growth regulators. The effect of BAP was also not significant. Steinitz and Lilien-Kipnis (1989) found that BA inhibited corm formation, whereas Ginzburg and Ziv (1973), Ziv (1979) and Sutter (1986) found that cytokinin induced cormel differentiation.

Table 3.8 **Growth response of *G. scabridus* cormel halves during stage II of *in vitro* propagation**

Growth regulator	% aseptic cultures	% explants dead or browned	Average number of		
			shoots [w]	roots [x]	cormlets [y]
0 mg l^{-1} NAA [z] 0 mg l^{-1} BAP	97.46	4.27	2.59	3.57 b	0.65
0.5 mg l^{-1} NAA 0.5 mg l^{-1} BAP	96.16	2.67	3.27	3.35 b	1.33
0.5 mg l^{-1} NAA 1.0 mg l^{-1} BAP	96.34	2.76	2.38	3.32 b	0.71
5.0 mg l^{-1} NAA 0.5 mg l^{-1} BAP	96.45	3.84	2.01	4.76 a	0.82
5.0 mg l^{-1} NAA 1.0 mg l^{-1} BAP	97.5	2.54	1.81	4.67 a	0.87

[w] not significant

[x] LSD (5%) = 0.659

[y] not significant

[z] control treatment

Letters a to b compare means in the column

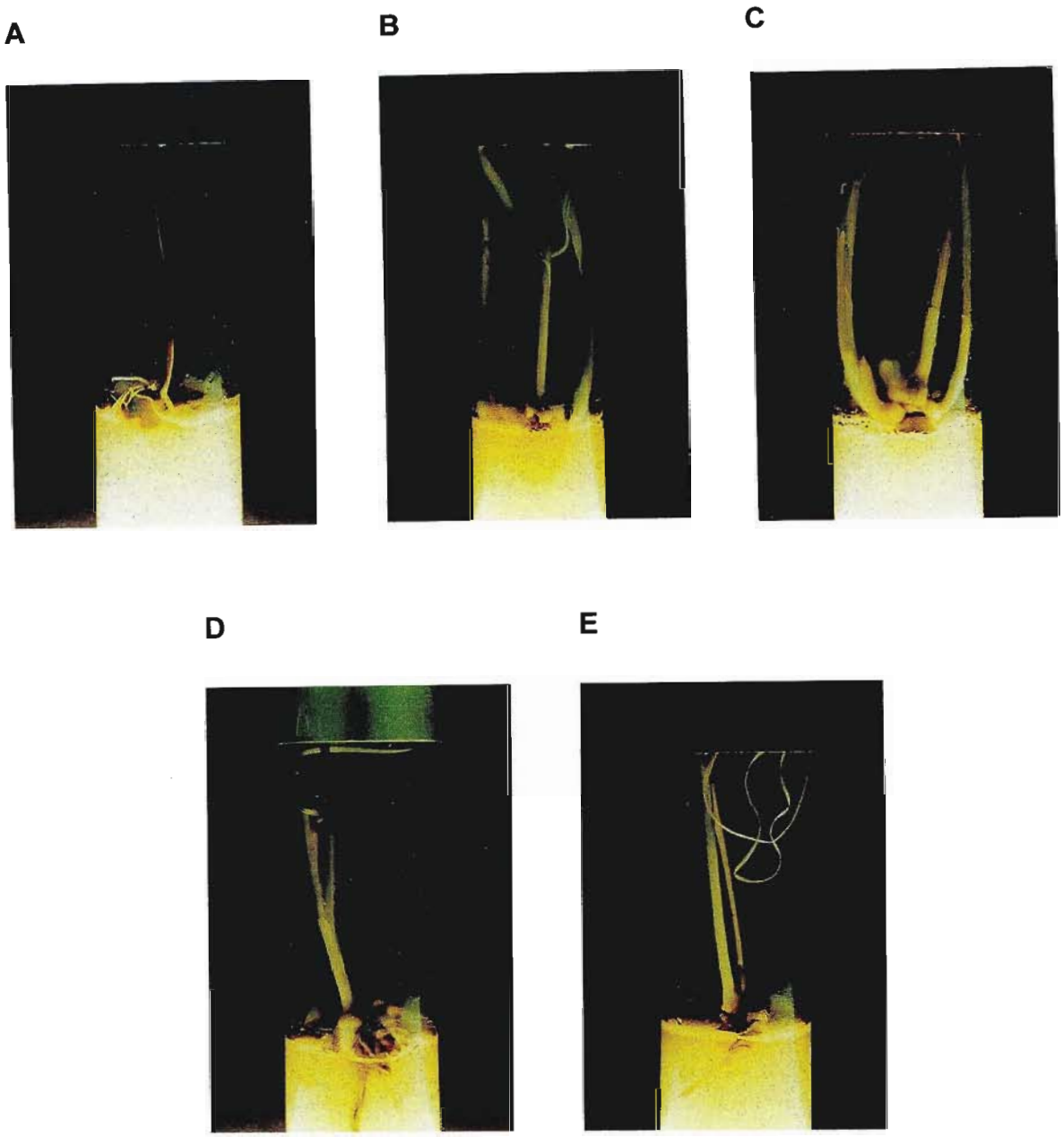


Plate 3.8 Growth response of *G. scabridus* cormel halves at the end of stage II cultured *in vitro* on MS medium with (A) 0 mg l^{-1} NAA and 0 mg l^{-1}BAP, (B) 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BAP, (C) 0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP, (D) 5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP and (E) 5.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP

3.2.3.3 Stage III - rooting

Axillary buds

Shoots: There was no significant interaction between the previous treatments from stage I and II and the levels of NAA and AC used during stage III ($p=0.504$). There was, however, a significant interaction between the new levels of NAA and AC ($p<0.001$). The greatest number of shoots (4.34) developed with 0 mg l^{-1} NAA and 0 % AC and the least number (1.70) with 1.0 mg l^{-1} NAA and 0 % AC. The higher the level of NAA in the rooting media, the lower the number of shoots at both levels of AC (Table 3.9), indicating that NAA inhibits shoot development of *G. scabridus* axillary bud explants in the rooting media.

Lilien-Kipnis and Kochba (1987) found that AC in the rooting medium has a beneficial effect on *Gladiolus* shoot growth. At the lower NAA levels of 0 and 0.5 mg l^{-1}, *G. scabridus* explants produced significantly more shoots without AC, (4.24 and 3.04 respectively), however, at 1.0 mg l^{-1} NAA, significantly more shoots developed with AC (2.05).

Roots: It is often very difficult to induce functioning root systems *in vitro* (Debergh & Maene, 1981) and plantlets need well developed roots to aid their survival during the hardening off phase (Ziv, 1979; Bertaccini & Marani, 1986). Lilien-Kipnis and Kochba (1987) found that *in vitro* rooting was greatly affected by the auxin content and the presence of AC in the medium. Both compounds influenced the number, length and morphology of these root systems.

In the *G. scabridus* cultures no roots developed in the absence of AC (Table 3.10). Media unsupplemented with AC became creamy brown in colour in many of the cultures. This could be as a result of a build up of toxic compounds on the wounded explant surfaces due to phenolic oxidation (Pierik, 1988). AC has the ability to eliminate toxic compounds thereby inhibiting this oxidation, thus providing a better culture environment for *in vitro* plantlets and improving rooting (Pierik, 1987; 1988). Roots that developed with 0.3 % AC were white, branched and healthy in appearance (Plate 3.9 A). Ziv (1979) added 0.3 % AC to pre-transplanting medium to improve the root growth of *Gladiolus* axillary bud explants under high light intensities.

G. scabridus root development was significantly affected by the interaction between the previous treatments from stages I and II and the new levels of NAA and AC ($p=0.013$). Explants exposed to 5.0 mg l^{-1} NAA during the initial stages, showed significantly better root development with 0.3 % AC at all three levels of NAA during stage III. Thus a prolonged exposure to NAA enhances root growth. During this third stage, increasing levels in NAA with 0.3 % AC resulted in a significant increase in root number. No roots formed when both NAA and AC were omitted from the media in all the previous treatments. However, Jager *et al.* (1998) successfully rooted explants on hormone free MS medium without AC. In the *G. scabridus* cultures rooting did take place without the addition of NAA to AC media, indicating that AC is more important than NAA for the rooting of these explants.

***Cormlets*:** The interaction between the previous treatments and NAA and AC on cormlet number was highly significant ($p<0.001$). Explants exposed to treatment four (5.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP) during stages I and II, produced significantly more cormlets than those exposed to the other four treatments (Table 3.11). A prolonged exposure to high NAA therefore improves cormlet development.

Sutter (1986) found that a medium with NAA or no growth regulators, promotes the formation of cormlets. *G. scabridus* cormlet development was significantly affected by NAA applied during stage III as the highest number of cormlets formed with the highest level of NAA (1.0 mg l^{-1}) and 0.3 % AC over all the previous treatments, indicating that NAA and AC both enhance cormlet formation (Plate 3.9 B). Takayama and Misawa (1980) suggest that an increase in bulb formation due to the presence of AC could be due to the regulation of internal physiological processes by AC. The effects of AC have also been attributed to establishing polarity by providing a dark environment in the medium, adsorption of plant growth regulators and other organic compounds and the release of substances naturally present in or adsorbed by AC which are beneficial to the growth and development of *in vitro* cultures (Pan & van Staden, 1998).

***Table* 3.9 Number of shoots produced *in vitro* by excised *G. scabridus* axillary buds during stage III**

NAA (mg l^{-1})	AC (%)	
	0	0.3
0	4.34 a	3.44 b
0.5	3.04 c	2.46 d
1.0	1.70 f	2.05 e

LSD (5%) = 0.316; Letters a to f compare means in the table

***Table* 3.10 Number of roots produced *in vitro* by excised *G. scabridus* axillary buds during stage III**

Growth regulator	Previous treatment from stages I and II				
	1 [v]	2 [w]	3 [x]	4 [y]	5 [z]
0 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
0 mg l^{-1} NAA 0.3 % AC	3.23 i	2.70 i	2.98 i	4.32 h	4.38 h
0.5 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
0.5 mg l^{-1} NAA 0.3 % AC	7.02 f	7.53 ef	6.08 g	9.62 bc	8.89 cd
1.0 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
1.0 mg l^{-1} NAA 0.3 % AC	11.22 a	9.27 c	8.26 de	11.37 a	10.28 b

[v] 0 mg l^{-1} NAA, 0 mg l^{-1} BAP [w] 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[x] 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} BAP [y] 5.0 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[z] 5.0 mg l^{-1} NAA, 1.0 mg l^{-1} BAP

LSD (5%) = 0.450; Letters a to j compare means in the table

Table 3.11 **Number of cormlets produced *in vitro* by excised *G. scabridus* axillary buds during stage III**

Growth regulator	Previous treatment from stages I and II				
	1 [v]	2 [w]	3 [x]	4 [y]	5 [z]
0 mg l^{-1} NAA 0 % AC	0.61 o	0.79 o	0.65 o	0.93 on	0.85 o
0 mg l^{-1} NAA 0.3 % AC	2.85 ij	2.13 klm	1.56 mn	2.93 ij	3.48 hi
0.5 mg l^{-1} NAA 0 % AC	1.89 ml	2.63 jkl	2.03 klm	2.19 jklm	2.18 klm
0.5 mg l^{-1} NAA 0.3 % AC	5.70 d	5.40 de	4.77 ef	6.76 c	5.54 d
1.0 mg l^{-1} NAA 0 % AC	3.90 gh	4.24 fg	3.42 hi	4.81 ef	3.50 ghi
1.0 mg l^{-1} NAA 0.3 % AC	7.08 c	6.53 c	7.82 b	8.70 a	8.46 ab

[v] 0 mg l^{-1} NAA, 0 mg l^{-1} BAP [w] 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[x] 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} BAP [y] 5.0 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[z] 5.0 mg l^{-1} NAA, 1.0 mg l^{-1} BAP

LSD (5%) = 0.737; Letters a to o compare means in the table

A

B

Plate 3.9 ***In vitro*** **(A) root development with AC (left) and without AC (right) and (B) shoot and cormlet (z) development of** ***G. scabridus*** **explants during stage III**

Cormel halves

Shoots: The combined effect of the previous treatments and NAA and AC during stage III on the number of shoots formed, was significant ($p=0.014$). Treatment 3 during stage I and II (0.5 mg l^{-1} NAA and 1.0 mg l^{-1} BAP) resulted in the significantly highest numbers of shoots (4.16) with 0 mg l^{-1} NAA and 0 % AC during stage III and the significantly lowest number of shoots (1.63) with 1.0 mg l^{-1} NAA and 0.3 % AC. Over all the previous treatments, an increase in NAA during stage III resulted in a decrease in shoot number, yet again indicating that high levels of NAA inhibit shoot production (Table 3.12).

Roots: The interaction between the previous treatments and NAA and AC, also had a significant effect on root number ($p=0.002$). As with axillary buds, no roots developed in the cultures without AC (Table 3.13). Roots already present from stage II, continued to elongate in the absence of AC but no new roots were formed. Logan and Zettler (1985) found that AC was necessary for successful rooting *in vitro* and Ziv (1979), Lilien-Kipnis and Kochba (1987) and Nicol (1993) observed that root number, root length and lateral branching was significantly increased by the addition of AC.

Those explants exposed to high levels of NAA (5.0 mg l^{-1}) in stages I and II (treatments four and five), showed significantly better root development than those exposed to lower NAA levels confirming that a prolonged exposure to NAA is beneficial for root production. An increase in NAA during stage III with 0.3 % AC, also showed an improvement in the root number over all the previous treatments.

Cormlets: There was no significant interaction between the previous treatments, NAA and AC ($p=0.184$) on cormlet development. However, there was a significant secondary interaction between the previous treatments and NAA ($p=0.002$) and NAA and AC during stage III ($p<0.001$).

An increase in the levels of NAA (0 to 1.0 mg l^{-1}) over all the previous treatments, improved the number of cormlets formed (Table 3.14). Explants exposed to treatments four and five (5.0 mg l^{-1} NAA) during stages I and II had higher numbers of cormlets at the end of stage III. As with rooting, the prolonged exposure of the explants to high levels of NAA appears to aid in the formation of cormlets.

Significantly higher numbers of cormlets formed at the highest level of NAA (1.0 mg l^{-1}) with 0.3 % AC, and the lowest number with 0 mg l^{-1} and 0 % AC, indicating that both play a role in *in vitro* cormlet development during stage III (Table 3.15). This is confirmed by Lilien-Kipnis and Kochba (1987) who found that the use of an auxin during rooting, enhances the yield of cormels harvested. They suggest that AC could affect the accumulation of storage products in the plant. Mielke and Anderson (1986) found that *in vitro* bulbing in iris was not enhanced by adding AC or auxin to the medium whereas Steinitz and Yahel (1982) found AC essential for bulblet regeneration in *Narcissus*.

***Table* 3.12 Number of shoots produced *in vitro* by *G. scabridus* cormel halves during stage III**

Growth regulator	Previous treatment from stages I and II				
	1 [v]	2 [w]	3 [x]	4 [y]	5 [z]
0 mg l^{-1} NAA 0 % AC	3.88 ab	3.35 cd	4.16 a	3.99 ab	3.47 bc
0 mg l^{-1} NAA 0.3 % AC	3.65 bc	3.57 bc	3.58 bc	3.41 cd	3.77 abc
0.5 mg l^{-1} NAA 0 % AC	2.75 efg	2.87 ef	2.48 fgh	2.55 efg	2.70 efg
0.5 mg l^{-1} NAA 0.3 % AC	2.97 de	2.66 efg	2.85 ef	2.75 efg	2.45 fgh
1.0 mg l^{-1} NAA 0 % AC	1.78 j	2.29 ghi	1.80 j	1.80 j	1.95 ij
1.0 mg l^{-1} NAA 0.3 % AC	1.90 ij	2.02 hij	1.63 j	1.97 ij	1.67 j

[v] 0 mg l^{-1} NAA, 0 mg l^{-1} BAP
[w] 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BAP
[x] 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} BAP
[y] 5.0 mg l^{-1} NAA, 0.5 mg l^{-1} BAP
[z] 5.0 mg l^{-1} NAA, 1.0 mg l^{-1} BAP

LSD (5%) = 0.473; Letters a to j compare means in the table

***Table* 3.13 Number of roots produced *in vitro* by *G. scabridus* cormel halves during stage III**

Growth regulator	Previous treatment from stages I and II				
	1 [v]	2 [w]	3 [x]	4 [y]	5 [z]
0 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
0 mg l^{-1} NAA 0.3 % AC	4.85 g	3.48 i	3.55 i	4.46 gh	4.35 h
0.5 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
0.5 mg l^{-1} NAA 0.3 % AC	7.10 e	6.16 f	6.11 f	7.22 e	8.15 d
1.0 mg l^{-1} NAA 0 % AC	0 j	0 j	0 j	0 j	0 j
1.0 mg l^{-1} NAA 0.3 % AC	10.64 b	9.8 c	10.89 b	11.69 a	11.97 a

[v] 0 mg l^{-1} NAA, 0 mg l^{-1} BAP
[w] 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BAP
[x] 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} BAP
[y] 5.0 mg l^{-1} NAA, 0.5 mg l^{-1} BAP
[z] 5.0 mg l^{-1} NAA, 1.0 mg l^{-1} BAP

LSD (5%) = 0.483; Letters a to j compare means in the table

***Table* 3.14 Number of cormlets produced *in vitro* by *G. scabridus* cormel halves during stage III**

NAA ($mg\ l^{-1}$)	Previous treatment from stages I and II				
	1 [v]	2 [w]	3 [x]	4 [y]	5 [z]
0	1.81 g	1.39 h	1.74 gh	1.78 gh	1.77 gh
0.5	3.26 e	2.59 f	2.65 f	3.67 cd	3.33 de
1.0	4.61 b	3.99 c	4.72 b	5.16 a	5.32 a

[v] 0 mg l^{-1} NAA, 0 mg l^{-1} BAP [w] 0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[x] 0.5 mg l^{-1} NAA, 1.0 mg l^{-1} BAP [y] 5.0 mg l^{-1} NAA, 0.5 mg l^{-1} BAP

[z] 5.0 mg l^{-1} NAA, 1.0 mg l^{-1} BAP

LSD (5%) = 0.404; Letters a to h compare means in the table

***Table* 3.15 Number of cormlets produced *in vitro* by *G. scabridus* cormel halves during stage III**

NAA (mg l^{-1})	AC (%)	
	0	**0.3**
0	1.09 e	2.31 d
0.5	2.27 d	3.93 b
1.0	3.05 c	6.48 a

LSD (5%) = 0.255; Letters a to e compare means in the table

3.2.3.4 Stage IV - hardening off

The most delicate and problematic step in the micropropagation of *Gladiolus*, is the transplantation and re-establishment of *in vitro* cultured plants in the field (Ziv *et al.*, 1970; Ziv, 1979; Ziv & Lilien-Kipnis, 1990; de Bruyn & Ferreira, 1992; Dantu & Bhojwani, 1995). Besides low survival rates, the plants show a strong tendency to become dormant at this stage (Hussey, 1977a; b; Sutter, 1986; Dantu & Bhojwani, 1995).

ESEM revealed that the *G. scabridus* explants had developed numerous stomata on their shoot leaf surfaces by the end of stage III (Plate 3.10). The presence of stomata aids plantlet survival during this last stage although stomata on plants grown *in vitro* do not always function properly (Hartmann *et al.*, 1997). The *G. scabridus* plantlets were therefore morphologically ready to be transplanted.

The only growth chamber available for the *in vitro* grown *G. scabridus* plantlets for stage IV, unfortunately had a lower light intensity than during stages I to III. Plantlets should be exposed to higher light intensities during this final stage to aid hardening off (Hartmann *et al.*, 1997). The influence of environmental conditions on dormancy has been studied in detail in numerous bulbous species because of its importance in horticultural practice (Ginzburg, 1973). External environmental factors have been shown to play a significant role on this process, any slight change inducing a response in the plants.

At both light levels of 17.3 µmol m^{-2} s^{-1} and 27.8 µmol m^{-2} s^{-1}, *G. scabridus* axillary bud and cormel halve explants remained green and healthy for about a week (Plate 3.11 A) after which a change became noticeable: most of the explants developed brown leaves from the tips, which eventually lead to leaf mortality. A small percentage of the plantlets, especially those transferred from stage III without roots, died in the growth chamber with no remaining cormlets in the media. A few plantlets survived the initial acclimatisation process until transfer to the misthouse, whereupon they also died down leaving resting cormlets. These plantlets had well-developed root systems when transplanted into the hardening off medium which appears to be an advantage and aids in a better chance of survival. Simonsen and Hildebrandt (1971) observed that *in vitro* plantlets with at least two or three roots, survived and grew in soil. However, roots that develop in agar, are often not functional in the soil environment (Lilien-Kipnis & Kochba, 1987) and can be damaged during the planting process (Debergh & Maene, 1981; George & Sherrington, 1984).

The remaining plantlets died down in the growth chamber and went into a dormant phase leaving small cormlets in the media which resembled *in vivo* corms except being much smaller. The longer the cormlets were allowed to dry in the hardening off medium, the more developed the outer papery covering around each cormlet became. The size of the cormlet produced depended on the size and vigour of the plantlets before dormancy as was found by Hussey (1977b). There was a high percentage survival of these cormlets after cold storage (Table 3.16) which is consistent with Dantu and Bhojwani (1995) who found that all cold treated cormlets germinated. The sprouted cormlets produced fairly large, firm and healthy leaves which is similar to the results of Dickens *et al.* (1986) (Plate 3.11 B).

Axillary buds

There were no significant interactions or main effects of the treatments used during stage III on percentage explant survival ($p=0.961$), percentage explant mortality ($p=0.962$), percentage explants that went into a dormant phase ($p=0.814$) or the number of explants that survived cold storage ($p=0.087$) during stage IV. However, the interaction between NAA and AC was significant on the number of viable cormlets that formed ($p<0.001$). Significantly more cormlets (4.60) were obtained with the highest levels of both NAA (1.0 mg l^{-1}) and AC (0.3 %) and the lowest number (0.99) when 0 mg l^{-1} NAA and 0 % AC were combined (Table 3.16). At each level of NAA, significantly more *G. scabridus* cormlets were produced in the presence of AC at 0.3 % than if it was omitted. In contrast, Dantu and Bhojwani (1995) found that AC, at the highest level of 3 %, inhibited both the frequency of corm formation and corm size.

Cormel halves

The results for the *G. scabridus* cormel halve explants follow a similar trend to those for axillary bud explants. The interactions of NAA and AC during stage III and their main effects, were not significant with respect to percentage explant survival ($p=0.613$), percentage explant mortality ($p=0.464$), percentage dormant explants ($p=0.478$) and the number of explants that survived cold storage ($p=0.357$). The interaction between NAA and AC was only significant on cormlet number ($p<0.001$). A significant increase in cormlet number was brought about by the presence of 0.3 % AC at each level of NAA, as well as an increase in the level of NAA. The highest yield of cormlets (6.02) was achieved at the highest levels of NAA (1.0 mg l^{-1}) and AC (0.3 %) and the lowest (1.06) in the absence of both compounds (Table 3.16).

Although stage IV is a difficult phase to control in *Gladiolus* (Dantu & Bhojwani, 1995), it is assumed that the low light levels in the growth chamber compared to previous culture stages had a role to play in the poor rate of plantlet survival. The slight change in photoperiod from 16 hours of light per day in stages I to III, to 14 hours of light per day in stage IV, may also have had a role to play in initiating this dormancy as well as the slight decrease in temperature from 25 ± 2 °C in stages I to III, to 24 ± 2 °C during stage IV. De Capite (1955) found that tissue cultures *in vitro* are extremely sensitive to changes in temperatures and the best results are obtained under constant temperature conditions throughout their life period. Being a summer flowering crop, *G. scabridus* becomes dormant during winter, a process triggered by the lower light intensities, shorter days and lower temperatures brought on by the colder months (De Hertogh & Le Nard, 1993). Therefore environmental conditions play an important role in their growth and development.

Several other bulbous species are difficult to acclimatize during stage IV. The successful transfer of tissue cultured Dutch iris propagules to the greenhouse has remained a problem. A cold treatment is required to synchronise the breaking of dormancy in the bulblets when planted from tissue culture into the greenhouse (Anderson *et al.*, 1990). In the culture of sparaxis hybrids, the corms are induced to form *in vitro* as the rooting of established young shoots under greenhouse conditions is difficult. These are then cold stored and transferred to greenhouse culture and grown as usual (Horn *et al.*, 1989). *Narcissus* cormlets are formed *in vitro*, cold treated and then planted out in the greenhouse (Hussey, 1982; Squires & Langton, 1990). However, rooted shoots of the Japanese garden iris can be successfully transplanted to pots in the greenhouse (Yabuya *et al.*, 1991).

Cormlets formed *in vitro* reduce the need for acclimatization and subsequent plant growth is improved (Nicol, 1993). Ziv (1979; 1989) avoided the hardening off process by allowing plantlets to dry *in vitro* and the cormlets were planted out after a period of cold storage. After drying, *in vitro* formed corms can be stored which allows for easy handling for transportation and sowing at anytime of the year (Steinitz *et al.*, 1991; Dantu & Bhojwani, 1995), which would be commercially advantageous when moving cormlets from a southern hemisphere to a northern hemisphere environment. Therefore, although the hardening off process will need to be investigated further in *G. scabridus*, the formation of cormlets *in vitro* is a time saving process that will allow for flexibility in planting date. Perhaps *G. scabridus* cultures can be induced to form numerous cormlets during stage III which can be dried, stored and planted out when desired, reducing the need for a final hardening off stage.

Table 3.16 **Cormel number from *G. scabridus* explants after cold storage at 4 °C at the end of stage IV**

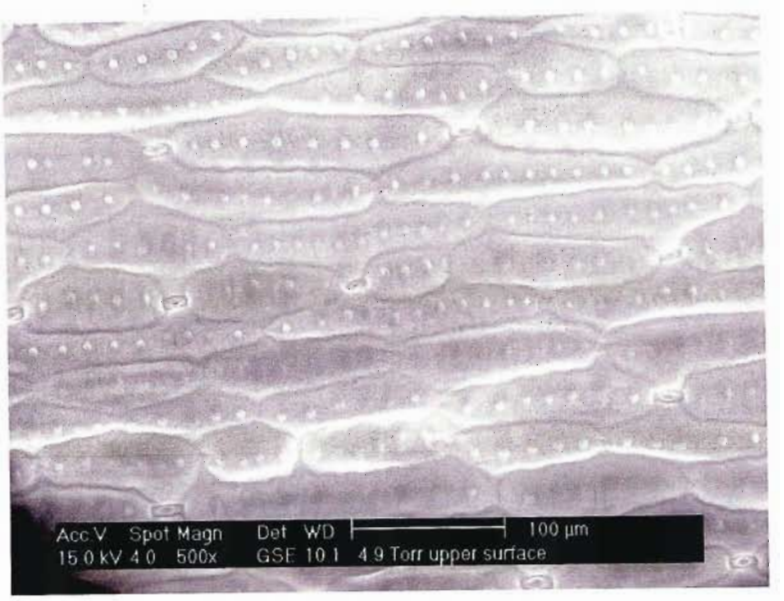
Growth regulator from stage III	Axillary buds [y]	Cormel halves [z]
0 mg l^{-1} NAA 0 % AC	0.99 e	1.06 e
0 mg l^{-1} NAA 0.3 % AC	2.20 d	2.80 d
0.5 mg l^{-1} NAA 0 % AC	2.19 d	2.68 d
0.5 mg l^{-1} NAA 0.3 % AC	3.16 b	4.05 b
1.0 mg l^{-1} NAA 0 % AC	2.76 c	3.42 c
1.0 mg l^{-1} NAA 0.3 % AC	4.60 a	6.02 a

[y] LSD (5%) = 0.226

[z] LSD (5%) = 0.183

Letters a to e compare means in each column

A



B

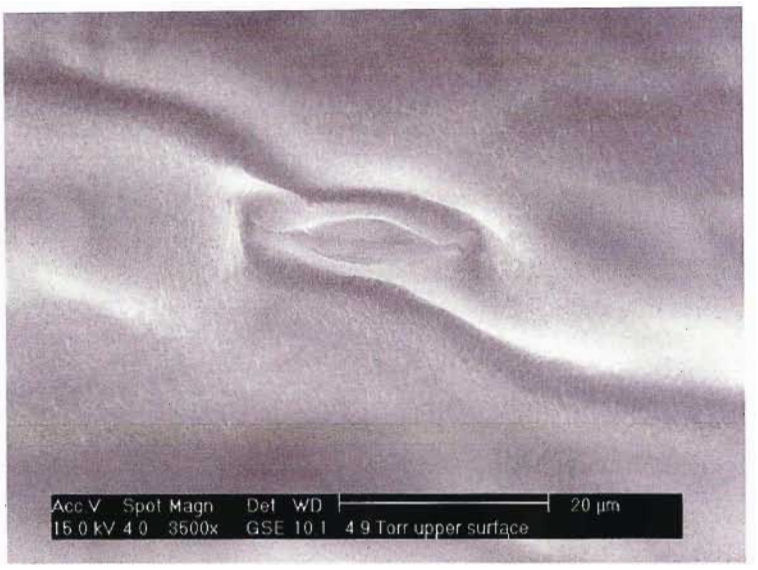
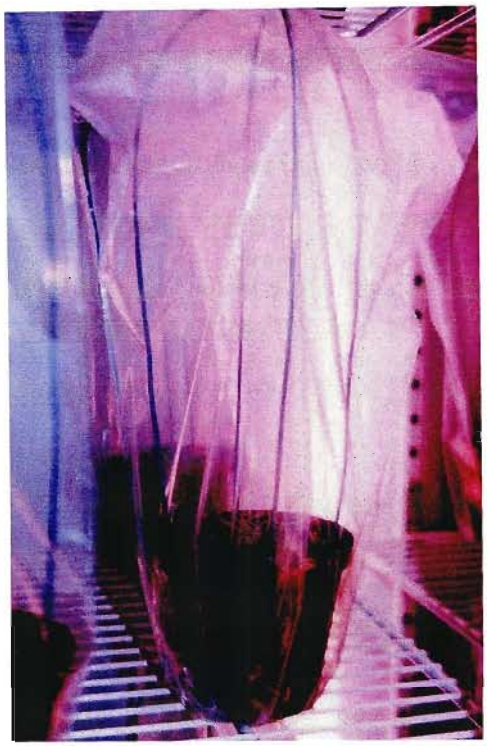


Plate 3.10 ESEM micrographs of *a G. scabridus* (A) leaf surface and (B) an enlargement of a stoma on *in vitro* cultured shoots at the end of stage III

A



B

Plate 3.11 ***G. scabridus*** **explants after** ***in vitro*** **culture (A) during the hardening off stage and (B) after cold storage at 4 °C**

3.2.4 Conclusions

G. scabridus has shown enormous potential for *in vitro* propagation and this has proved to be another successful method of producing large numbers of plants of this threatened species in a relatively short space of time. *G. scabridus* plantlets could be successfully regenerated from both axillary bud and cormel halve explants. Axillary buds provide the potential for numerous plants to be grown from one mother corm and cormels are convenient for experimental handling because of their size.

G. scabridus explant growth is not wholly dependant on the presence of applied growth regulators although the presence thereof does appear to enhance and improve growth. The control cultures produced higher numbers of shoots during stage I and II, however, it is advised that a combination of 1.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA be used for the shoot proliferation stages as shoots were stronger and healthier with this treatment. Axillary bud explants produced more shoots than cormel halve explants during stage I, while cormel explants showed a stronger tendency for root production during stage II. The number of *in vitro* cormlets formed by the cormel halve explants was on average greater than those formed by the axillary buds. *G. scabridus* cultures produced roots and cormlets much earlier in the micropropagation process than expected. This has the advantage of eliminating certain stages and reducing the time to hardening off. Future work could involve the use of further levels of BAP and NAA as well as other growth regulators at various concentrations.

G. scabridus root growth during stage III was severely retarded by the absence of AC. Cormlet development was improved in the presence of this compound. Hence it is essential to add AC to the rooting medium. A combination of 0.3 % AC with 1.0 mg l^{-1} NAA in the rooting medium allows for considerable root and cormlet development during this stage or alternatively 0.3 % AC with 0.5 mg l^{-1} NAA can be used if continued emphasis on shoot development is required as well. Perhaps AC could be added to media during stage II to enhance root and cormlet development in these early stages to save time.

It has been shown that the formation of a well developed root system before stage IV, is essential for improving transplantation success and cormlet formation for plantlet survival thereafter. High NAA and AC are important for cormlet development during stage IV. It is highly beneficial to induce the production of *in vitro* cormlets during stage IV as these can be

stored and planted out when required. This creates flexibility in the micropropagation process.

Future work could also explore the potential of the *in vitro* culture of other plant parts of *G. scabridus* as has been done with other species. However, a fairly large supply of stock plants will first need to be made available. The long term effects of the use of rifampicin will also need to be ascertained and perhaps other methods of sterilization explored.

CHAPTER 4

GROWTH AND NUTRITIONAL STUDIES OF *GLADIOLUS SCABRIDUS*

4.1 Introduction

The requirement for growing bulbous plants from seed is to produce plants as fast as possible with an emphasis on strong, healthy growth ultimately with good flower production (Rix, 1983). It is important to ensure that the plant is supplied with sufficient reserves to enhance the formation of the storage organ, the most important part or 'control centre' of a bulbous plant.

Growing media serves as a storehouse for plant nutrients and normally provides a substantial amount of the plant's nutrient requirements. Under most conditions, however, growth is enhanced by the proper application of supplemental nutrients (Soil Improvement Committee California Fertilizer Association, 1990) and for satisfactory plant growth, all nutrients must be in adequate supply and in balance with each other (Salinger, 1985).

Along with temperature, light and water, mineral nutrition is one of the main factors that affects the growth and flowering of *Gladiolus* hybrids (Deswai & Patil, 1983; Salem & Tahar, 1989). Contradictory reports exist regarding the nutrient requirements of gladioli and they exhibit a great variation (Potti & Arora, 1986). Therefore, no single fertilizer recommendation can be made for the various growing conditions of gladioli (De Hertogh & Le Nard, 1993) and the benefit of fertilizer is often only seen during the second season of growth (Wilfret, 1992).

In the wild, *G. scabridus* is restricted to well-drained, quartzite outcrops with shallow soil (Goldblatt & Manning, 1998) which is poor in nutrition. The plants will only utilize what is available in the soil and appear to thrive accordingly (Chapter 2). The need for nutrient amendment is thus uncertain, hence fertilizer trials were undertaken to observe the effects of the addition of nutrients to the growing environment of these plants, and to evaluate such amendments in relation to the growth cycle of *G. scabridus*.

4.2 Materials and Methods

4.2.1 Season 1

Seeds of *G. scabridus* were collected in February from the Biodiversity Research Gardens, KwaZulu-Natal Wildlife, Queen Elizabeth Park, Pietermaritzburg. They were immediately germinated in 40 x 30 x 6 cm black, plastic germination trays filled with pine bark seedling mix which had been drenched with Sporekill® prior to seed placement. Dewinged seeds were sown 1.5 cm deep and 3 cm apart and kept evenly moist. The trays were left in a glasshouse at 25 °C (day) and 18 °C (night).

When the seedlings were 10 cm tall, about 4 to 5 weeks after sowing, they were carefully transplanted into 3 litre black plastic bags filled with the same media, as any damage to the young plant can mean seedling death (McLean, 1941). The plants were maintained in a glasshouse set at 25 °C (day) and 18 °C (night) and were automatically drip irrigated twice a day (60 ml / plant / day). By late June, the plants had all died down and the cormels were removed and stored at 4 °C for 8 weeks during the winter period.

In spring (mid-September), the cormels were planted out into 3 litre black bags with a steam pasteurized pine bark : perlite : river sand medium, 2:1:0.2 (v/v/v). Bark is used extensively in greenhouse media and functions to reduce costs and improve aeration. It can yield excellent results provided it is prepared and used properly (Reed, 1996). Pine bark mixtures result in good root growth and at the same time retain enough water (Beardsell, 1999). The composted pine bark used in the trial contained 2 kg dolomitic lime per 6 m^3 bark (National Plant Food, pers. commun., 2000). The use of well composted pine bark reduces the presence of phenolics which can severely inhibit root growth (Marschner, 1995). Perlite is a stable, sterile, chemically inert, lightweight material with a good water holding capacity and provides good aeration to the medium. Sand generally has little effect on the chemical characteristics of a medium. It is useful in stabilizing tall plants and provides good drainage and aeration (Reed, 1996; Hartmann *et al.*, 1997).

The trial consisted of 12 treatments (Table 4.1) set up as a randomised block design and replicated six times. During season 1, treatment 7 was used as the control treatment as this correlates with the general industry standard used for the commercial production of gladioli. Monocalcium phosphate ($2Ca(H_2PO_4)_2.H_2O$) (Ocean Agriculture) was mixed into all the media

at the start of the trial at a rate of 60 kg ha^{-1}. A preplant application of P lasts for the entire crop (Reed, 1996). Ammonium nitrate (NH_4NO_3) and potassium sulphate (K_2SO_4) were applied once a week as a liquid soil drench. A liquid fertilizer application was used to minimize soluble salt buildup by watering to cause some leaching (Ball, 1998). The NH_4NO_3 was applied at rates of 50, 100, 150 and 200 kg ha^{-1}. One of the main benefits of the addition of this fertilizer, is the simultaneous application of ammonia and nitrate to the media (D. Haynes, pers. commun., 2000) and positive results have been attained using this fertilizer for *Gladiolus* production (Hilal *et al.*, 2001). The K_2SO_4 was applied at rates of 50, 100 and 150 kg ha^{-1}. This fertilizer is generally used on plants that are sensitive to chloride ions, like gladioli, or where chloride may build up in the soil or media (Ball, 1998). Omnispoor® (Nutrifert), a micro nutrient fertilizer, was also applied in the weekly drench. It contains iron (87 g kg^{-1}), manganese (18 g kg^{-1}), zinc (10.7 g kg^{-1}), copper (1.2 g kg^{-1}), boron (22 g kg^{-1}) and molybdenum (2 g kg^{-1}), and was applied at a rate of 3 kg ha^{-1}.

Plants were grown in a glasshouse at 25 °C (day) and 18 °C (night) and were automatically drip irrigated 3 times a day (90 ml / plant / day). Plants were weeded by hand once a week and Confidor® (1 ml l^{-1}), Malathion® (1.25 ml l^{-1}) and Cypermethrin® (1 ml l^{-1}) were used for pest control when necessary.

Plant characteristics measured were plant height, number of leaves, change in corm mass, change in corm size, and number and mass of cormels produced. Plants grown during season 1 were not large enough to provide sufficient leaf material for mineral analysis. The chemical properties of the soil were investigated at the beginning and end of the growing season. Nitrogen analysis was performed by Allerton Provincial Veterinary Laboratory, Department of Agriculture and Environmental Affairs, Pietermaritzburg and all other chemical analyses were carried out at the Department of Soil Science, Cedara Agricultural College, KwaZulu-Natal Department of Agriculture, Howick.

At the end of autumn, the corms were removed and stored at 4 °C for 8 weeks during the winter period and were planted out again the following spring.

4.2.2 Season 2

The trial was repeated as before with the same twelve fertilizer treatments in a randomized block design with 6 replications per treatment. However, the control during this season was

plants grown in the same media without an application of NH_4NO_3 or K_2SO_4 (Table 4.2). Control plants received an initial application of $2Ca(H_2PO_4)_2.H_2O$ (60 kg ha^{-1}) as before and weekly applications of Omnispoor® equal in volume to that of the other fertilizer applications.

Plant characteristics measured were plant height, number of leaves, change in corm mass, change in corm size, number and mass of cormels produced, flowering percentage, number of floral branches and floral spike length.

Prior to flowering, the most recently matured, fully expanded leaf (Cresswell & Weir, 1997; Ball, 1998) was removed from each plant and used for mineral analysis to determine the nutrient status of the plants. The growing media was again chemically analysed prior to and at the end of the growing season. Nitrogen analysis in both leaf and soil samples was again carried out at Allerton Provincial Veterinary Laboratory and all other analyses by the Soil Science Department at Cedara Agricultural College. Plant uptake of N and K was calculated as the initial value in the media plus the amount of N added to the media from which the final level in the media was deducted.

4.2.3 Season 3

A third trial was conducted during another growing season. Mature *G. scabridus* corms were planted out as before into 3 litre plastic brown pots with an initial application of $2Ca(H_2PO_4)_2.H_2O$ (60 kg ha^{-1}) (no addition of lime). The trial was again set up as a randomised block design. The NH_4NO_3 was applied weekly to the plants as a liquid drench at rates of 50, 150 and 250 kg ha^{-1} each supplemented with 50 kg ha^{-1} K_2SO_4, resulting in 3 treatments (30 replications per treatment). At the onset of flowering each of these treatments was further split into 3 new applications of K_2SO_4 at rates of 50, 150 and 250 kg ha^{-1} together with 50 kg ha^{-1} NH_4NO_3 (10 replications per treatment). Fertilizer applications throughout the season resulted in a total of 9 treatments (Table 4.3). Omnispoor® was applied with each fertilizer application at a rate of 3 kg ha^{-1}.

Plant characteristics measured were plant height, number of leaves, change in corm mass, change in corm size, number and mass of cormels produced, floral spike length and number of floral branches on each spike.

The most recently matured, fully expanded leaves were removed and used for mineral analysis in October (3 plants per treatment) in December just prior to flowering (9 plants per treatment) and in March (3 plants per treatment). Leachate samples were taken in November and January to determine the extent of nutrient leaching from the media. Only 40 ml were taken from each of 10 pots per treatment so as not to over flush the media. These were combined to allow for sufficient sample for analysis but resulting in too few samples for statistical analysis. The chemical properties of the soil were investigated at the beginning, middle (just prior to the new fertilizer applications) and end of the growing season. All mineral analyses were carried out by the Soil Science Department at Cedara Agricultural College. Plant uptake of N and K was calculated as before.

All results were statistically analysed using the Genstat statistical package (8th Edition, 8.1 Release, Lawes Agricultural Trust, Rothamsted Experimental Station) by means of analysis of variance (ANOVA) at a 5 % level of significance. Data between treatments was compared as well as the separate effects of the nitrogen and potassium applications. Some of the data was also subjected to covariate and regression analyses to determine any relationships between growth characteristics. This will be highlighted in the appropriate sections.

During the fourth season, all corms and cormels were planted out and once established, the plants were returned to the Biodiversity Division at KwaZulu-Natal Wildlife.

***Table* 4.1 Soil amendment treatments used during season 1 of the *G. scabridus* fertilizer trial**

Treatment	NH_4NO_3 (kg ha^{-1})	K_2SO_4 (kg ha^{-1})
1	50	50
2	100	50
3	150	50
4	200	50
5	50	100
6	100	100
7 [z]	150	100
8	200	100
9	50	150
10	100	150
11	150	150
12	200	150

[z] control

***Table* 4.2 Soil amendment treatments used during season 2 of the *G. scabridus* fertilizer trial**

Treatment	NH_4NO_3 (kg ha^{-1})	K_2SO_4 (kg ha^{-1})
1	50	50
2	100	50
3	150	50
4	200	50
5	50	100
6	100	100
7	150	100
8	200	100
9	50	150
10	100	150
11	150	150
12	200	150
control	0	0

***Table* 4.3 Soil amendment treatments used during season 3 of the *G. scabridus* fertilizer trial**

Treatment	Applied from Sep - mid Dec		Applied from mid Dec - Apr	
	NH_4NO_3 (kg ha^{-1})	K_2SO_4 (kg ha^{-1})	NH_4NO_3 (kg ha^{-1})	K_2SO_4 (kg ha^{-1})
1	50	50	50	50
2	50	50	50	150
3	50	50	50	250
4	150	50	50	50
5	150	50	50	150
6	150	50	50	250
7	250	50	50	50
8	250	50	50	150
9	250	50	50	250

4.3 Results and Discussion

4.3.1 Season 1

4.3.1.1 Seed germination and seedling growth

G. scabridus seed germination in the pine bark seedling mix was slow, uneven and erratic (Plate 4.1 A). Even under the most favourable conditions gladioli seeds are slow to germinate (McLean, 1941) and this process can be delayed, the seeds coming up at various stages throughout the season (Pickell, 1972). However, sufficient seedlings became available for use in the fertilizer trial. The first seedlings appeared 18 days after sowing and were thin and grass-like in appearance (Pickell, 1972; Rix, 1983) (Plate 4.1 B). The largest and most healthy seedlings were selected for transplanting and use in the fertilizer trial.

G. scabridus seedlings responded well to the transplantation process and continued to elongate from their bases for several weeks, eventually reaching a height of 20 to 30 cm. Most seedlings only developed a single shoot, however, a few formed a small second leaf a few weeks after the first one appeared. After a period of about 2 months, the shoots began to develop brown, dry tips. It is uncertain as to whether this is due to a seasonal change in light and temperature or a common response from these young plants. The media was kept moist at all times so this was not due to a lack of water. McLean (1941) noted that this usually occurs in *Gladiolus* seedlings at any time throughout the year and a similar process occurs in *Babiana* seedlings (P. Shanahan, pers. commun., 2000). The brown tips of the *G. scabridus* seedlings led to eventual above ground death. Aerial leaves had died completely by the end of autumn, and substantial sized corms of about 10 mm in diameter were removed from the growth medium.

4.3.1.2 Plant growth in general

Once planted out again, corms took between 12 and 18 days to sprout. The first sign of this was the appearance of 2 or 3 sheath leaves, which were initially green in colour, shortly afterwards changing to purple (Plate 4.2 A). These varied in length depending on the final height of the plant. The true leaves emerged one at a time from the sheath leaves 15 to 20 days after placement, each leaf appearing from the split in the edge of the previous one. These leaves varied in length and were thin in appearance due to the juvenility of the plants

and formed an erect, loose fan from the base (Plate 4.2 B). A number of the plants displayed axillary bud development from the corm as evidenced by smaller, side shoots appearing alongside the main axillary shoot. As the season progressed, the *G. scabridus* plants were drawn into the media, a phenomenon that occurs as a result of the contractile roots dragging the older corm deeper into the media to allow space for the formation of the daughter corm above it, especially if the old corm is too close to the surface (Pickell, 1972; du Plessis & Duncan, 1989).

As expected, no flowering took place this season (Plate 4.2 C). Leaf development reached a maximum, two thirds into the season after which it remained quiescent. In response to the seasonal change in temperature and light at the end of the growing season, the leaves became brown and dry starting from the tips, eventually leading to complete above ground mortality. Substantial sized corms were removed at the end of the season.

4.3.1.3 Nutritional effects on plant growth

Plant height: There were no significant differences in *G. scabridus* plant height when comparing the 12 fertilizer treatments (p=0.995). The mean plant height was 43.3 cm. There was a tendency for treatment 4 (45.9 cm), treatment 12 (45.4 cm) and the control (46.4 cm) to result in taller plants, all with high levels of nitrogen (N) and varying levels of potassium (K) applied to the media. All soil nutrients play a role in plant growth and development (Raven *et al.*, 1992), however, N is one of the most important for vegetative growth, plant vigour and health (Parnes, 1990) thereby affecting plant height. Jhon *et al.* (1997), working on gladioli, found that high N and low P and K produced the tallest plants. *G. scabridus* plant height appears to be improved with higher N applications which promotes growth, resulting in taller plants.

Leaf number: There was no significant difference in leaf number between the treatments (p=0.794). Perhaps the plants were too young for any considerable difference to occur in this regard. Overall, the plants produced between 2 to 7 leaves during season 1 which varied in size and length. The control produced a mean leaf number of 3.33. No leaf abnormalities, deficiency or toxicity symptoms were noted during the growing season, indicating that nutrient levels were sufficient for normal plant and leaf growth to occur.

Corm size: Corms that were removed at the end of the first growing season, were much larger than those planted out at the beginning of the season, indicating a large accumulation of storage products for next year's growth (De Hertogh & Le Nard, 1993). Corm diameter increased by a mean value of 9.30 mm. There were no significant differences between the fertilizer applications (p=0.140), both N and K usually influencing the formation and size of the daughter corms (Hancke & Verryn, 1989b). Control corms increased by 8.01 mm.

The new corms showed a mean increase in height of 11.46 mm with no significant differences between the treatments (p=0.519). Control corms showed an increase of 12.77 mm. The new corms were generally oblong in shape with 'raised centres', a phenomenon also noted in gladioli by Salinger (1985). This indicates that the growth of the *G. scabridus* daughter corms during season 1 is mainly longitudinal.

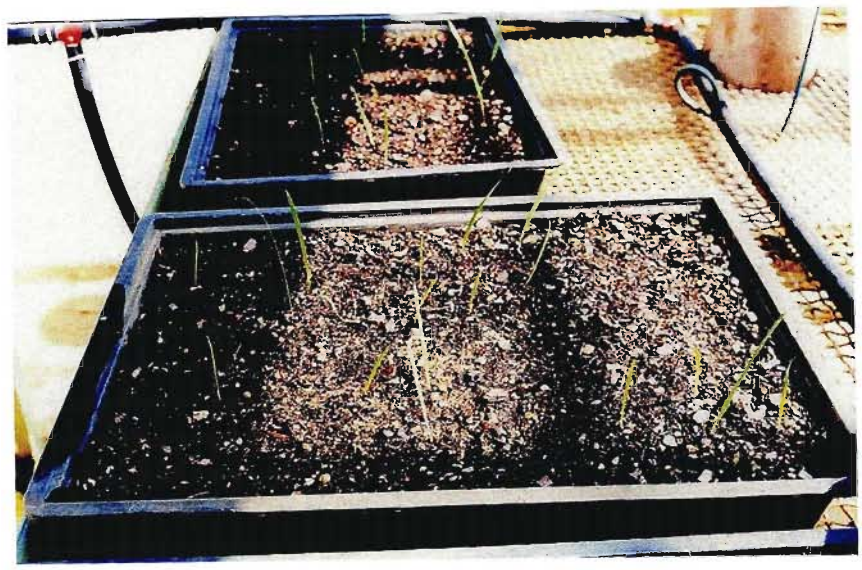
Corm mass: The change in corm mass over the growing season was also non significant between treatments (p=0.511) and appeared to develop independently of the fertilizer applications. The total mean increase in corm mass was 2.84 g and that of the control was 2.59 g. At the beginning of the season, the corms all weighed less than 1.0 g. Most of them doubled in mass some almost reaching 9.0 g by the end of the season, yet again indicating a considerable accumulation of reserves during the growing season. The enhanced nutritional environment certainly may play an important role in this regard. Covariate analysis showed that there was no significant effect of *G. scabridus* leaf number on the change in corm mass (p=0.267).

Cormel number: There were no significant effects of treatment on the cormel numbers (p=0.539), although there was a tendency for the highest number of cormels to develop at the highest levels of K with varying levels of N. The control resulted in an average of 9.8 cormels.

Growing cormels is a relatively inexpensive method of increasing plant stock, especially of a new variety, providing one is prepared to wait a few years for the first blooms (Pickell, 1972). Growth during season 1 indicates that *G. scabridus* is a prolific producer of cormels as the plants produced between 2 and 28 cormels, resulting in about 700 cormels from this season's growth. This is ideal with regards to an endangered species as it allows for the production of large amounts of plant material from a relatively small population in a short space of time. A nutrient rich environment should aid in the production of this important source of plant material.

Cormel mass: There were no significant differences between the mass of the cormels from each treatment (p=0.778). The overall mean cormel mass was 1.30 g and the control treatment produced the highest cormel mass of 1.67 g.

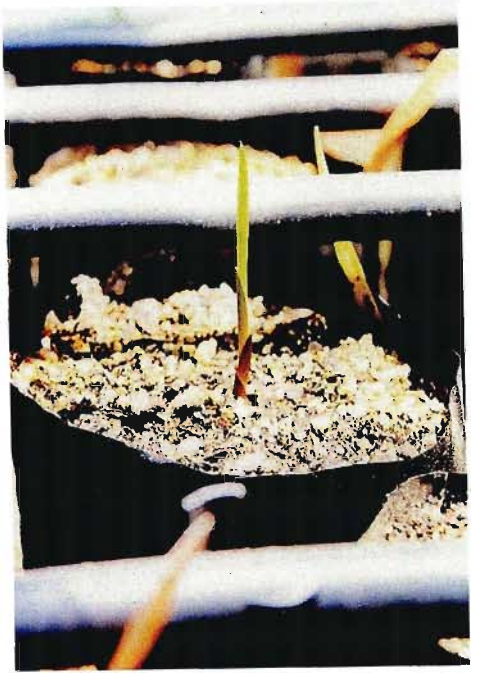
A



B

Plate 4.1 (A) Germination of *G. scabridus* seedlings in pine bark seedling mix and (B) a *G. scabridus* seedling after germination showing its thin, grass-like appearance

A



B

C

Plate 4.2 (A) A young, sprouting *G. scabridus* plant at the beginning of season 1 showing the purple sheath leaves, (B) a fully grown *G. scabridus* plant and (C) the juvenile *G. scabridus* plants during season 1

4.3.1.4 Growing media changes

Nitrogen (N): The level of N in the media at the start of the trial (0.276 %) had significantly depleted by the end of the growing season (0.204 %) ($p<0.001$). N was added in the form of mineral NH_4^+ and NO_3^- both of which get absorbed by plants, NO_3^- more so than NH_4^+, the latter becoming attached to clay mineral particles (Salinger, 1985). The reduction in N indicates that the plants absorb available N as found by Groen and Slangen (1990). This could of course be due to some leaching of mobile NO_3^- as nitrogen can be lost from the soil in this way (Salinger, 1985) and is not bound by the media (Reed, 1996). However, this would not have occurred in excessive quantities as the irrigation water was insufficient to completely flush the media and the liquid fertilizer application only occurred once a week, just enough to drench the soil, yet prevent the buildup of salts. The slight loss of nutrients in such a way would have been uniform throughout the trial as each plant received the same amount of irrigation water and liquid fertilizer each week.

There was no significant difference between the N levels in the control (0.215 %) and any of the other 11 treatments ($p=0.305$).

Phosphorus (P): There was a highly significant reduction in the level of P in the growing media ($p<0.001$). P is characterized in the soil by a lower mobility than any other nutrient (Parnes, 1990). The media initially contained 262.8 mg l^{-1} P and even with the addition of $2Ca(H_2PO_4)_2.H_2O$ to each plant, the level decreased to a mean value of 154.1 mg l^{-1}, implying that *G. scabridus* plants readily take up available P. There were no significant differences between the final P levels in each treatment ($p=0.664$). The control treatment resulted in 160.3 mg l^{-1} P.

Potassium (K): There were no significant differences between the final levels of K in the media ($p= 0.519$).The initial level was 457.3 mg l^{-1} and was significantly reduced to an overall mean level of 111.3 mg l^{-1} ($p= 0.001$) presumably through uptake by the *G. scabridus* plants. The final level of K in the control media was 118.7 mg l^{-1}.

Calcium (Ca): This element is usually supplied when liming soils (Salinger, 1985) and hence would have been released from the dolomitic lime in the pine bark media. However, it is very slowly available from this source (Salinger, 1985). Ca was also present in the application of $2Ca(H_2PO_4)_2.H_2O$. Plants display a low ability to take up Ca as plant roots are inefficient at

absorbing Ca from the soil despite the large quantity that may be present (Parnes, 1990). The initial level of 1465 mg l^{-1} in the growing media did not significantly change (p=0.877). This decreased slightly in some treatments, whilst increasing in others, as in the control (1549 mg l^{-1}). Even though these results indicate that *G. scabridus* plants do not take up much Ca from the media, the slight increase shows that Ca was released from the two Ca sources and the release thereof exceeded the uptake by the plants. Smaller plants would have taken up less Ca.

A higher concentration of any ion in the soil will tend to displace others (Soil Improvement Committee California Fertilizer Association, 1990), hence, the higher amount of Ca in the media would have meant less binding sites for other exchangeable cations such as Mg and K, thus a lower level of these elements. Soils with a pH between 6.0 and 8.0 have predominantly Ca ions (Tisdale *et al.*, 1993) as in the fertilizer trial.

Magnesium (Mg): This element would also have been available from the dolomitic lime (Salinger, 1985). Cation exchange is the only means of holding Mg against losses. An antagonistic relationship exists among Ca, Mg and K as they compete for uptake by plant roots (Parnes, 1990). Hence higher levels of Ca, as observed in the trial, could mean lower levels of Mg. No significant differences in the final levels of Mg were observed (p=0.621), however, it decreased in all treatments from the initial level of 415.2 mg l^{-1} to a mean value of 366.1 mg l^{-1} (p=0.165) implying that *G. scabridus* plants did take up some Mg.

Zinc (Zn): The availability of Zn decreases with an increase in media pH (Tisdale *et al.*, 1993). Media pH was acidic in the fertilizer trial therefore conditions were suitable for Zn uptake by the *G. scabridus* plants. During the trial, Zn levels decreased from an initial level of 26.40 to 20.31 mg l^{-1} (p=0.166), with no significant differences between the final levels (p=0.828).

Zinc is not readily leached from the soil (Cresswell & Weir, 1997). Available Zn is absorbed and held by clay minerals in the soil, its availability also being determined by competition with other cations for these binding sites (Soil Improvement Committee California Fertilizer Association, 1990). There was a non-significant decrease in both the clay percentage (p=0.156) and the total cation content (p=0.315) during the fertilizer trial, correlating with the decrease in the Zn content of the media as less binding sites and thus cations become available for uptake.

Manganese (Mn): The solubility and availability of Mn in the soil solution is greatly increased under acidic conditions (Tisdale *et al.*, 1993). The initial Mn level of 47.83 mg l^{-1} was significantly lower by the end of the trial at 8.82 mg l^{-1} ($p<0.001$). There were no significant differences between the final levels ($p=0.623$) with the lowest level in the control media (7.17 mg l^{-1}). Mn is fairly mobile in the soil and the addition of acid-forming NH_4^+, enhances Mn uptake (Tisdale *et al.*, 1993). Therefore, at higher levels of NH_4^+, less Mn could have been expected to be present in the media at the end of the trial.

pH (in KCl): Growth medium pH influences the availability and plant uptake of all essential plant nutrients (Reed, 1996; Ball, 1998). The pH significantly increased from 5.24 before the trial ($p<0.001$) to values ranging from 6.02 to 6.10. These final pH values were not significant to each other ($p=0.251$) and the pH of the control medium was 6.05. Gladioli do not require a high pH (Salinger, 1985), hence, this was presumably a satisfactory pH range for *G. scabridus* plant growth.

The fertilizer applications would have affected the pH. Ammonium nitrate decreases the pH, pottasium sulfate has a neutral effect, monocalcium phosphate is basic (Ball, 1998), the Omnispoor® has a pH of 6.50 and the pH of the irrigation water was 5.26. The overall effect was an increase in media pH. There was a highly significant decrease in the exchangeable acidity ($p<0.001$) and the acid saturation ($p<0.001$) of the media which is a reflection of how many hydrogen (H) and aluminium (Al) ions are attached to the clay particles (Marschner, 1995). The clay percentage also decreased, these changes all indicating the presence of less H and Al ions, therefore reflecting a higher final pH value.

4.3.1.5 Pests and diseases

No problems were experienced with the common diseases associated with *Gladiolus*. The only pests that made an occasional appearance were aphids and whitefly, however, these were easily eradicated through spot spraying with the insecticides.

4.3.2 Season 2

4.3.2.1 Plant growth in general

The performance of gladioli corms depends upon their size, cultural practices and the growing conditions of the previous year (Sharga & Basario, 1976). Once the *G. scabridus* corms were planted out, they sprouted within 10 to 15 days, slightly faster than the previous season as larger corms become less dormant and therefore sprout faster (Salinger, 1985). The application of fertilizer or the lack thereof did not exhibit any noticeable effects on sprouting. However, this may have been due to the use of uniform corms for planting (Potti & Arora, 1986).

The sheath leaves were yet again the first organs to appear and were much longer and thicker than the previous season. The true leaves were also much larger and wider in the second season due to the age of the corms. Overall, *G. scabridus* plants in season 2 were much taller, fuller and stronger in appearance and most needed staking (Plate 4.3 A). Unlike season 1, the bases of these plants were much thicker and it could be predicted from early on that many of the plants would flower. Floral spikes developed on most of the plants, even those that were fairly small in size and from which flowering was not expected. Flowers appeared after 6 to 7 foliage leaves had developed, about 90 days after sprouting.

Axillary bud development was again evident, with the appearance of smaller side shoots. Once the leaves had all died down at the end of the growing season and the plants had commenced their dormant seasonal phase, the corms and cormels were removed. The resulting corms were much larger than the previous season and many more cormels were produced around their perimeters.

4.3.2.2 Nutritional effects on plant growth

Plant height: The control produced significantly shorter *G. scabridus* plants ($p=0.036$) of 57.1 cm, compared to the tallest plants in treatments 4 (82.3 cm), 6 (80.4 cm), 7 (81.5 cm), 8 (79.5 cm) and 12 (79.4 cm) (Figure 4.1), all with higher levels of N with varying levels of K as in season 1. Haider *et al.* (1981) and Potti and Arora (1986) found no significant effect on plant height due to the application of N, P and K, although a tendency to taller plants results from higher levels of these elements (Kosugi & Sano, 1961; Potti & Arora, 1986). The results

confirm that higher levels of N are important for vegetative plant growth in *G. scabridus* and should be carefully considered when applying fertilizer.

Leaf number: There were no significant differences in leaf numbers between the treatments (p=0.306), although there was a tendency for higher levels of N with various levels of K to result in plants with more leaves. Control treatments produced the lowest mean number of leaves (8.17). This implies that the addition of nutrients enhances the vegetative growth of the plants with higher levels of N, improving *G. scabridus* plant leaf numbers.

Corm size: At the end of season 2, corm diameter had increased substantially, although no significant increases between the fertilizer treatments were noted (p=0.408). Mean final corm diameter was 36.28 mm. Corm height did not significantly change (p=0.676) and decreased in many of the corms resulting in a negative change in corm height. *G. scabridus* daughter corms from season 2, were more swollen and compressed in shape than season 1, giving the corms the vertically orientated look characteristic of mature gladioli (du Plessis & Duncan, 1989). Therefore the growth of the daughter corms during this season was mainly horizontal.

Corm circumference was measured at the end of the season and differences between treatments were found to be significant (p=0.029). The circumference is a good indicator of the size of the corms of which the significantly largest corms in this regard developed with treatments 4 (11.37 mm), 7 (11.72 mm) and 8 (11.28 mm), all with higher levels of N and lower levels of K yet again (Figure 4.2). Thus, N also seems to play a role in the development of *G. scabridus* corms during the growing season.

Corm mass: Corm mass prior to planting out at the beginning of season 2, had decreased slightly from the end of season 1, possibly due to slight dessication during the cold storage period. The corms all weighed less than 9.0 g at the start of season 2 and most of them doubled in mass, some attaining 34.0 g. The total mean corm mass change was 11.36 g.

There were no significant differences between the treatments (p=0.524). However, there was a tendency for the highest changes to occur in treatment 4 (15.21 g), 7 (14.43 g), 8 (14.19 g) and 12 (13.96 g) with high levels of N and varying levels of K. Das (1998) found that corm mass increased with increasing rates of K, yet Singh (1995) and (1998) found that the highest rate of N produced significantly more large-sized corms, similar to the results of the *G. scabridus* fertilizer trial during the second season. A lack of fertilizer application in the

control resulted in the smallest change in corm size and mass (7.55 g) confirming that a nutrient rich environment is beneficial to the formation of *G. scabridus* storage organs. Covariate analysis showed a significant effect of the leaf number ($p=0.001$) and plant height ($p<0.001$) on the increase in corm mass. The higher the number of leaves and the taller the plants, the greater the increase in mass. This could be related to the level of N. Higher N means enhanced vegetative growth which means a greater supply of reserves to the daughter corm hence a larger increase in corm mass.

Cormel number: The *in vivo* propagation rate of *Gladiolus* depends on the ability of the cultivar to form cormels (van der Linde, 1992). There were no significant differences between the cormel numbers of the different treatments ($p=0.800$), although the control plants produced the lowest number of cormels (13.7) with the largest number of cormels (39.2) from treatment 9, at a low level of N and a high level of K. Results from season 1 and 2 thus indicate that nutrients enhance *G. scabridus* cormel formation and that K may play a role in the production thereof. Das (1998) found that cormel number was not affected by K.

Cormel mass: The lowest number of cormels in the control treatment meant a lower final mean cormel mass of 2.67 g, which was highly significant ($p<0.001$) from treatments 6 (8.61 g), 7 (8.14 g) and 8 (9.96 g), with the highest cormel mass at various levels of N with higher levels of K (Figure 4.3). Potti and Arora (1986) found that P and K resulted in a marginal increase in the number of cormels and that the highest yield occurred at the highest level of N. This was attributed to an increase in growth which results in an increase in dry matter production and hence higher numbers of corms and cormels. Thus an enhanced nutritional environment significantly increases the weight of *G. scabridus* cormels at the end of the growing season especially with higher application levels of K. The control from season 1 (treatment 7), produced positive results in both growing seasons in this regards, hence would be a recommended ratio to use to enhance cormel formation in the growth of *G. scabridus*.

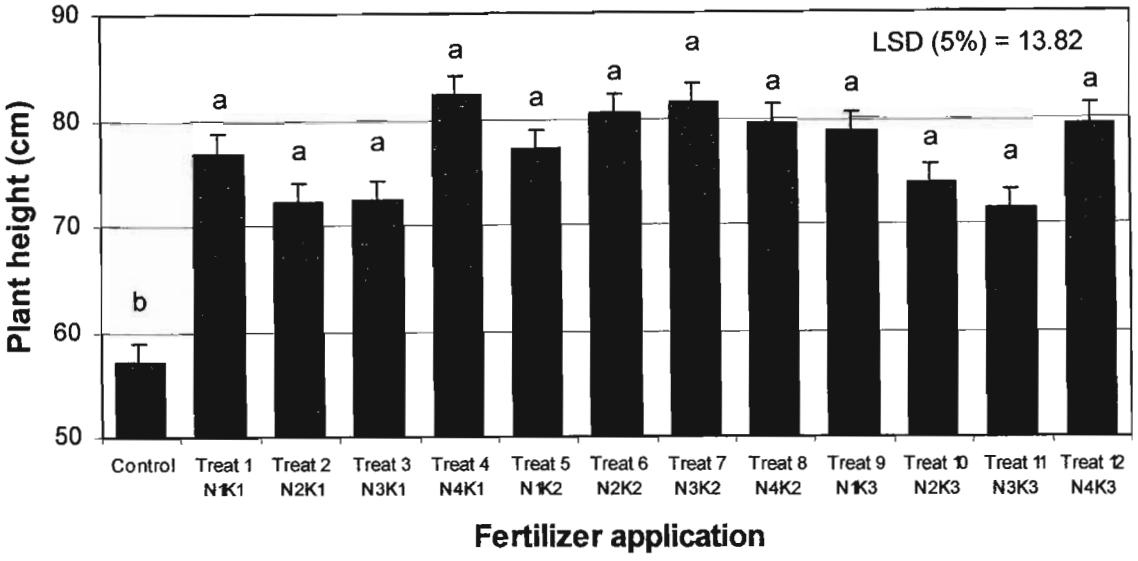


Figure 4.1 ***G. scabridus*** **plant height as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)**

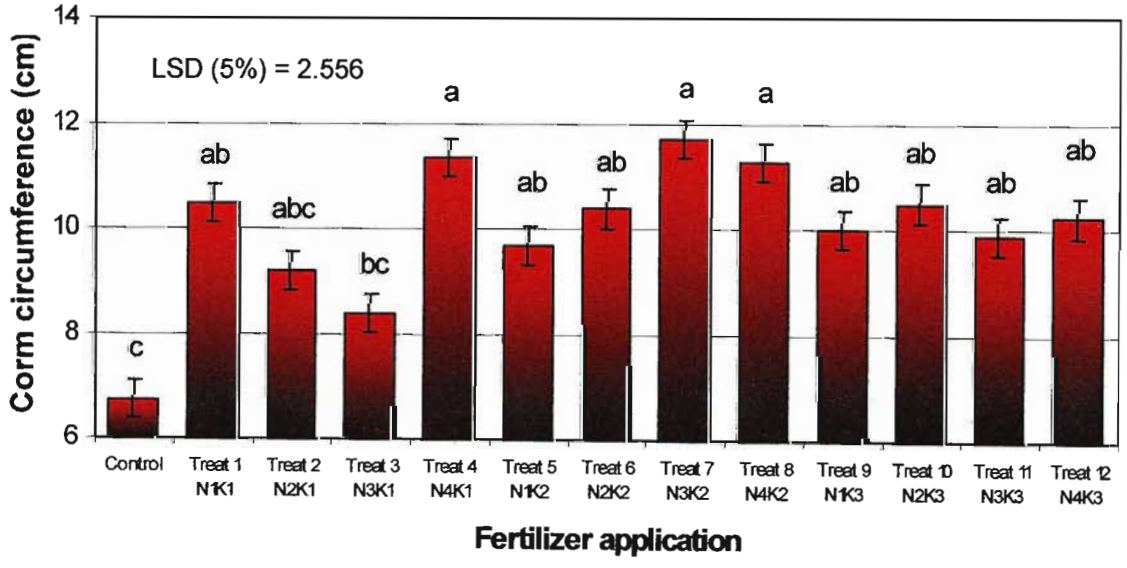


Figure 4.2 ***G. scabridus*** **corm circumference as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)**

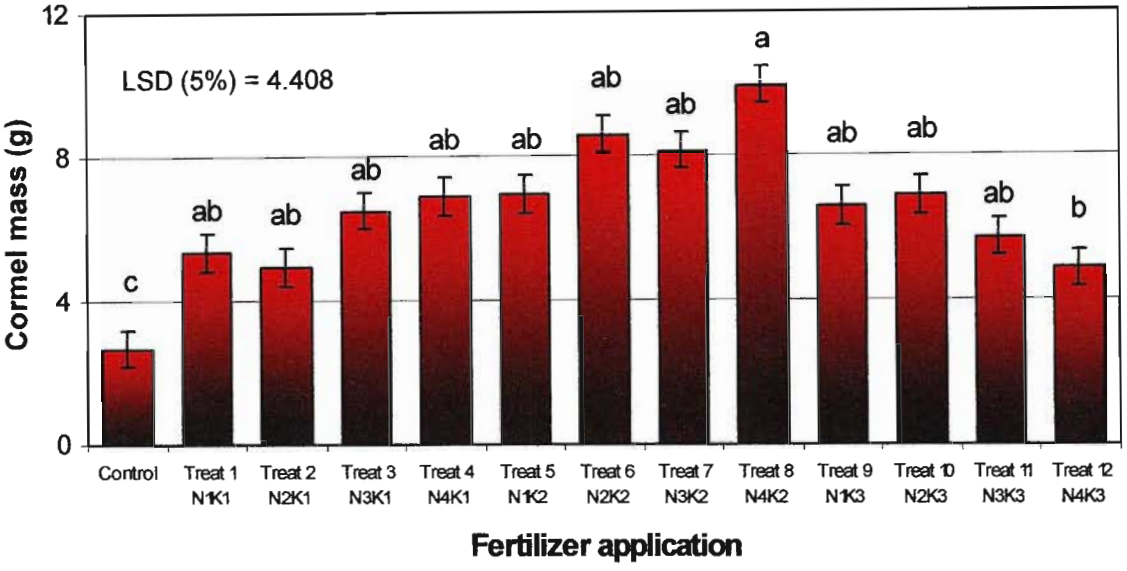


Figure 4.3 ***G. scabridus*** **cormel mass as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)**

Flowering: It has been estimated and assumed that in the wild, *G. scabridus* takes approximately 3 years (seasons) to flower from seed (C.R. Scott-Shaw, pers. commun., 2001). However, most of the plants grown during season 2 produced flowering spikes, confirming that the nutrient rich environment enhanced growth and shortened the juvenility phase of the species. The flowers were attractive and striking in colour (Plate 4.3 B) and would certainly be of interest to producers and consumers alike as a new ornamental product for the floricultural market. The fact that growth of this wild species can be enhanced in a nutrient rich environment to attain this product, is highly advantageous and would save production costs and time.

There was a highly significant effect of the treatments on the flowering percentage of the *G. scabridus* plants ($p<0.001$). Treatments 4 (80 %), 7 (90 %), 8 (80 %) and 12 (80 %) resulted in significantly more flowering plants at higher levels of N with varying levels of K (Figure 4.4). Salem and Tahar (1989) showed that K applications during the growing period have a considerable effect on flower emergence of some *Gladiolus* hybrids and therefore K applications should be taken into account and considered as an important factor in gladioli flower emergence. However, no significant differences were observed between the different applications of K_2SO_4 ($p=0.173$). Thus the wild species respond differently to the common hybrids as N appears to be important for flower development in *G. scabridus*. A lack of N can produce blind plants (Woltz, 1955).

The results have indicated that treatments with higher application levels of N at varying levels of K, are also important for *G. scabridus* plant height and leaf production. This implies that the improvement of vegetative growth has a positive effect on the flowering of the plants. Sixty percent of the control plants did flower. However, these flowers were much smaller in size and not as prolific as flowers from plants with fertilizer applications as found by Pimpini and Zanin (2002) and certainly would not meet the export requirement of long, sturdy stems with plenty of florets. The export requirement for gladioli stems is 75 cm or more (Salunke *et al.*, 1990). Thus the smaller size of these plants as noted by height and leaf number, meant smaller floral spikes. A change in *G. scabridus* corm mass has also been noted to be improved by treatments with higher N and varying levels of K. Thus, the enhancement of vegetative growth, not only improves flowering but also results in larger *G. scabridus* corms. Above ground reserves are important for the formation and development of the underground storage organ (du Plessis & Duncan, 1989). A further season's growth could be expected to result in larger spikes and thus larger corms.

Potti and Arora (1986) observed that the length of *Gladiolus* spikes was significantly influenced by the application of N at higher levels in combination with P and K and Kawarkhe *et al.* (2001) found that the number of spikes per plant and spike length increased with the increase in application rates of N and P fertilizers. There were no significant differences between *G. scabridus* floral stalk lengths ($p=0.203$) and number of flowering branches on each spike ($p=0.064$) between each treatment. However, the different levels of N had a significant effect ($p=0.029$) on the number of branches that developed (Figure 4.5). The highest level of 200 kg ha^{-1} NH_4NO_3 resulted in the highest number of branches (1.70) with 50 kg ha^{-1} and 100 kg ha^{-1} NH_4NO_3 resulting in significantly lower numbers (1.07 and 0.87 respectively). Yet again, high levels of N have a positive effect on the flowering of *G. scabridus* as the more floral branches produced by each spike, the more florets there will be thus improving the marketability of the floral spike.

Even though the plants during season 2 were staked because of their size, some still grew at a slight angle and these floral spikes displayed the common phenomenon in gladioli of geotropism, a response to gravity which is mainly a postharvest problem experienced during horizontal transport (Philosoph-Hadas *et al.*, 1995). This therefore is an important consideration in the production of *G. scabridus*.

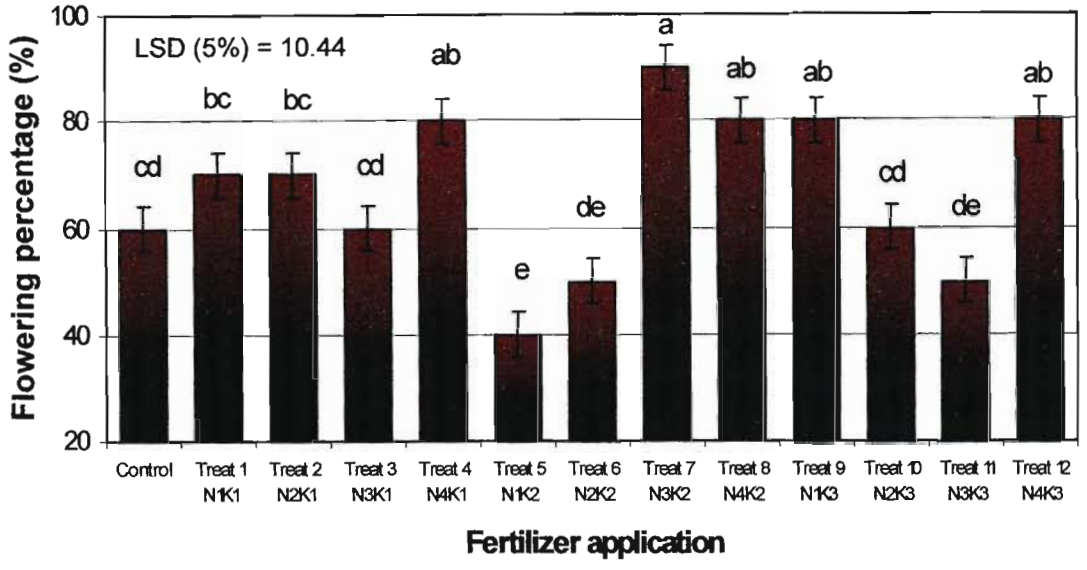


Figure 4.4 ***G. scabridus*** **flowering percentage as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)**

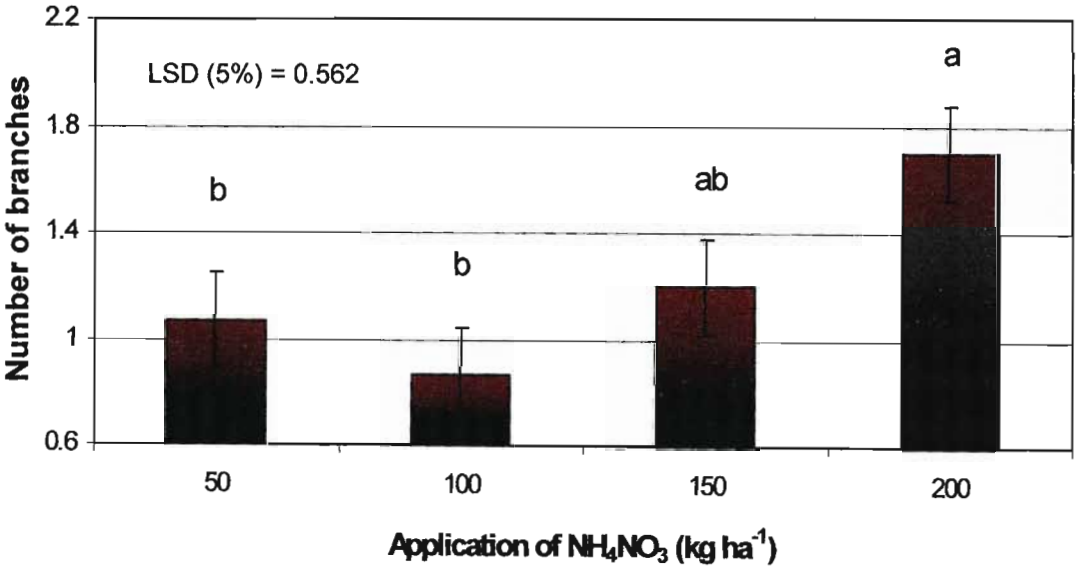


Figure 4.5 Number of ***G. scabridus*** **floral branches as influenced by NH_4NO_3 application during season 2 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)**

A

B

Plate 4.3 (A) The *G. scabridus* fertilizer trial during season 2 and (B) a *G. scabridus* floral spike that developed as a result of this season's growth

4.3.2.3 Growing media changes

Nitrogen (N): The results yet again indicate that *G. scabridus* plants take up N. This is shown by the significant decrease ($p=0.008$) in the N percentage in the media from the start of the growing season (0.271 %) to the levels at the end of the trial (0.160 %). Some loss would have been as a result of leaching (Groen & Slangen, 1990) but is unlikely to explain the results.

There were no significant differences in the final levels of N in the growing media between the different treatments ($p=0.582$). The control media showed the highest N level of 0.210 %. Control plants were smaller in size as indicated by plant height, leaf number and corm size data, and therefore would have taken up less N (Figure 4.6). Nitrogen was continually added to the other treatments and they show lower final N levels in the media indicating an uptake of N by these plants. Figure 4.6 confirms *G. scabridus* plant uptake of N for each fertilizer treatment ($p<0.001$). This indicates that the higher the application level of NH_4NO_3, the more N was taken up. Pine bark acts as a slow release fertilizer as far as nitrogen is concerned (Grzeszkiewicz, 1978) so it would also have supplied N to the plants. N uptake has also been confirmed by the increase in plant height, leaf number, corm size and improved flowering using fertilizer treatments with higher levels of N at varying levels of K.

Phosphorus (P): There was a highly significant decrease ($p<0.001$) in the level of P in the media from the start of the trial (458.0 mg l^{-1}), again indicating that *G. scabridus* plants took up available P. There were no significant differences ($p=0.576$) between the final levels of P (164.5 mg l^{-1}). The control, however, had the lowest final level of P in the media, unlike N. N and P usually compete for uptake by the plant roots (Parnes, 1990) so less N absorbed by the control *G. scabridus* plants might have meant a higher uptake of P.

Potassium (K): As with N and P, K was significantly reduced ($p<0.001$) in the growing media from before the trial (663.3 mg l^{-1}) to the end (134.8 mg l^{-1}). There were no significant differences in these final levels between the treatments ($p=0.057$). There was, however, a significant difference between levels in the media when comparing the three K_2SO_4 treatments ($p=0.006$). The highest application level of 150 kg ha^{-1} K_2SO_4 resulted in the highest medium content of 153.2 mg l^{-1} and the lowest application level of 50 kg ha^{-1} K_2SO_4 resulted in the significantly lowest K level of 113.2 mg l^{-1} (Figure 4.7). Thus higher application levels of K were unused by the plants as more was left in the media. This trend is confirmed

in Figure 4.8 which shows *G. scabridus* plant uptake of K. The higher the level of K_2SO_4 applied to the media, the less was taken up by the plants. Therefore, higher levels of K application are wasted on these plants. The results have shown that higher K is important for cormel development thus there is a fine balance between plant use and excess wastage.

Calcium (Ca): The initial level of Ca in the growing media was 1701 mg l^{-1} and as in season 1, the final concentrations of some treatments were higher and others lower than the start of the growing season, with no significant differences between the treatments ($p=0.412$). Ca was yet again available from the dolomitic lime in the pine bark media and the $2Ca(H_2PO_4)_2.H_2O$ applications, the release of which appeared to exceed its uptake by many of the plants.

Magnesium (Mg): Growing media levels were significantly reduced at the end of the trial from 423.8 to 359.4 mg l^{-1} ($p<0.001$). There were, however, no significant differences between the final Mg levels in each of the treatments ($p=0.544$). K and Ca are able to displace Mg from cation exchange sites in the soil (Cresswell & Weir, 1997) which may have occurred in the trial. There were significant differences between Mg levels in the growing media when comparing the four NH_4NO_3 treatments ($p=0.005$). At 150 kg ha^{-1} NH_4NO_3, the highest level of Mg was noted (384.3 mg l^{-1}) and this was significantly different to the other NH_4NO_3 treatments (Figure 4.9). This follows the same trend as the total cations in the media (Figure 4.10) which was also significantly higher at 150 kg ha^{-1} ($p=0.030$). Thus at this level of NH_4NO_3 more cations, especially Mg, become available for *G. scabridus* plant growth.

Zinc (Zn): This element was also significantly reduced in the growing media from 23.17 mg l^{-1} at the start of the trial to 16.51 mg l^{-1} at the end ($p<0.001$). There were no significant differences between the final levels of Zn ($p=0.152$), however, the control media resulted in the highest level of Zn (18.55 mg l^{-1}) compared to the other 12 treatments. Yet again, the smallest *G. scabridus* plants in the control would have taken up less Zn hence resulting in the highest level in the media at the end. High rates of N fertilizers may induce zinc deficiencies especially if the zinc supply is marginal (Cresswell & Weir, 1997). As in season 1, there was a non-significant decrease in the clay percentage ($p=0.101$) correlating with the decrease in zinc. The pH was suitable for Zn absorption.

Manganese (Mn): There was a non significant decrease in the level of Mn in the growing media from 10.00 mg l^{-1} to an overall mean value of 7.31 mg l^{-1} ($p=0.117$). There were no significant differences between the control (7.00 mg l^{-1}) and any of the 12 treatments ($p=0.842$), although some of the treatments showed higher Mn levels than the control and others lower, indicating different rates of uptake.

pH (in KCl): The pH of the medium was acidic before the trial commenced (5.44) but as a result of the addition of nutrients and the growth of *G. scabridus*, the pH significantly increased to 6.42 ($p<0.001$). There were no significant differences between the treatments ($p=0.386$) although the control resulted in the lowest pH. The control showed a higher final level of N in the media probably due to a greater presence of NH_4^+ attached to the soil particles and hence more H^+ ions contributing to a lower growth medium pH value.

There were significant differences in the media pH values between the individual NH_4NO_3 applications ($p=0.012$). The higher the application level of NH_4NO_3, the lower the pH. A level of 50 kg ha^{-1} resulted in the highest pH (6.51) which was significantly different to the 2 lowest levels at 150 kg ha^{-1} (6.42) and 200 kg ha^{-1} (6.35) (Figure 4.11). Yet again, the more H^+ ions added to the soil, the lower the pH. As in season 1, the exchangeable acidity and the acid saturation significantly decreased from the start of the trial ($p<0.001$) correlating with the increase in growing media pH.

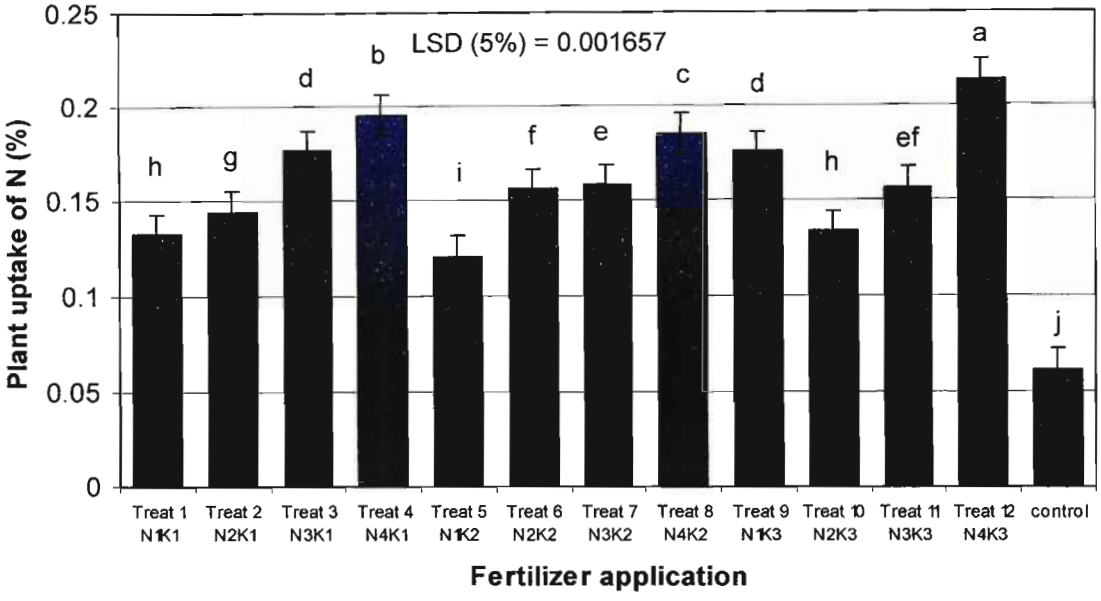


Figure 4.6 ***G. scabridus*** **uptake of nitrogen as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)**

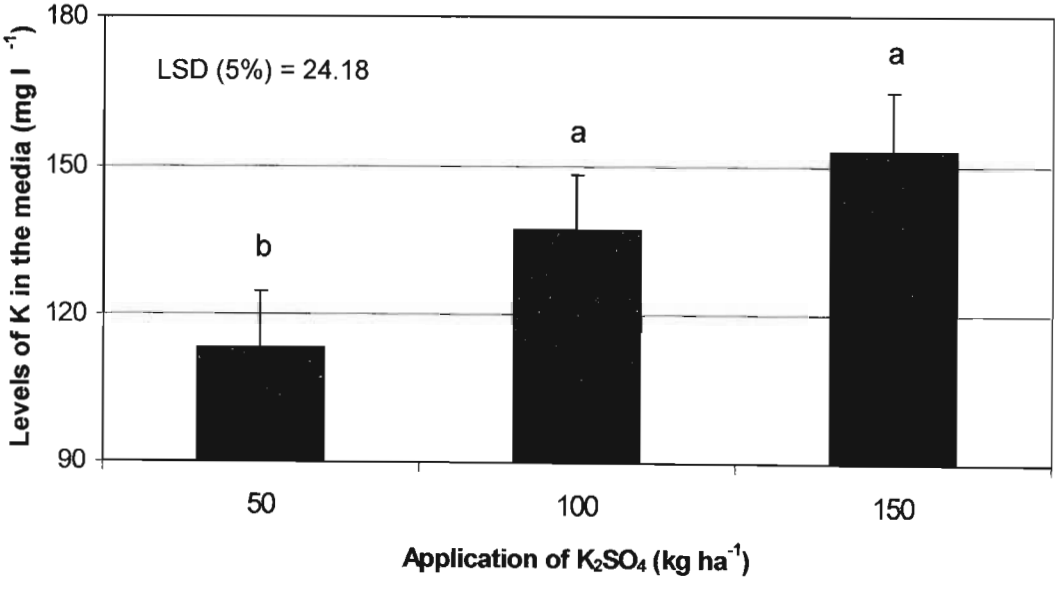


Figure 4.7 Potassium in the growing media at the end of season 2 of the ***G. scabridus*** **fertilizer trial as influenced by K_2SO_4 application. (Different letters indicate significant differences between treatments according to the LSD value.)**

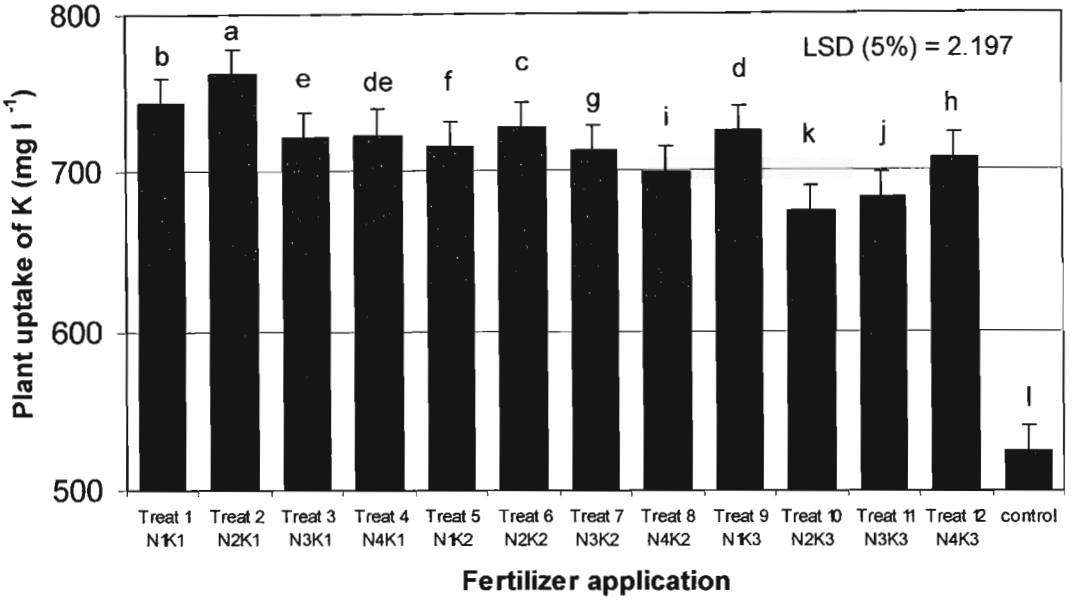


Figure 4.8 ***G. scabridus*** **uptake of potassium as influenced by fertilizer treatment during season 2 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 100 kg ha^{-1}, 3 = 150 kg ha^{-1}, 4 = 200 kg ha^{-1}). (Different letters indicate significant differences between treatment according to the LSD value.)**

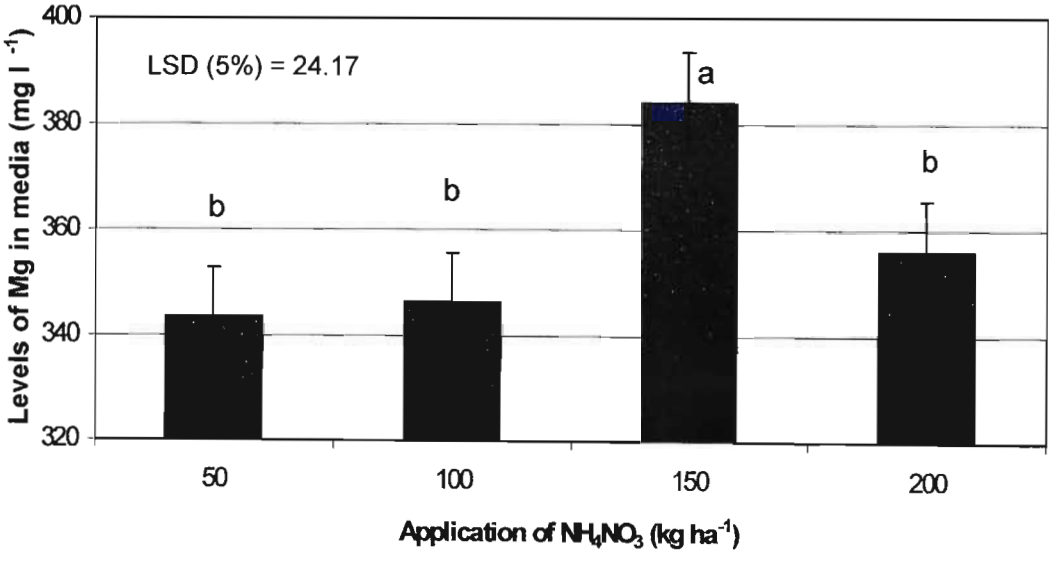


Figure 4.9 Magnesium in the growing media at the end of season 2 of the ***G. scabridus*** **fertilizer trial as influenced by NH_4NO_3 application. (Different letters indicate significant differences between treatments according to the LSD value.)**

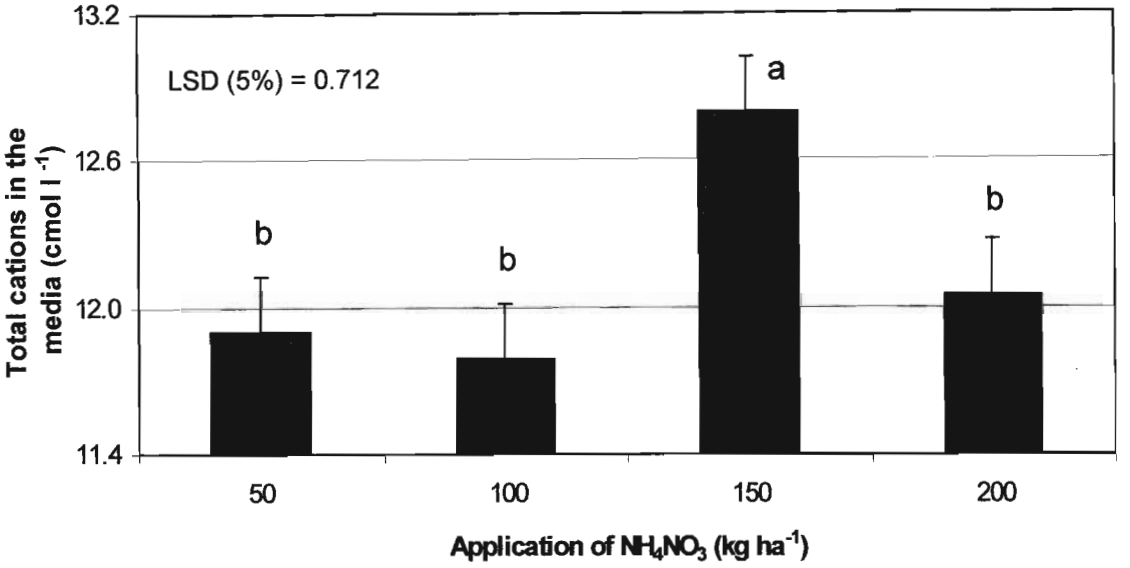


Figure 4.10 Total cations in the growing media at the end of season 2 of the *G. scabridus* fertilizer trial as influenced by NH_4NO_3 application. (Different letters indicate significant differences between treatments according to the LSD value.)

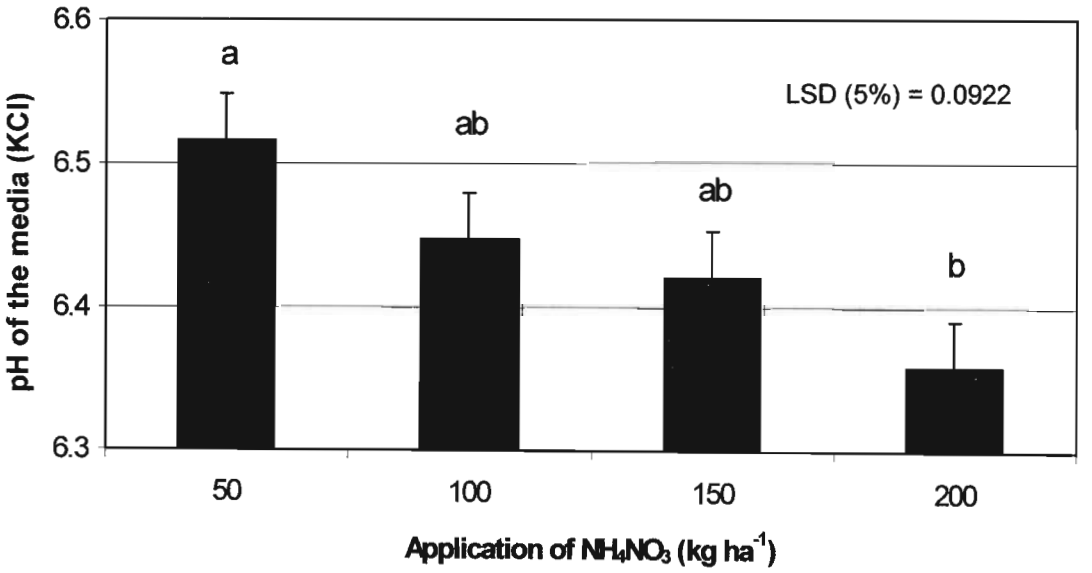


Figure 4.11 The pH of the growing media at the end of season 2 of the *G. scabridus* fertilizer trial as influenced by NH_4NO_3 application. (Different letters indicate significant differences between treatments according to the LSD value.)

4.3.2.4 Leaf analysis

An analysis of the above-ground portion of gladioli will give an indication of how much of each element was absorbed by these organs as well as assessing the nutrient status of the crop (Groen & Slangen, 1990). This can be done by comparing nutrient concentrations in specific plant tissues with predetermined ranges or standards for healthy, productive crops of the same species (Cresswell & Weir, 1997). However, it is also useful to perform a comparative study by evaluating a crop in relation to its performance. Appendix 1 shows the leaf nutritional standard values for commercially grown *Gladiolus* hybrids. A regression analysis was performed to see whether a relationship exists between the soil and leaf nutrient data of each element and no significant results were obtained.

Nitrogen (N): A comparison of the gladioli leaf standard levels with the leaf analysis values of *G. scabridus* from the fertilizer trial (Table 4.3), indicated that N in the *G. scabridus* leaves was deficient. Of all the soil nutrients, N is the most likely to be deficient (Parnes, 1990). However, throughout the trial, the leaves remained healthy with no visible deficiency symptoms and growth was enhanced in this nutrient rich environment. Soil analysis results indicated that the plants took up a substantial amount of N from the growing media. These plants have adapted to a low nutrient environment in the wild which will mean that tolerance levels in the leaves will be lower. Thus deficient levels in *Gladiolus* hybrids do not indicate deficient levels in the wild species and will be lower as indicated by the results.

There was a higher level of N in the control plant leaves. These plants were smaller and took up less N because of their size but would not have utilized as much N for plant growth processes as the other plants.

Soil analysis has shown that nitrogen in *G. scabridus* plants is important for vegetative growth, corm growth and flowering. Figure 4.12 shows that a leaf nitrogen level of about 1.4 % N is the optimum level for maximum plant height and 1.45 % N for maximum leaf growth (Figure 4.13). The optimum level for the highest corm weight change is about 1.5 % N (Figure 4.14) and that for maximum flowering is 1.3 % N (Figure 4.15). These are important considerations when applying nitrogen to the media for *G. scabridus* plant growth as a leaf nitrogen level between 1.3 and 1.5 % seems to be the optimum for the best plant growth.

Phosphorus (P): According to the leaf analysis norms (Appendix 1), P levels in the leaves were also very low indicating yet again that under hybrid conditions the leaves would be deficient in this element which could have affected the growth and appearance of the plants. *G. scabridus* growth occurred normally and the plants thrived, indicating that the leaf norms for *Gladiolus* hybrids are not indicative of those of a wild species like *G. scabridus*. It was assumed from the soil analysis that the control plants had taken up more P than plants in the other treatments and the leaf analysis results confirm that this was indeed so.

Potassium (K): All leaf K values fall below the low range (Appendix 1) but yet again it must be assumed that these levels do not apply to *G. scabridus*. Plants displayed no deficiency symptoms or abnormalities in growth as a result of a lack of K. Soil results indicated that plants took up K and that it is important for cormel growth in *G. scabridus*. Figure 4.16 shows that leaf levels between 0.800 and 0.900 % allow for maximum cormel development. However, this best fit curve was almost flat, thus indicative that at the time of sampling the effect of K on cormel development was not pronounced. Sampling was done just before flowering, and cormel development only begins after this process (Buch, 1972; Rees, 1992). Thus an important consideration when looking at the *G. scabridus* leaf analysis results, is the timing of the sampling. Samples were only taken at one point in the growing season, however, if accurate assumptions and recommendations are to be made, leaf samples should be taken at various stages throughout the growing season, together with soil analyses. Bulbous plants use minerals for different purposes at different times in the growing season. Sampling can also become difficult when dealing with an endangered plant, as all plant material is valuable. Regular sampling was not viable for the purpose of this trial as there were not sufficient plants to allow for destructive sampling without affecting the growth cycle. This may have affected the development of flowers, the daughter corm and the new cormels.

Calcium (Ca): Parnes (1990) considered that no relation exists between the amount of calcium in the soil and the amount in the plant. Soil values indicated that the plants did not take up much Ca. However, the leaf analysis values indicated that the plants took up enough Ca to be within the sufficient and high range for hybrid gladioli (Appendix 1). The control plants appear to have taken up more Ca than the other plants or used less of it for growth processes, as with N.

Magnesium (Mg): Most of the values fall in the sufficient range for hybrid gladioli, a few just above this and the control in the upper level of the low range. This indicates that the plants had adequate supplies of Mg and it is assumed that the control plants took up less Mg.

Sodium (Na): Although Na is not an essential element, it can benefit the growth of some plants by replacing K in the osmotic regulation of cells (Cresswell & Weir, 1997). The results were varied but were all quite low despite healthy plant growth.

Manganese (Mn): Most of the treatments and the control were within the sufficient range (Appendix 1), the control resulting in the highest level. All other levels can be classed as low thus indicating that the levels of Mn in the leaves of *G. scabridus* compared to the published norms are not indicative of deficiencies within these leaves.

Zinc (Zn): Zn is required by plants in small quantities and Zn deficiencies are uncommon on ornamental and greenhouse crops (Reed, 1996; Cresswell & Weir, 1997). The levels of Zn varied greatly between treatments, however, all were in the sufficient range (Appendix 1). The control plants showed the lowest level of Zn.

Copper (Cu): Applications of NPK fertilizers can induce Cu deficiencies as well as the use of acid-forming N fertilizers (Tisdale *et al.*, 1993). Cu levels were all in the sufficient range indicating *G. scabridus* plants absorbed a substantial amount of Cu for growth. The control showed the lowest level of Cu which is just above the low range. Cu deficiencies are less common than deficiencies of other micronutrients. The concentration of soil solution Cu decreases with increasing pH, and its supply to plants is reduced because of decreased solubility and increased adsorption (Cresswell & Weir, 1997).

Iron (Fe): Plants differ in their abilities to absorb and translocate Fe (Tisdale *et al.*, 1993). Fe becomes more available at a lower pH (Cresswell & Weir, 1997). Levels were all in the sufficient range with treatment 1 showing the lowest level followed closely by the control.

4.3.2.5 Pests and diseases

The only problems encountered were aphids and whitefly which were eradicated with spraying. A few *G. scabridus* plants were grown in the open under shadecloth and these were attacked quite prolifically by rust. The protected glasshouse environment was thus conducive to protecting the plants from any major problematic pests and diseases.

***Table* 4.4 Mineral analysis of the most recently matured *G. scabridus* leaves sampled during the second growing season as related to fertilizer treatment**

Treatment	Element (%)						Element (ppm)			
	N	P	K	Ca	Mg	Na	Mn	Zn	Cu	Fe
1	1.130	0.162	0.837	1.447	0.275	0.04	40.5	81	9.5	74.5
2	1.175	0.160	0.758	1.547	0.248	0.02	54.5	136	11.7	135.8
3	1.285	0.230	1.008	1.442	0.340	0.03	41.7	51	10.0	96.7
4	1.240	0.185	1.130	1.225	0.210	0.12	36.2	66	9.7	86.0
5	1.135	0.187	0.870	1.517	0.320	0.03	48.5	66	10.7	98.5
6	1.620	0.127	0.840	1.775	0.340	0.06	54.0	108	8.5	91.2
7	1.430	0.167	1.007	1.382	0.275	0.03	41.7	76	10.0	140.8
8	1.315	0.157	0.845	1.612	0.165	0.04	53.5	45	10.7	142.3
9	1.200	0.155	0.650	1.512	0.215	0.03	52.5	51	7.7	85.0
10	1.080	0.150	0.745	1.665	0.223	0.03	39.2	28	9.2	97.0
11	1.620	0.227	0.850	1.735	0.240	0.02	48.2	77	10.2	105.5
12	1.223	0.142	0.935	1.375	0.330	0.04	41.5	65	9.7	170.0
control	2.200	0.200	1.120	1.855	0.145	0.06	59.0	40	8.0	76.5

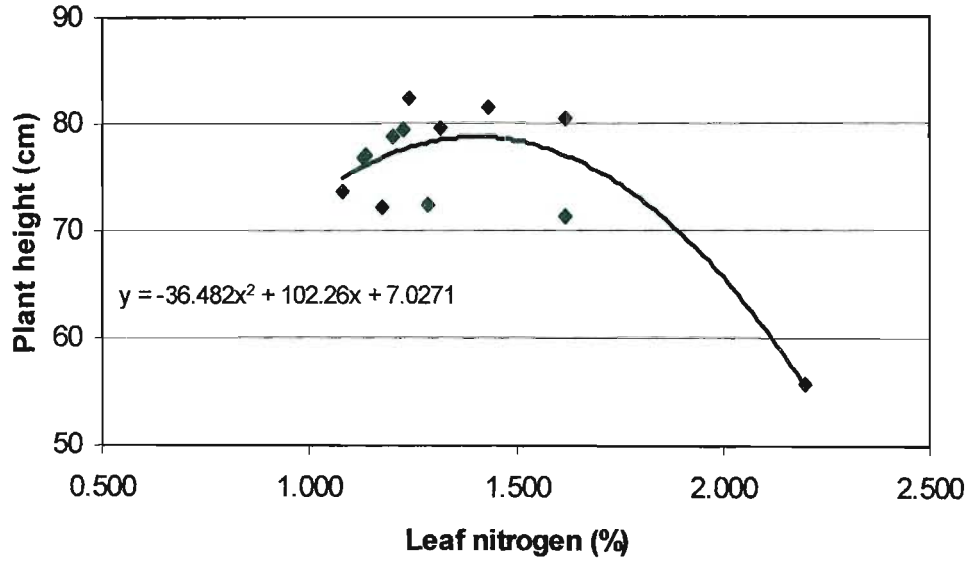


Figure 4.12 ***G. scabridus*** **plant height in relation to the nitrogen level in the leaves during season 2 of the fertilizer trial**

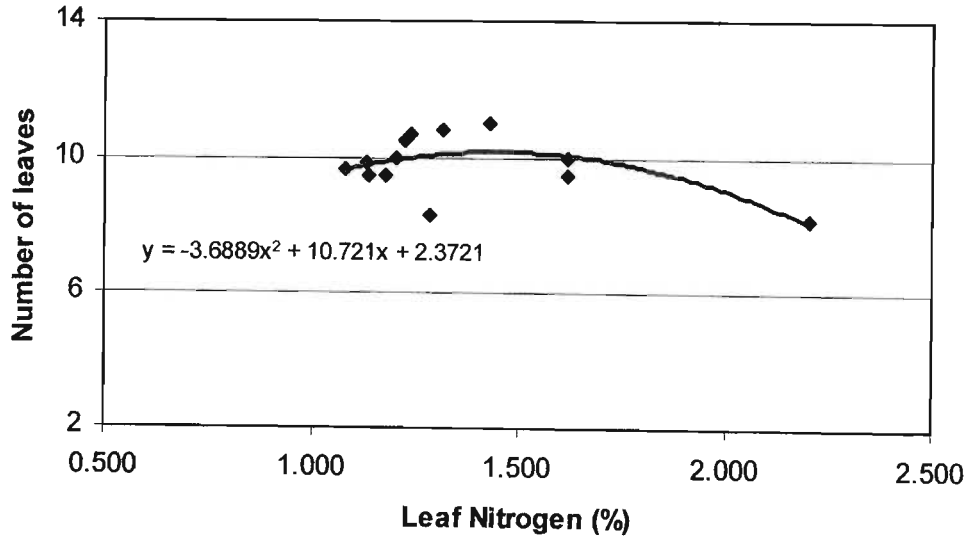


Figure 4.13 ***G. scabridus*** **leaf number in relation to the nitrogen level in the leaves during season 2 of the fertilizer trial**

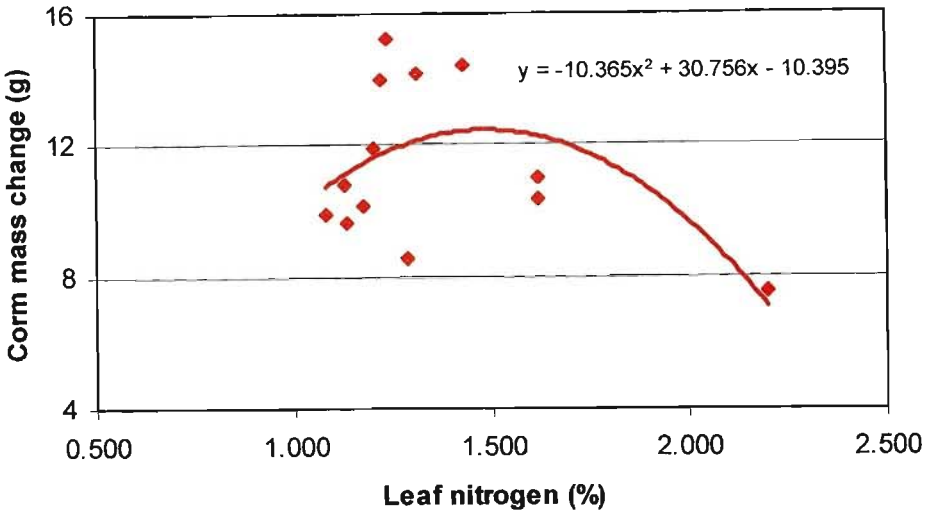


Figure 4.14 ***G. scabridus*** **corm mass change in relation to the nitrogen level in the leaves during season 2 of the fertilizer trial**

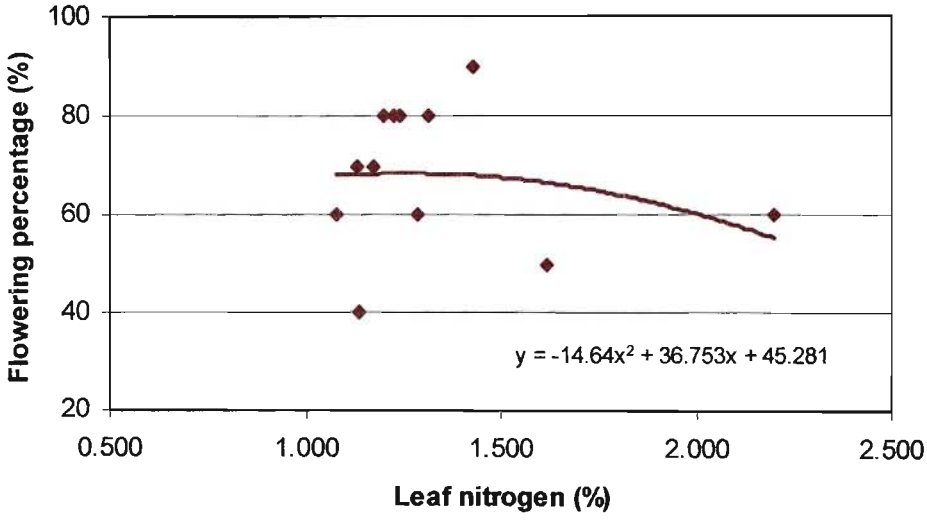


Figure 4.15 ***G. scabridus*** **flowering percentage in relation to the nitrogen level in the leaves during season 2 of the fertilizer trial**

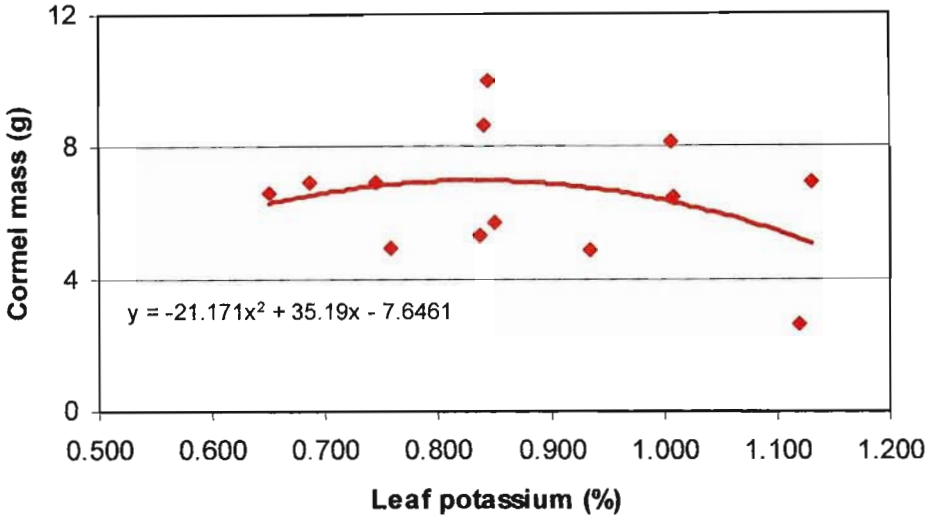


Figure 4.16 ***G. scabridus*** **cormel mass in relation to the potassium level in the leaves during season 2 of the fertilizer trial**

4.3.3 Season 3

4.3.3.1 Plant growth in general

The *G. scabridus* corms sprouted within 10 to 14 days of planting out. Growth was rapid and the resulting plants during this season were all tall and healthy in appearance, due to the fact that slightly larger / more mature corms were used this season (Remotti *et al.*, 2002). The base of the shoots were thick, all plants needed staking and all produced flowering spikes. Flowers appeared once 6 to 7 foliage leaves had fully matured which was about 85 to 90 days after sprouting.

Axillary bud development was evidenced with the appearance of a number of smaller side shoots, which appears to be a very characteristic trait of *G. scabridus* corms as this was also observed in the previous seasons. At the end of this season, healthy, large daughter corms had developed with the production of numerous cormels.

4.3.3.2 Nutritional effects on plant growth

Plant height: Results from the previous seasons indicated that N is important for the vegetative growth of *G. scabridus* plants. The 3 applications of NH_4NO_3 during the first half of season 3 had a highly significant effect on plant growth and hence plant height ($p<0.001$). The highest application rate of 250 kg ha^{-1} NH_4NO_3 resulted in the tallest plants (105.6 cm), that of 150 kg ha^{-1} NH_4NO_3 produced plants with a mean height of 86.2 cm, whilst plants receiving 50 kg ha^{-1} NH_4NO_3 had the lowest mean height of 77.7 cm (Figure 4.17). This clearly confirms the importance of a high initial application of N to the growing media of developing *G. scabridus* plants as found by Jhon *et al.* (1997) and Sehrawat *et al.* (2003).

Leaf number: There was also a highly significant effect of N application on the average number of leaves ($p<0.001$). As with plant height the highest level of NH_4NO_3 resulted in the most number of leaves (9.97) which was significantly different to the leaf number of plants receiving 150 kg ha^{-1} NH_4NO_3 (9.24) and those with 50 kg ha^{-1} NH_4NO_3 (8.18). This confirms results from season 2 that N plays a vital role in the vegetative growth of *G. scabridus* (Figure 4.18). Pradhan *et al.* (2004) also noted that the average number of leaves per plant was highest with higher levels of N.

Corm size: There were no significant differences in the change in corm width when comparing corms between the N treatments (p=0.130), the final K treatments (p=0.378) and the combined effects of all the treatments during the season (p=0.467). Overall final mean corm width was 50.43 mm. There was, however, a tendency for higher levels of both N and K to result in slightly wider / fatter corms. It appears that the growth of the new corms is more horizontal in nature than vertical. As with season 2, daughter corms were compressed in shape. Final mean corm height was 25.96 mm. No significant differences were observed between corms when comparing the N treatments (p=0.319), the K treatments (p=0.746) and the treatment combinations (p=0.169) although, yet again, higher levels of both elements resulted in slightly bigger corms as found by Hancke and Verryn (1989b).

Corm mass: The total mean change in corm mass this season was 20.21 g. This change in mass was significantly affected by the initial 3 NH_4NO_3 applications (p=0.023), as well as the 3 K treatments during the second half of the growing season (p=0.019). The highest application of 250 kg ha^{-1} NH_4NO_3 resulted in a corm mass increase of 26.4 g (Figure 4.19). This was not significant to applications of 150 kg ha^{-1} NH_4NO_3 (21.2 g) but was significantly different to the changes in corm mass with 50 kg ha^{-1} NH_4NO_3 (15.9 g). K applied at a rate of 250 kg ha^{-1} resulted in the highest change in corm mass of 26.2 g (Figure 4.20). This was not significantly different to the change in mass with 150 kg ha^{-1} K (20.4 g) but was significantly different to corms receiving 50 kg ha^{-1} K (14.9 g).

This yet again confirms previous results that a higher application of N during the first half of the season is important not only for improved vegetative growth, but this in turn provides a greater supply of reserves to developing daughter corms from the above ground plant parts. Covariate analysis showed that there was no significant effect of *G. scabridus* leaf number (p=0.150) or plant height (p=0.108) on corm mass change. High levels of K are important from the onset of flowering as this is when daughter corm development starts to take place (Delpierre & du Plessis, 1973; Salinger, 1985; Greving, 1992) and thus enhances this new corm formation. Previous authors have also noted the importance of both N and K in corm development (Hancke and Verryn, 1989b; Sehrawat *et al.*, 2003; Shankar & Dubey, 2005).

Cormel number: There were no significant differences in the final cormel numbers between the N treatments (p=0.070), the K treatments (p=0.121) and when comparing the combination thereof (p=0.261). The mean number of cormels was 14.1. There were, however, tendencies for the highest number of cormels to be produced under conditions of

higher levels of K, confirming results from the previous seasons that this element is important for this stage of growth.

Cormel mass: The results of cormel mass followed similar trends to the results for cormel number with no significant differences between the N treatments (p=0.355), the K treatments (p=0.157) and when comparing all 9 treatments (p=0.552). Mean cormel mass was 3.65 g. The quality of the cormels produced is of great importance, since the cormel crop is the first step in corm production (De Hertogh & Le Nard, 1993). From an observational point of view, it appears that the more cormels the *G. scabridus* plants produce, the smaller they are in size. Treatments with higher levels of K yet again showed tendencies for a larger cormel mass.

Flowering: Every *G. scabridus* plant used in the fertilizer trial this season produced a flowering spike resulting in 100 % flowering percentage for each treatment. Generally, plants produced tall floral spikes with healthy, large, open florets (Plate 4.4) and clearly thrived as before in the nutrient rich environment. It is certainly advantageous choosing large, healthy corms when planting out as this certainly aids in producing larger, healthy *G. scabridus* spikes.

Bhattacharjee (1981) observed that increasing levels of N greatly increased flower spike length. There was a significant effect of NH_4NO_3 on the length of *G. scabridus* floral spikes (p=0.005). An application rate of 250 kg ha^{-1} NH_4NO_3 resulted in the tallest spikes (133.9 cm) which was significantly different to heights of 115.6 cm with 150 kg ha^{-1} NH_4NO_3 and 107.1 cm when applying 50 kg ha^{-1} NH_4NO_3 (Figure 4.21). There was no significant difference in floral spike length between the 2 lower N treatments. This confirms that N clearly plays an important role in *G. scabridus* floral spike development as shown this season.

As in season 2, the number of flowering branches on each spike was also significantly affected by applications of NH_4NO_3 following similar trends to that of spike length (Figure 4.22). The greatest number of branches developed with 250 kg ha^{-1} NH_4NO_3 (2.56). This was significantly different to 150 kg ha^{-1} NH_4NO_3 (1.88) and 50 kg ha^{-1} NH_4NO_3 (1.79). There was no significant difference in branch number between the two lower N treatments. It is highly advantageous to produce the greatest number of floral branches on each spike as the greater the number of branches, the more florets will develop and will mean more colour on each spike. Hybrid gladioli are normally sold as spikes with single branches. If this is desired, then lower N levels will need to be applied to *G. scabridus* spikes. Growth of the floral spike can clearly be manipulated using different levels of N as noted by Pradhan *et al.* (2004).

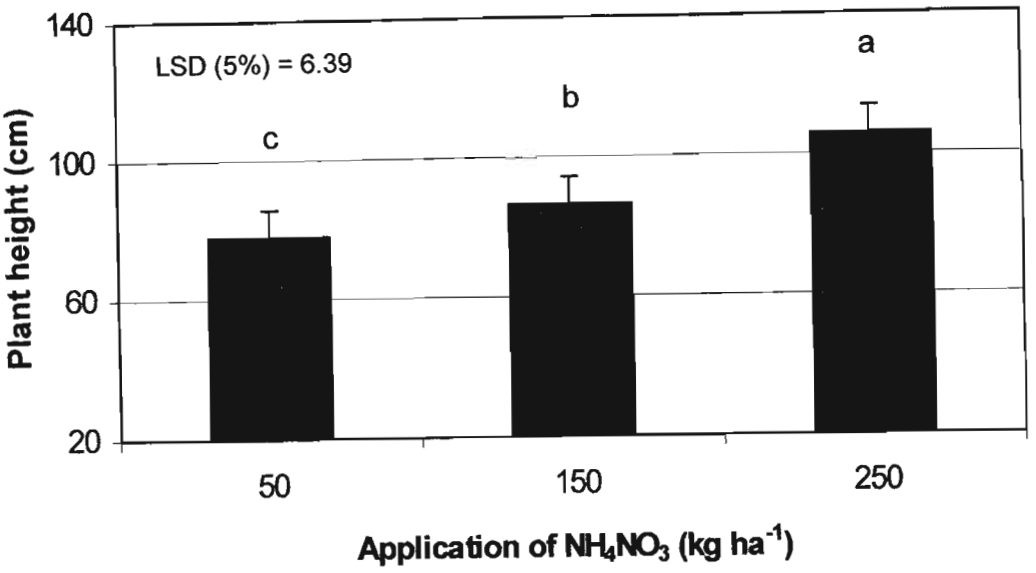


Figure 4.17 *G. scabridus* plant height as influenced by NH_4NO_3 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)

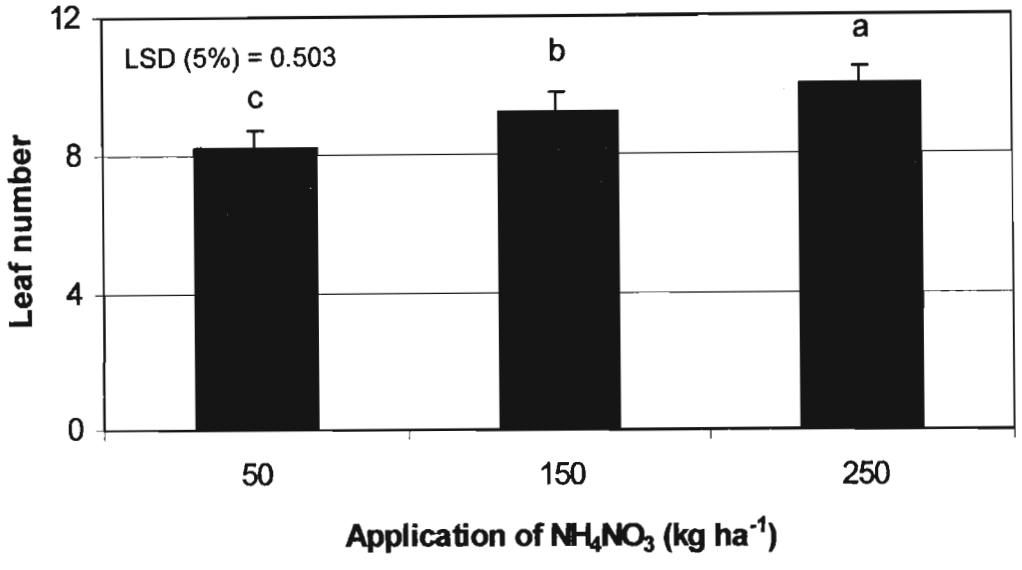


Figure 4.18 *G. scabridus* leaf number as influenced by NH_4NO_3 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)

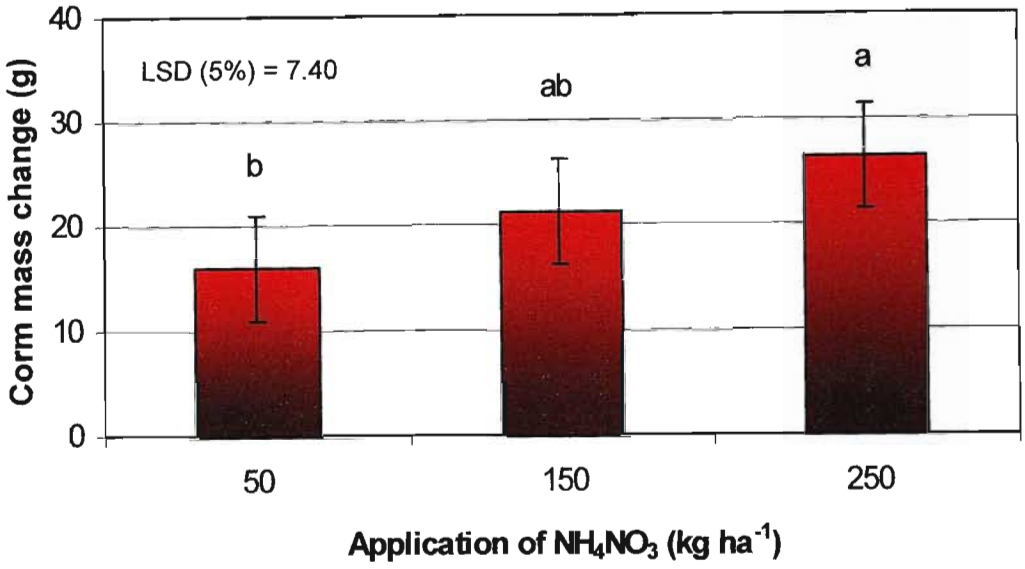


Figure 4.19 ***G. scabridus*** **corm mass change as influenced by NH_4NO_3 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)**

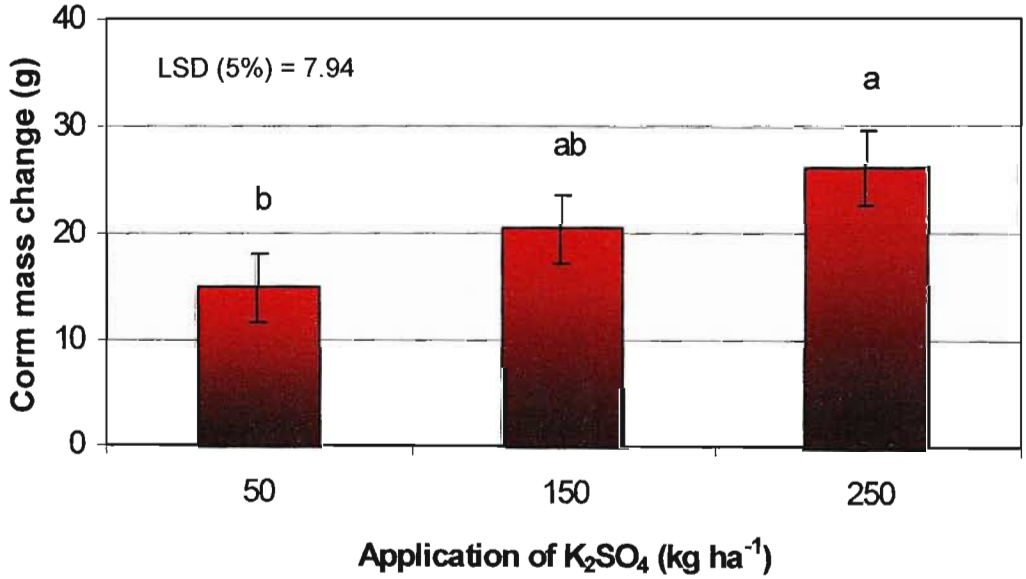


Figure 4.20 ***G. scabridus*** **corm mass change as influenced by K_2SO_4 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)**

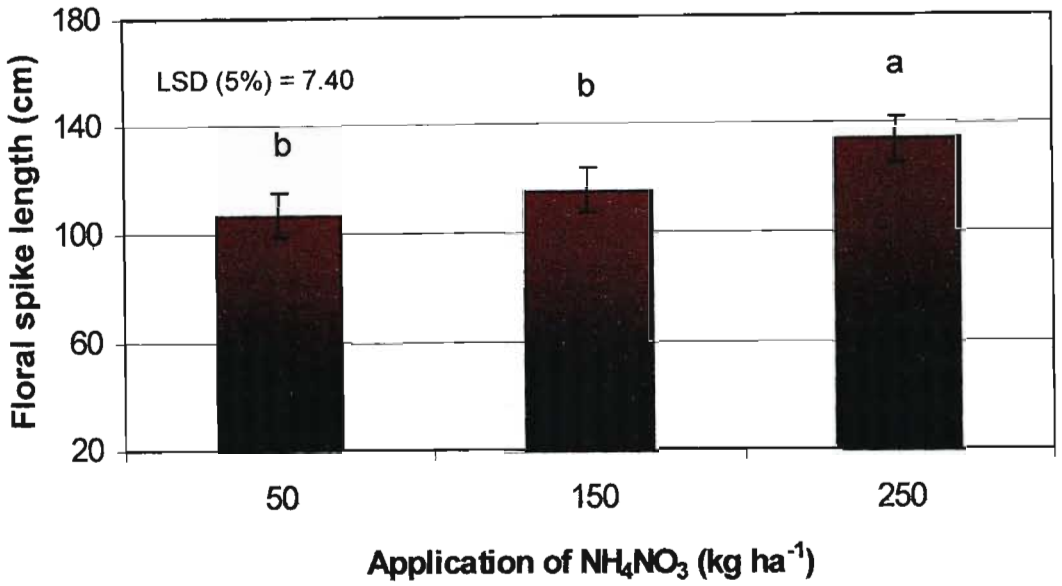


Figure 4.21 ***G. scabridus*** **floral spike length as influenced by NH_4NO_3 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)**

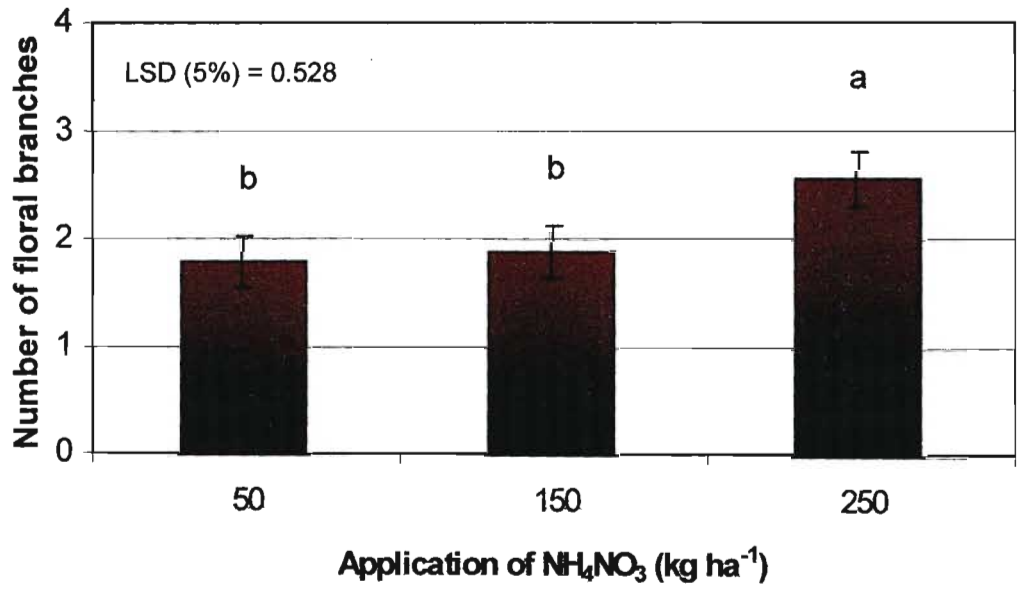


Figure 4.22 ***G. scabridus*** **floral branch number as influenced by NH_4NO_3 application during season 3 of the fertilizer trial. (Different letters indicate significant differences between treatments according to the LSD value.)**

Plate 4.4 The *G. scabridus* fertilizer trial during season 3 showing the healthy, large flowering spikes

4.3.3.3 Growing media changes

***Soil leachate analysis*:** The results from the mineral analysis of the leachate samples indicate that very low concentrations of the elements were lost through irrigation water and the application of liquid fertilizers. Levels of N were undetectable. It can thus be clearly confirmed that nutrient depletion in the media is mainly as a result of the uptake by the *G. scabridus* plants (Table 4.5).

Nitrogen (N): The initial level of N in the media (0.443 %) had decreased significantly by the termination of the trial with a final mean value of (0.400 %). There were no significant differences when comparing growing media results between the initial N treatments ($p=0.320$) or the final treatment combinations ($p=0.910$).

There were also no significant differences in the plant uptake of N between any of the treatment combinations. The overall mean uptake of N was 0.061 %. However, plants which received the highest level of 250 kg ha^{-1} NH_4NO_3 during the first half of the growing season, showed tendencies to take up the least N implying that this level may have been too high if extra N was not taken up. The overall uptake of N was lower than the previous growing season perhaps due to the use of larger corms which contain more storage products and hence do not rely as much on an external source of N. However, plant data has shown the importance of this external applied N.

Phosphorus (P): There was a highly significant ($p<0.001$) decrease in P in the media when comparing mean values from the start of the trial (175.0 mg l^{-1}) to the end of the trial (136.5 mg l^{-1}). This clearly shows, yet again, that the *G. scabridus* plants take up P during the growing season. P has several functions in the plant including energy distribution and it acts as a stimulus for root development but it is involved in every metabolic process affecting healthy growth and flowering (Parnes, 1990). There were no significant differences in growing media P levels when comparing treatments at any of the sampling dates.

Potassium (K): The K level in the growing media at the start of the trial was 610.3 mg l^{-1}. This had decreased significantly ($p<0.001$) by the end of the trial to a mean value of 368.4 mg l^{-1}. There were also highly significant differences in the final K content in the media when comparing the final 9 treatments ($p<0.001$) and the 3 separate K_2SO_4 treatments applied during the second half of the growing season ($p<0.001$) (Figure 4.23 and Figure 4.24). These show the more K that was added, the higher the final media level as was found in season 2.

As with N, results from this season show that less K was taken up compared to the previous season, presumably due to the larger corms that were planted out with higher quantities of storage reserves. There was no significant difference in the plant uptake of K midway ($p=0.805$) or at the end ($p=0.511$) of the growing season although there was a tendency for higher application levels that resulted in higher final K levels in the media (as was found in season 2).

Calcium (Ca): There was no significant difference in the overall change in Ca in the growing media ($p=0.075$). The initial level was 1803 mg l^{-1} and this increased in all the treatments to a mean value of 1942 mg l^{-1}. There were also no significant differences when comparing final Ca levels between treatments. This confirms previous results that *G. scabridus* plants do not readily take up Ca. There were no observed deficiency or toxicity symptoms on the leaves and plant health was excellent which indicates that they do not require large amounts of Ca for plant development. No dolomitic lime was added to the media this season, hence Ca would have been released from cations sites in the pine bark media (Salinger, 1985) and from the initial application of $2Ca(H_2PO_4)_2.H_2O$.

Magnesium (Mg): The initial mean level of Mg in the media was 655.8 mg l^{-1} and this had been significantly reduced to 586.1 mg l^{-1} by the end of the trial ($p<0.001$) indicating that the plants do take up Mg for growth and development. There were no significant differences between the final Mg levels in all the treatments.

Zinc (Zn): Results from season 1 and 2 both showed a decrease in the levels of Zn in the media. However, the levels of Zn in the media during this season significantly increased ($p=0.017$) from an initial level of 14.20 mg l^{-1} to an overall final mean level of 16.37 mg l^{-1}. This implies that very little, if any, Zn was taken up by the plants during this growing season. The total cation content and clay percentage of the media showed non-significant changes, however, did increase in some of the treatments. This meant that more binding sites became available for cations, such as zinc, to become attached to.

There was a significant difference ($p=0.027$) in the levels of Zn when comparing media between the three K_2SO_4 treatments applied during the second half of the growing season. The level of Zn was highest in media to which 250 kg ha^{-1} K_2SO_4 had been applied (17.82 mg l^{-1}). This was significantly different to the other 2 treatment levels of 150 kg ha^{-1} K_2SO_4 (15.76 mg l^{-1}) and 50 kg ha^{-1} K_2SO_4 (15.54 mg l^{-1}), although these 2 lower treatments were not

significantly different to each other (Figure 4.25). Perhaps when high levels of K ions are readily available, the uptake of these ions is favoured over others like Zn resulting in higher levels of Zn in the media. Zn uptake is strongly affected by plant species and varieties (Follett *et al.*, 1981).

Manganese (Mn): There was a highly significant ($p<0.001$) decrease in the level of Mn in the growing media from the start of the trial (69.86 mg l^{-1}) to the final mean value of 34.90 mg l^{-1} indicating that the plants readily take up Mn. The only significant differences, when comparing the treatment combinations, were between media analysed midway through the growing season as a result of the 3 NH_4NO_3 applications ($p=0.030$). There was a significant difference between Mn levels in media to which 250 kg ha^{-1} NH_4NO_3 had been applied (49.8 mg l^{-1}) and the lowest level of 50 kg ha^{-1} NH_4NO_3 (40.4 mg l^{-1}). Both these levels were not significant to the Mn level in growing media to which 150 kg ha^{-1} NH_4NO_3 had been added (Figure 4.26). These results imply that the higher the application of N, the less Mn is taken up by the plants. Perhaps as with K and Zn, *G. scabridus* plants favour the uptake of N over Mn.

Copper (Cu): Cu levels showed a non-significant ($p=0.736$) change from the start of the trial (2.409 mg l^{-1}) to the end of the trial (2.438 mg l^{-1}). The levels increased in some of the treatments and decreased in others showing variable rates of uptake. Cu and Zn often compete for the same absorption sites on roots (Follett *et al.*, 1981).

pH (in KCl): The pine bark based growing media had a pH value of 5.53 at the start of the trial which showed a non-significant increase ($p=0.280$) to 5.60 when comparing all the treatments. This can be correlated with the mean significant decrease ($p<0.001$) in the acid saturation of the growing media. Although increasing, the pH level was still conducive to healthy plant growth as it remained acidic. There was, however, a highly significant difference in the pH values of media analysed in the middle of the growing between the 3 NH_4NO_3 treatments ($p<0.001$). Media to which 250 kg ha^{-1} NH_4NO_3 had been applied had the significantly lowest pH value of 5.35 (Figure 4.27). As before, the higher the level of applied NH_4NO_3, the lower the pH due to the presence of more H^+ ions.

***Table* 4.5 Mineral analysis of leachate samples taken during the third growing season of the *G. scabridus* fertilizer trial**

Treatment	Sample Date	E.C. (mS/m)	PH	Cations (me/L)				Anions (me/L)	
				Na	Ca	Mg	K	P	Cl
control[v]	Nov	10.07	7.20	0.19	0.47	0.20	0.04	0	0.47
1[w]	Nov	13.41	6.72	0.39	0.42	0.40	0.19	3.7	0.85
2[x]	Nov	16.16	6.85	0.44	0.50	0.49	0.23	3.0	0.83
3[y]	Nov	21.06	6.96	0.56	0.62	0.59	0.29	2.6	0.71
Control[z]	Jan	11.81	7.44	0.19	0.64	0.23	0.04	0	0.55
1	Jan	11.41	6.58	0.35	0.32	0.30	0.15	2.0	0.65
2	Jan	11.49	6.85	0.35	0.36	0.33	0.17	1.6	0.65
3	Jan	14.89	6.63	0.45	0.41	0.39	0.19	2.1	0.82

[v] irrigation water before application to the growing media

[w] 50 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[x] 150 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[y] 250 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[z] irrigation water before application to the growing media

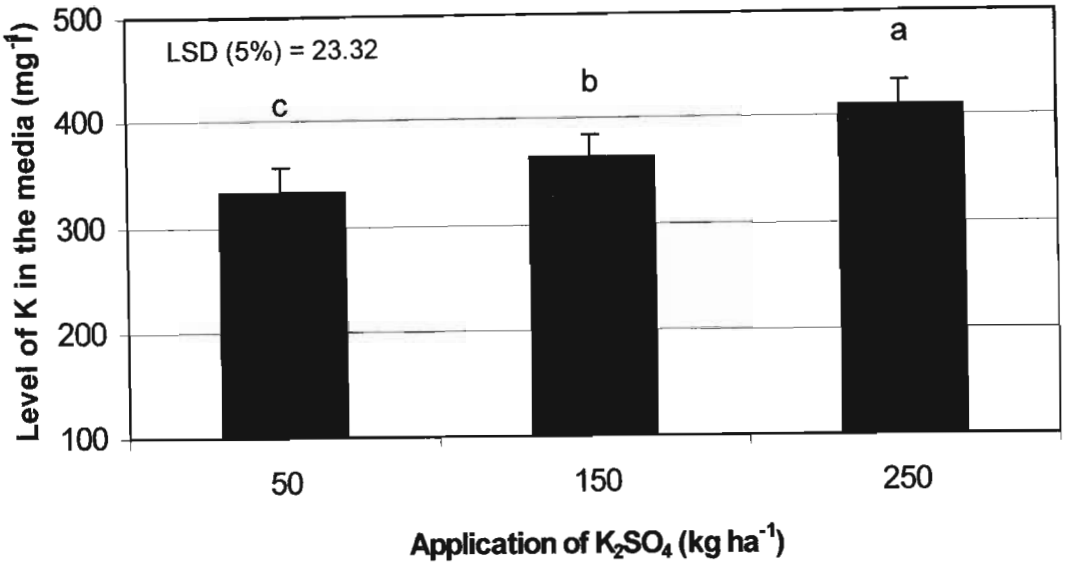


Figure 4.23 Potassium in the growing media at the end of season 3 of the *G. scabridus* fertilizer trial as influenced by K_2SO_4 application. (Different letters indicate significant differences according to the LSD value.)

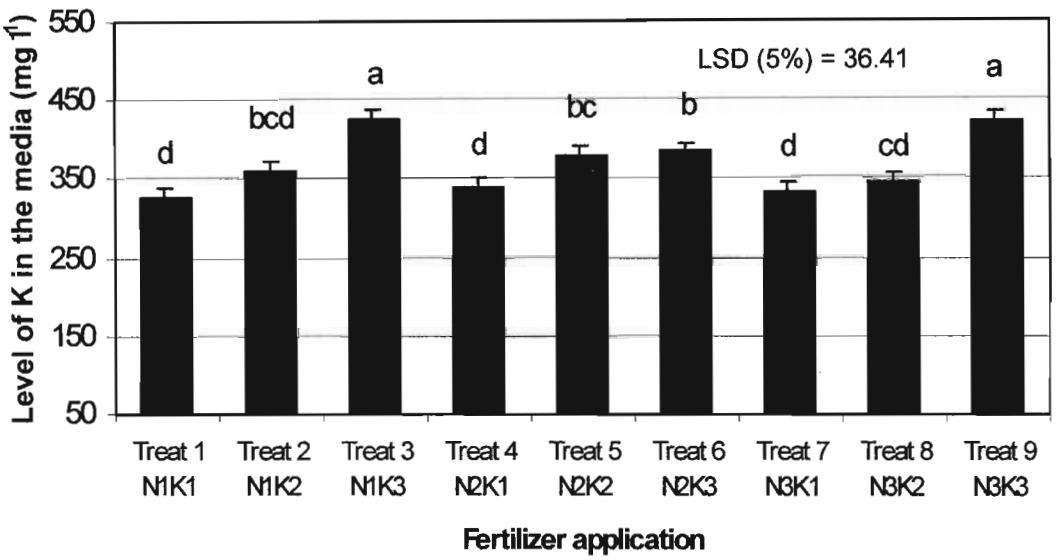


Figure 4.24 Potassium in the growing media as influenced by fertilizer treatment during season 3 of the fertilizer trial (1 = 50 kg ha^{-1}, 2 = 150 kg ha^{-1}, 3 = 250 kg ha^{-1}). (Different letters indicate significant differences between treatments according to the LSD value.)

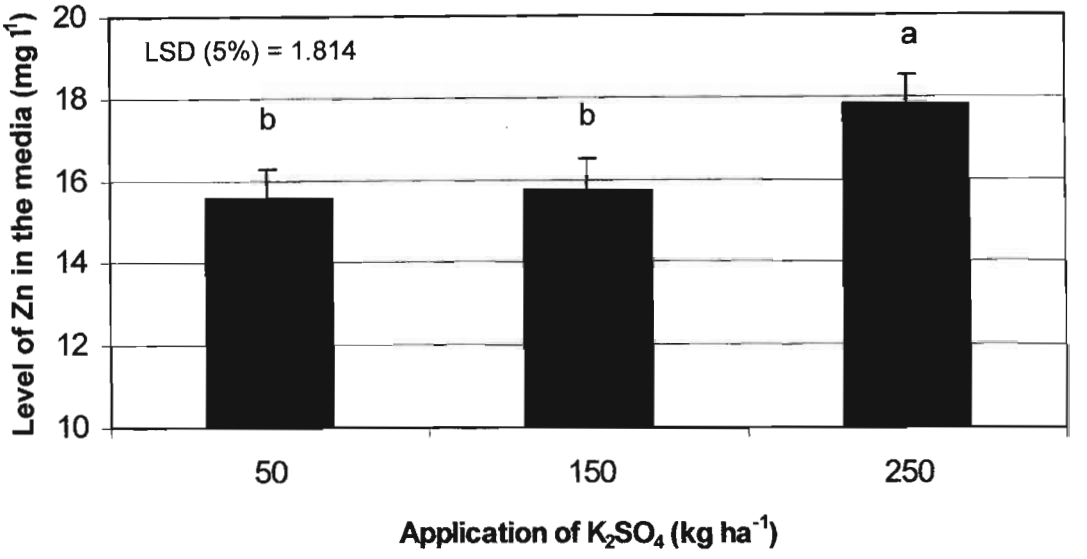


Figure 4.25 **Zinc in the growing media at the end of season 3 of the *G. scabridus* fertilizer trial as influenced by K_2SO_4 application. (Different letters indicate significant differences according to the LSD value.)**

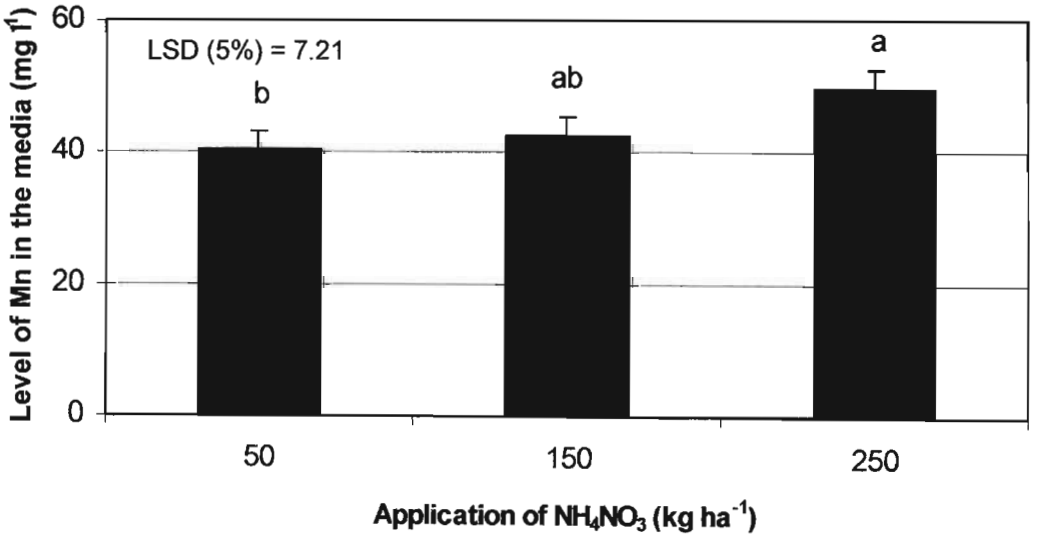


Figure 4.26 **Manganese in the growing media midway through season 3 of the *G. scabridus* fertilizer trial as influenced by NH_4NO_3 application. (Different letters indicate significant differences according to the LSD value.)**

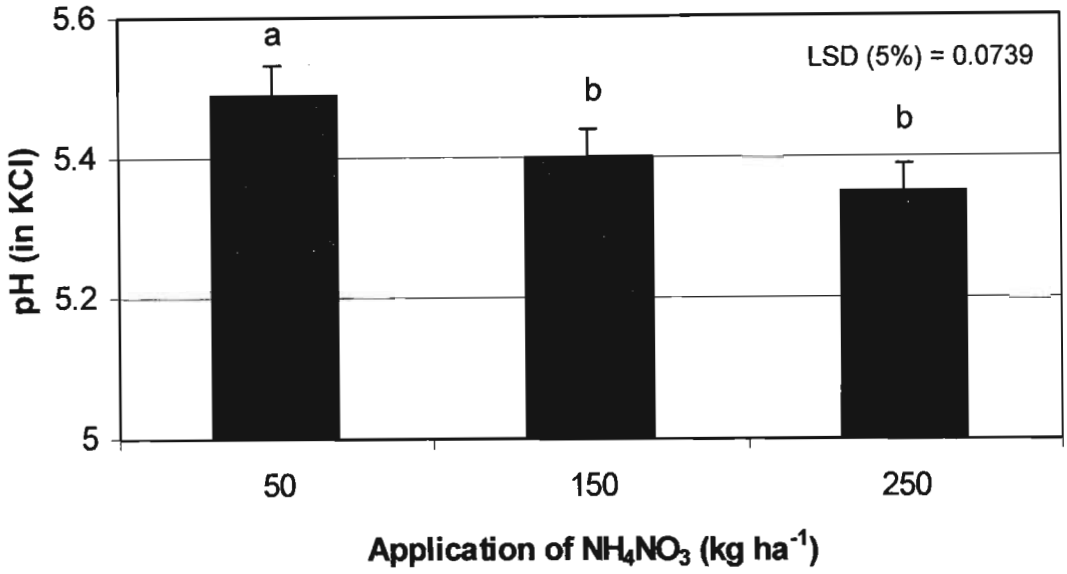


Figure 4.27 The pH of the growing media midway through season 3 of the the *G. scabridus* fertilizer trial as influenced by NH_4NO_3 application. (Different letters indicate significant differences according to the LSD value.)

4.3.3.4 Leaf analysis

There were no significant differences when comparing the leaf nutrient values between the treatments and a regression analysis between soil and leaf data also revealed no significant results. Statistical analysis showed that the removal of leaves at the various growth stages for nutrient analysis, had no significant effect on the final results.

Nitrogen (N): The levels of N in the *G.scabridus* leaves decreased over time (Tables 4.6 to 4.8). On the first sample date, during the second month of growth, the levels of N in the leaves already showed a proportional relationship to the amount of N being added to the soil, thus indicating that the young plants act as sinks and rapidly take up N from the growing media. These levels were in the low to sufficient range for *Gladiolus* leaf norms (Appendix 1), although showing healthy, vigorous growth, confirming results from season 2 that these wild species tolerate and thrive at lower nutrient levels than the hybrids. These N levels then decreased over time, as shown on the second sampling date, still showing an increase in leaf N with increasing N treatment. On the final sampling date leaves still appeared healthy and the N levels in the leaves were far below the low level for hybrid leaf norms. These levels would be expected to be lower nearer the end of the growing season as the plants begin senescing and the leaves become sources of nutrient reserves for the developing daughter corm and cormels underground.

N growth curves show different optimum levels of N in *G.scabridus* leaves at different times in the growing season. A higher application of 250 kg ha^{-1} NH_4NO_3 meant that a leaf nitrogen level of about 3 % early in the season and then 1.75 to 1.825 % 2 months later gives the maximum plant height, number of leaves and corm mass change, with the tallest floral spikes with the highest number of floral branches (Figures 4.28 to 4.34). The difference in N levels clearly indicates the importance of the timing of leaf sampling, an important consideration in the commercial production of this species. When comparing the growth curves of December leaf sampling results between season 2 and 3, the optimum levels are slightly higher during season 3. Bulbous plants have a complex growth habit with continual source : sink competition for reserves (De Hertogh & Le Nard, 1993). The corms used during season 3 were physiologically more mature and thus would have had higher photosynthetic demands from the different plant parts resulting in different optimum leaf levels. Thus, no specific optimum growth level can be given for *G. scabridus* in general as this is affected by so many growth factors. However, the results obtained serve as a guide for the growth of the species,

and are considered valuable in that they have been shown to be very different to the norms for hybrids.

Phosphorus (P): Leaves sampled in October show levels of P in the sufficient range. This then decreased with successive sampling dates to levels below the low range. However, plant growth remained healthy and there were no visible signs of P deficiency. The plants clearly use P for growth and development as the levels from the December sampling data were half those obtained from sampling 2 months earlier.

Potassium (K): October leaf sampling data revealed that K in the leaves was highest at this time of the growing season, although falling into the low range for gladioli leaf norms. These levels then decreased over time as shown with December sampling and then remained fairly constant between December and March. This confirms yet again that the plants readily use available K in the soil as it was during this final stage that the plants were supplied with higher levels of K. When comparing K leaf values between the 3 final K treatments, there was a proportional relationship between how much K was added to the media and how much was taken up and present in the leaves.

Figure 4.25 shows that leaf potassium levels 0.8 to 1.2 % K allow for maximum cormel development. This confirms results from the previous season as the same values were obtained during season 2.

Calcium (Ca): The actual amount of Ca most plants take up is relatively small (Parnes, 1990). The results indicate that *G. scabridus* plants do take up Ca from the media and store it in their leaves. This was evidenced by the fact that with each sampling analysis, the levels of Ca in the leaves increased. Levels were always within the sufficient range for hybrid gladioli (Appendix 1). The maintenance of these high levels must contribute to the continued plant health throughout the growing season. Ca is needed in cell membranes and a relatively large part of the Ca content of plants is located in the leaves (Follett *et al.*, 1981).

Magnesium (Mg): Levels of Mg in the *G. scabridus* leaves remained fairly constant throughout the growing season and remained within the sufficient range of the gladioli leaf analysis norms. As no Mg was added to the media throughout the trial, the plants clearly do not use much of it for plant growth and this is not an important element when considering fertilizer applications for *G. scabridus* plant growth.

Sodium (Na): Although low, the levels of Na remained fairly constant throughout the trial and do not appear to have any major effects on plants growth.

Manganese (Mn): Nutrient analysis revealed that the levels of Mn within the leaves increased over time. Levels from the initial sampling were in the low range and some remained within this level whilst others increased enough to be in the sufficient range. Mn was applied to the media every week with the fertilizer applications in the Omnispoor®. The plants clearly take it up and store it in the leaves. It is interesting to note that an increase in the application of K meant an increase in the level of Mn in the leaves (Table 4.9).

Zinc (Zn): Zn levels within the leaves were in the sufficient range during October. These had increased by the December sampling and then decreased to be in the low range at the final sampling date. Zn was added when Omnispoor® was applied to the media and the results indicate that the plants readily took up this available Zn and used it for plant growth processes. The increase in Zn coincided with floral development. Singh and Singh (2004) found that Zn increased the number of flowers per spike and spike length so this element may also play a role in *G. scabridus* spike growth. The decrease in Zn occurred simultaneously to the development of the daughter corm and cormels after flowering, thus implying that Zn plays a role in corm development.

Copper (Cu): Levels of Cu remained in the low to sufficient ranges throughout the trial. These results followed similar trends to that of Zn with an increase between the first 2 sampling dates followed by a decrease on the final sampling date. Thus Cu may also play a role in corm formation. Cu functions in plants are largely associated with enzymes (Follett *et al.*, 1981).

Iron (Fe): Fe is of great importance in flowering, corm growth and cormel formation (Chen *et al.*, 1982/83). As with Zn and Cu, levels of Fe showed a large decrease during the time when daughter corm and cormel development is taking place. This micronutrient clearly plays a role in this regard.

4.3.3.5 Pests and diseases

No diseases occurred this season and the only pests that appeared were whitefly and green aphids. These were soon eradicated with the insecticides and never became a major problem.

Table 4.6 **Mineral analysis of the most recently matured *G. scabridus* leaves sampled in October during the third growing season growth as related to fertilizer treatment**

Treatment	Element (%)						Element (ppm)			
	N	P	K	Ca	Mg	Na	Mn	Zn	Cu	Fe
1[x]	2.51	0.37	2.42	0.67	0.25	0.02	39.0	39.3	6.77	88.3
2[y]	2.79	0.32	2.22	0.55	0.23	0.03	30.0	47.3	7.27	122.3
3[z]	3.07	0.32	2.29	0.65	0.26	0.02	33.3	34.0	6.20	68.0

[x] 50 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[y] 150 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[z] 250 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

Table 4.7 **Mineral analysis of the most recently matured *G. scabridus* leaves sampled in December during the third growing season as related to fertilizer treatment**

Treatment	Element (%)						Element (ppm)			
	N	P	K	Ca	Mg	Na	Mn	Zn	Cu	Fe
1[x]	1.61	0.14	1.08	0.83	0.26	0.02	51.0	92	18	214
2[y]	1.67	0.12	1.05	0.71	0.21	0.03	43.5	394	69	1530
3[z]	1.82	0.15	1.15	0.81	0.23	0.02	44.8	50	10	139

[x] 50 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[y] 150 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

[z] 250 kg ha^{-1} NH_4NO_3 and 50 kg ha^{-1} K_2SO_4

Table 4.8 **Mineral analysis of the most recently matured *G. scabridus* leaves sampled in March during the third growing season as related to overall fertilizer treatment**

Treatment[z]	Element (%)						Element (ppm)			
	N	P	K	Ca	Mg	Na	Mn	Zn	Cu	Fe
1	1.02	0.20	1.54	1.02	0.24	0.03	43.0	12.0	3.80	107.0
2	1.09	0.09	1.15	1.16	0.22	0.02	64.8	14.2	3.15	146.0
3	1.12	0.18	0.81	1.22	0.27	0.07	67.0	17.0	0.40	51.0
4	1.28	0.17	1.00	1.14	0.21	0.03	57.0	14.5	2.00	46.0
5	1.16	0.08	1.19	1.05	0.19	0.03	41.0	13.0	3.70	132.0
6	0.94	0.07	1.13	1.10	0.19	0.06	79.0	17.0	3.20	104.0
7	0.76	0.06	0.53	1.05	0.20	0.05	40.0	10.0	1.30	37.0
8	0.98	0.08	1.08	1.41	0.35	0.04	60.0	14.0	4.50	158.0
9	1.14	0.24	1.42	1.28	0.24	0.02	64.5	11.0	2.00	59.5

[z] as per Table 4.3

Table 4.9 **Mineral analysis of the most recently matured *G. scabridus* leaves sampled in March during the third season of growth as related to K_2SO_4 fertilizer treatment**

Treatment	Element (%)						Element (ppm)			
	N	P	K	Ca	Mg	Na	Mn	Zn	Cu	Fe
1[x]	1.08	0.15	1.06	1.09	0.21	0.03	49.2	12.7	2.27	59.0
2[y]	1.07	0.10	1.14	1.19	0.24	0.03	57.6	13.8	3.62	146.0
3[z]	1.24	0.18	1.24	1.22	0.23	0.04	68.8	14.0	1.90	68.5

[x] 50 kg ha^{-1} K_2SO_4 and 50 kg ha^{-1} NH_4NO_3

[y] 150 kg ha^{-1} K_2SO_4 and 50 kg ha^{-1} NH_4NO_3

[z] 250 kg ha^{-1} K_2SO_4 and 50 kg ha^{-1} NH_4NO_3

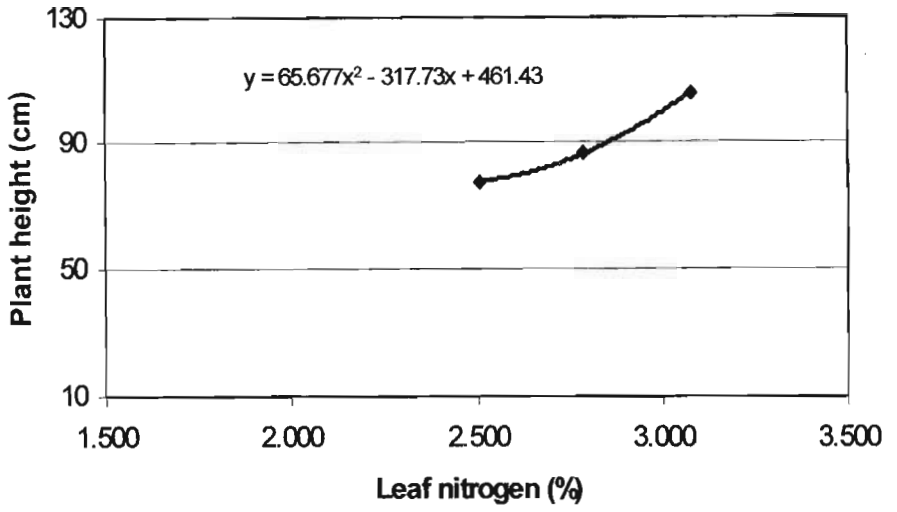


Figure 4.28 *G. scabridus* plant height in relation to the nitrogen level in the leaves sampled in October during season 3 of the fertilizer trial

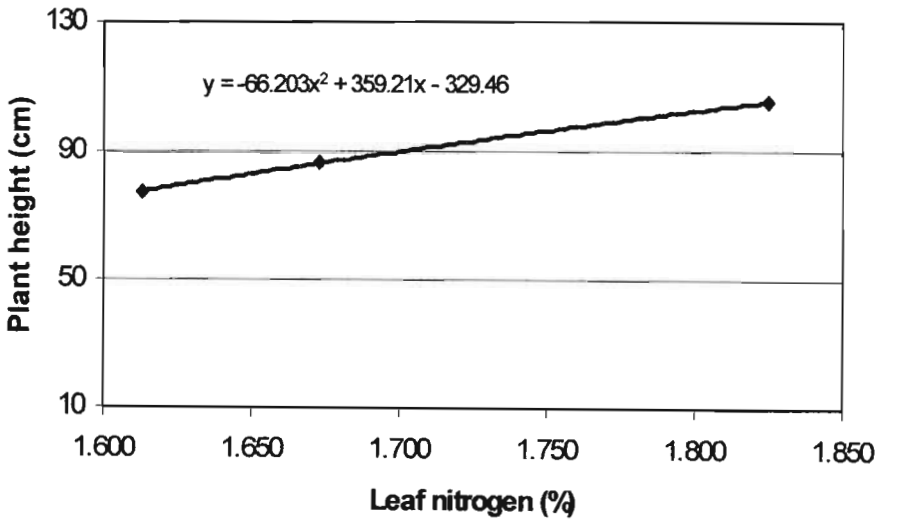


Figure 4.29 *G. scabridus* plant height in relation to the nitrogen level in the leaves sampled in December during season 3 of the fertilizer trial

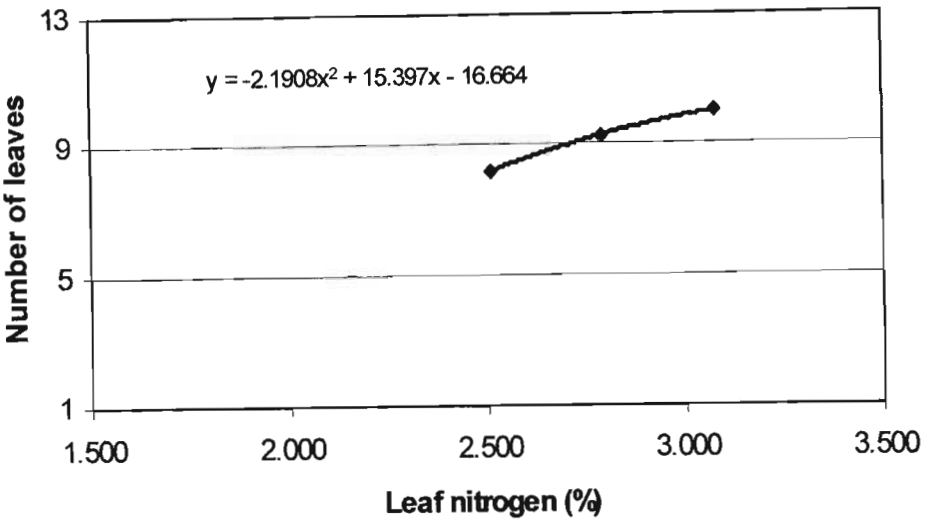


Figure 4.30 *G. scabridus* leaf number in relation to the nitrogen level in the leaves sampled in October during season 3 of the fertilizer trial

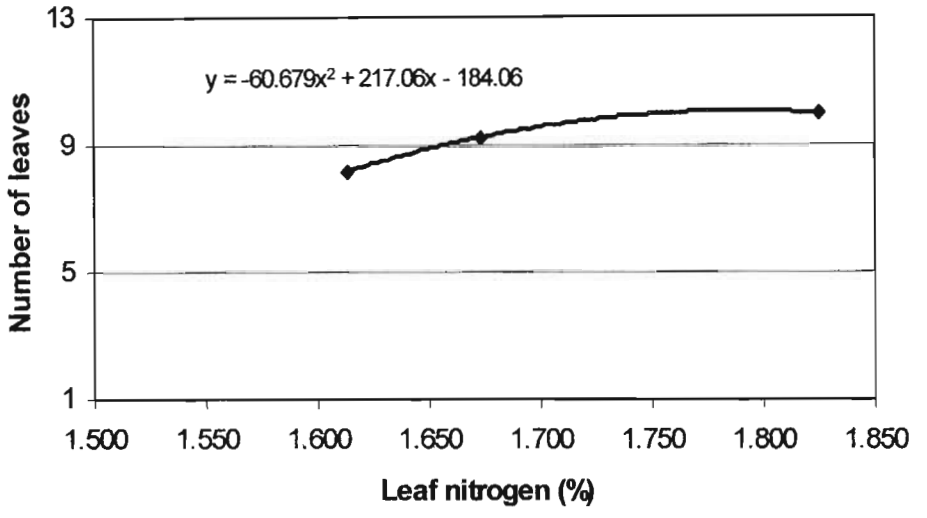


Figure 4.31 *G. scabridus* leaf number in relation to the nitrogen level in the leaves sampled in December during season 3 of the fertilizer trial

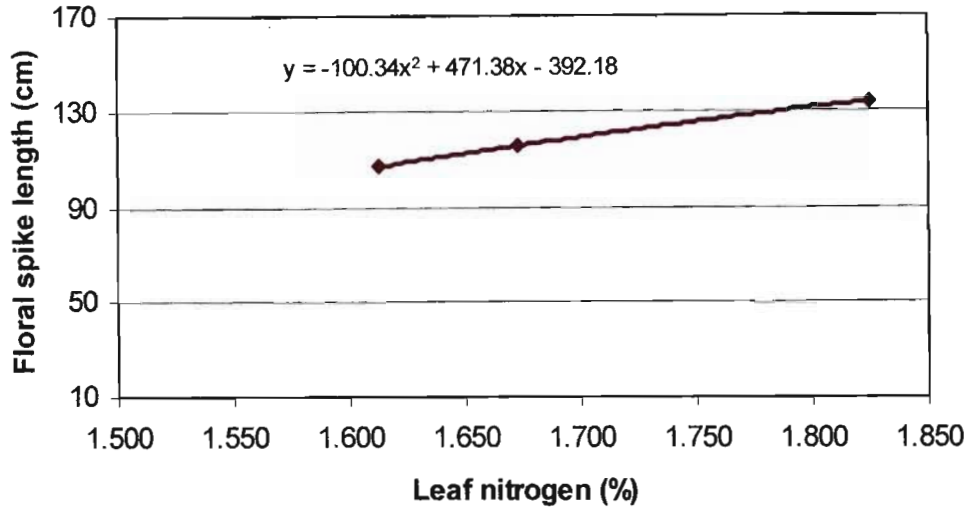


Figure 4.32 *G. scabridus* floral spike length in relation to the nitrogen level in the leaves sampled in December during season 3 of the fertilizer trial

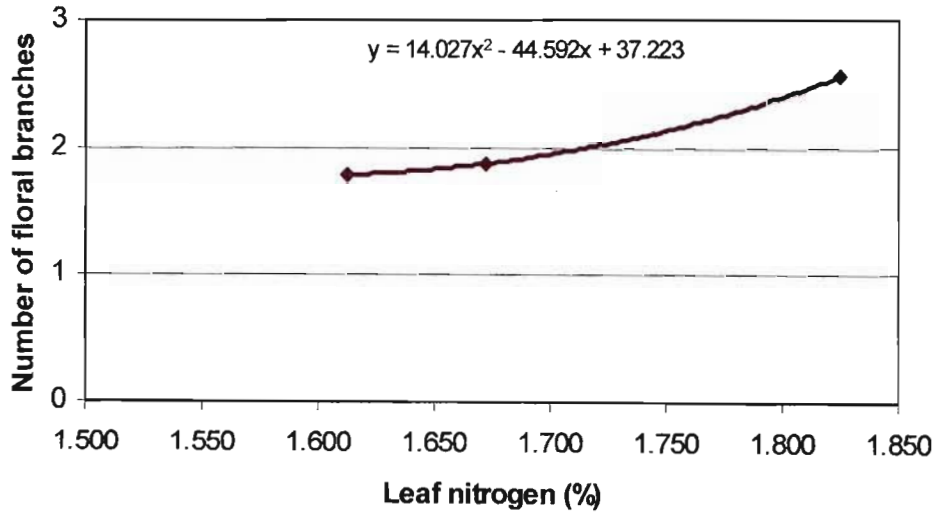


Figure 4.33 *G. scabridus* floral branch number in relation to the nitrogen level in the leaves sampled in December during season 3 of the fertilizer trial

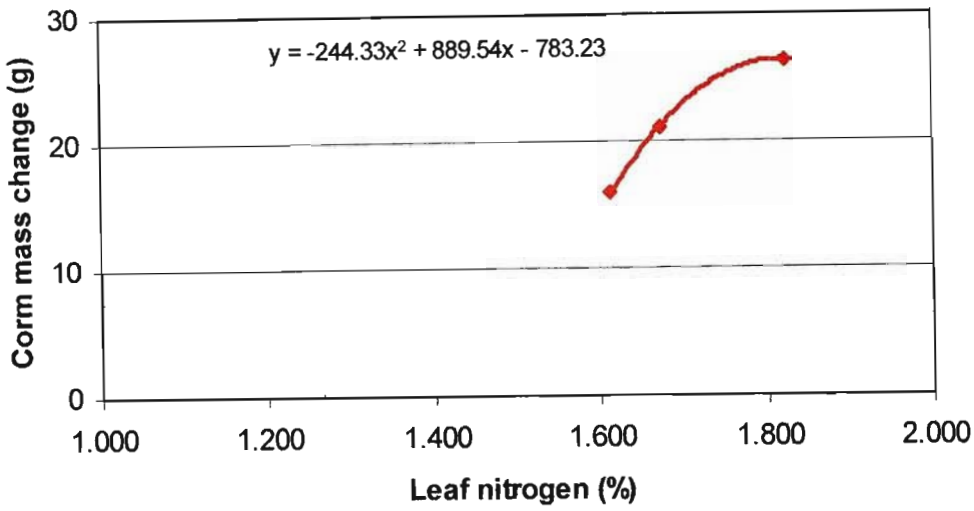


Figure 4.34 *G. scabridus* corm mass change in relation to the nitrogen level in the leaves sampled in December during season 3 of the fertilizer trial

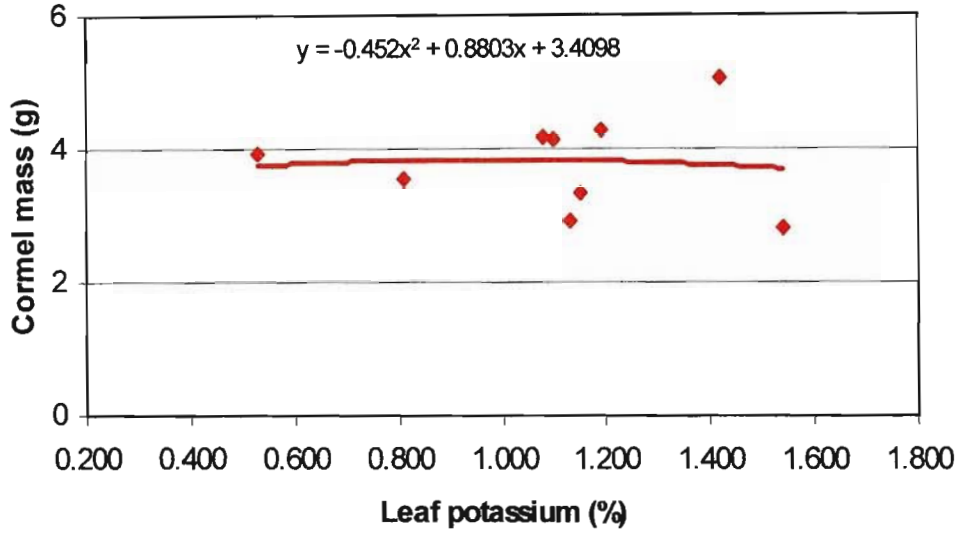


Figure 4.35 *G. scabridus* cormel mass in relation to the potassium level in the leaves sampled in March during season 3 of the fertilizer trial

4.4 Conclusions

G. scabridus plants show capacities for improved growth under controlled conditions as the plants thrived in this new environment and took up provided nutrients. This became more evident in the second fertilizer trial, as the plants flowered in their second season which was unexpected and the control plants were smaller in size. This is a significant result as it means that flowers and flowering sized corms can be produced from seeds or cormels in a shorter time which is ideal for the commercial floriculture industry as well as for the restoration of wild populations. In a controlled environment, other factors will also have an effect on improved growth such as temperature, water, soil type and the control of pests and diseases which these wild plants are not used to. These plants are adapted to dry soils low in nutrition and thus under controlled growth, the addition of water and nutrients provides 'luxury' conditions to which the plants respond very well.

The third fertilizer trial confirmed previous results that nitrogen plays an important role in the growth and development of *G. scabridus* as higher N applications result in taller plants with more leaves, taller flowering spikes with more flowering branches and a greater increase in corm mass. K aids in the formation of the cormels resulting in a higher final cormel mass. This is significant in that both these nutrients play an important role in the growth and development of the species but at different points in the growth cycle. As shown in season 3, more emphasis should be placed on each element according to when it affects plant growth. A fertilizer with higher N and lower K is appropriate for the beginning and middle of the growing season (150 kg ha^{-1} NH_4NO_3 and 100 kg ha^{-1} K_2SO_4) and then adjusted to a lower N and higher K fertilizer to promote cormel formation (100 kg ha^{-1} NH_4NO_3 and 150 kg ha^{-1} K_2SO_4). The addition of different levels of these elements at the correct time of plant growth will thus enhance plant development. A preplant application of $2Ca(H_2PO_4)_2.H_2O$ at a rate of 60 kg ha^{-1} is sufficient for healthy plant growth. Leaf analysis results indicate that micronutrients such as Zn, Cu and Fe play a role in daughter corm and cormel development, so future work could focus on the effects of these elements. This also indicates the importance of applying a general micronutrient fertilizer containing these elements.

The published leaf norms for hybrid gladioli do not necessarily refer to those of wild species such as *G. scabridus*. The latter plants are presumably selected to survive on lower levels of nutrients and thus have adapted their growth accordingly (Chapter 2). This proves to be a cost effective factor when fertilizing this species due to these lower nutrient requirements.

An important consideration for a grower is the timing of the leaf sampling to determine optimum growth. Growth curves are affected by physiological corm maturity and source : sink relationships. However, at this stage it can be concluded that a leaf N level of between 1.5 to 2 % and 0.8 to 1.2 % for K seems to result in the optimum *G. scabridus* plant growth. Whatever the growing situation, the leaf values should correlate to those of optimal growth in order for the plant to perform at its best.

CHAPTER 5

FLOWERING OF *GLADIOLUS SCABRIDUS*

5.1 CORM FORCING

5.1.1 Introduction

Gladioli have a year round marketing season worldwide (De Hertogh & Le Nard, 1993), therefore it is crucial to develop sound forcing techniques to allow a constant availability of cut gladioli flowers to satisfy consumer demands. This becomes particularly important for new species, like *G. scabridus*. By creating a continuous supply of cut flowers, one hopes to increase the awareness of the availability of a plant as well as increasing demand for new products. New flower crops are vital to the industry to overcome changing market demands and external competition (Shillo, 1997).

To ensure profitability in the forcing of bulbous plants, a thorough understanding of the current and future marketing requirements is needed by forcers, exporters and growers alike (Nelson, 1985).

Forcing requirements have been determined for hybrid gladioli but it is uncertain whether these techniques can be applied to wild species and if these species will respond to floral induction out of the normal flowering season, which is normally in summer from late December to mid-late January for *G. scabridus* flowers (Goldblatt & Manning, 1998; Bryan, 2002). Cold temperatures used during forcing treatments are supposed to break dormancy and delay sprouting (Shillo & Simchon, 1973) thus in theory manipulation of the corms could occur at any time of the year.

Thus methods were undertaken in an attempt to force this species to flower out of season which if successful, would make it extremely viable commercially and create year round demand and potential for its production.

5.1.2 Materials and Methods

G. scabridus corms were obtained from the KwaZulu-Natal Wildlife Biodiversity Research Gardens, Queen Elizabeth Park, Pietermaritzburg.

5.1.2.1 Initial study

In June, 5 *G. scabridus* corms were buried in perlite in each of 4 plastic trays (30 x 20 x 8.5 cm) (Plate 5.1 A). Two trays were placed in a cold storage chamber set at 10 °C and the other 2 at 5.5 °C in continual darkness. One tray per treatment was cold stored for 6 weeks and the other for 8 weeks respectively. After storage the corms were planted out at the beginning of August into 15 cm pots filled with a steam pasteurized pine bark : perlite : river sand medium, 2:1:0.2 (v/v/v). The pots were placed in a controlled environment growth chamber. This was initially set at 21 °C (day) and 15 °C (night) and changed to 26 °C (day) and 18 °C (night) 6 weeks later. Light was provided by Osram flourescent lamps (298.14 $\mu mol\ m^{-2}\ s^{-1}$). Photoperiod was originally set for a 10 hour light / 14 hour dark day, then changed to 12 hour light / 12 hour dark 4 weeks later and finally set for 14 hour light / 10 hour dark a further 4 weeks later. The corms were watered and fertilized by hand and received 100 ml water every day and 50 ml Chemicult® (10 mg l^{-1}) once a week. The plants were moved to a greenhouse set at the same final temperatures just prior to flowering. Plant characteristics measured were days to sprouting, number of leaves, plant height, flowering percentage, length of flowering spike and number of flowering branches.

5.1.2.2 Second study

The following April, 10 freshly harvested *G. scabridus* corms were placed into 1 of 4 plastic trays (30 x 20 x 8.5 cm) and buried in perlite. (More corms were used during the second study due to a greater availability of plant material.) Two trays were placed in a cold storage chamber set at 5.5 °C and the other 2 at 2 °C in continual darkness. One tray at each temperature was stored for 6 weeks and the other for 8 weeks respectively. After cold storage the corms were planted out in June into 18 cm plastic pots filled with a steam pasteurized pine bark : perlite : river sand medium, 2:1:0.2 (v/v/v). The pots were placed in a controlled environment growth room which was initially set at 21 °C (day) and 15 °C (night) and changed to 26 °C (day) and 18 °C (night) 6 weeks later. Light was provided by Osram flourescent lamps (289.82 $\mu mol\ m^{-2}\ s^{-1}$). Photoperiod was originally set for a 10 hour light /

14 hour dark day, then changed to 12 hours light / 12 hours dark 4 weeks later and finally set for a 14 hour light / 10 hour dark day a further 4 weeks later. The corms were watered and fertilized by hand and received 100 ml water every day and 50 ml Chemicult® (10 mg l^{-1}) once a week. Plant characteristics measured were days to sprouting, number of leaves, plant height, flowering percentage, length of flowering spike, number of florets and number of flowering branches. The entire study was conducted in a controlled environment and the plants were never exposed to outside conditions during growth.

Data was analysed using the Genstat ANOVA programme (8th Edition, 8.1 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

5.1.3 Results and Discussion

5.1.3.1 Initial study

The initial forcing treatments resulted in no significant differences between treatments in any of the measured variables. Plants took an average of 16.45 days to sprout after cold storage ($p=0.613$). Eighty percent of the corms sprouted ($p=0.537$). The plants produced an average number of 7.3 leaves ($p=0.673$), grew to a mean height of 119.8 cm ($p=0.512$) and 75 % of the corms produced a flowering spike ($p=0.899$). The spikes were on average 151 cm in length ($p=0.508$) with a mean number of 1.75 flowering branches per spike ($p=0.697$). Some wild *Gladiolus* species are dependant on specific temperatures and daylengths to stimulate flowering, while others will flower irrespective of these variables (Littlejohn & van der Walt, 1992). As a result of both these forcing treatments, the *G. scabridus* corms began flowering from early November which is 6 to 8 weeks earlier than the normal flowering time, showing that they have potential to be forced to flower earlier through cold treatment and the manipulation and control of their growing temperature and daylength.

5.1.3.2 Second study

The treatments had a significant effect ($p=0.002$) on the number of days to sprouting from planting out. Corms stored at 5 °C for 8 weeks took an average of 10.2 days to sprout. This was not significantly different to corms stored at 2 °C for 6 weeks which took 11.9 days to sprout but was significantly different to the other treatments. Corms stored at 5.5 °C for 6

weeks sprouted after 12.89 days and those stored at 2 °C for 8 weeks, after 14.25 days. These two treatments were significantly different to each other (Table 5.1).

There was also a significant difference in the percentage of corms that sprouted (p=0.024). Corms stored at 5 °C for 8 weeks and 2 °C for 6 weeks both resulted in 100 % sprouting. These treatments were significantly different to the other 2 treatments which both resulted in 60 % sprouting (Table 5.1).

The forcing treatments also had a significant effect on vegetative growth with similar trends to that for sprouting percentage. There were significantly higher average leaf numbers (p=0.016) and plant heights (p=0.035) for corms stored at 5 °C for 8 weeks (7.8 and 78.0 cm) and 2 °C for 6 weeks (7.6 and 82.5 cm). These treatments were not significantly different to each other but were significantly different to corms stored at 5.5 °C for 6 weeks and 2 °C for 8 weeks which produced an average of 4.2 and 4.4 leaves and plant heights of 48.1 cm and 48.7 cm respectively (Table 5.1). These latter 2 treatments were not significantly different to each other.

The forcing treatments had no significant effect on spike length (p=0.588), flowering percentage (p=0.674), floret number (p=0.825) and number of floral branches (p=0.890).

The corms were successfully forced to flower out of season as the corms that did sprout, began flowering from the beginning of September (Plate 5.1 B) when the corms are naturally just beginning to sprout in the wild. This is an important result, confirming that *G. scabridus* corms can be manipulated to flower out of season. However, treatments will need to be avoided which result in reduced sprouting as with corms stored at 5.5 °C for 6 weeks and 2 °C for 8 weeks.

The same 2 treatments had a consistently significant effect on corm sprouting and vegetative growth showing that forcing the corms to grow out of season requires a shorter time (6 weeks) at a lower temperature (2 °C) or a longer time (8 weeks) at a higher temperature (5.5 °C). Waithaka (1986) also found that *Gladiolus* corms require at least 8 weeks at 3 to 5 °C for dormancy release and Littlejohn and van der Walt (1992) note that a cold treatment at 5 to 8 °C for 8 to 10 weeks after harvesting promotes flowering out of season in gladioli. However, a shorter time period for forcing is more suitable as this will mean the earlier production of flowers. Hence, 2 °C would be more the appropriate temperature to use for *G. scabridus* corms.

A lack of treatment effect on floral development indicates that the forcing treatments merely promote the initial sprouting and growth of the corms and that the flowering process follows naturally once this has been achieved. However, during this second trial an average of 60 % of the corms that sprouted, produced flowering spikes. Lower light intensities can cause blindness (failure to flower) in *Gladiolus* plants (Shillo & Halevy, 1975; Imanishi & Imae, 1990) so an increase in light intensity may increase the flowering percentage of forced *G. scabridus* plants. The ultimate aim of forcing the corms is to produce cut spikes (Waithaka, 1986). Thus future work will need to be undertaken to determine the exact effect of external factors, such as light intensity, temperature and daylength on improving flowering as inflorescence development is under the control of these environmental factors (Shillo & Halevy 1976b; c; d).

***Table* 5.1 Effect of storage temperature and time on *G. scabridus* corm sprouting and subsequent growth out of season**

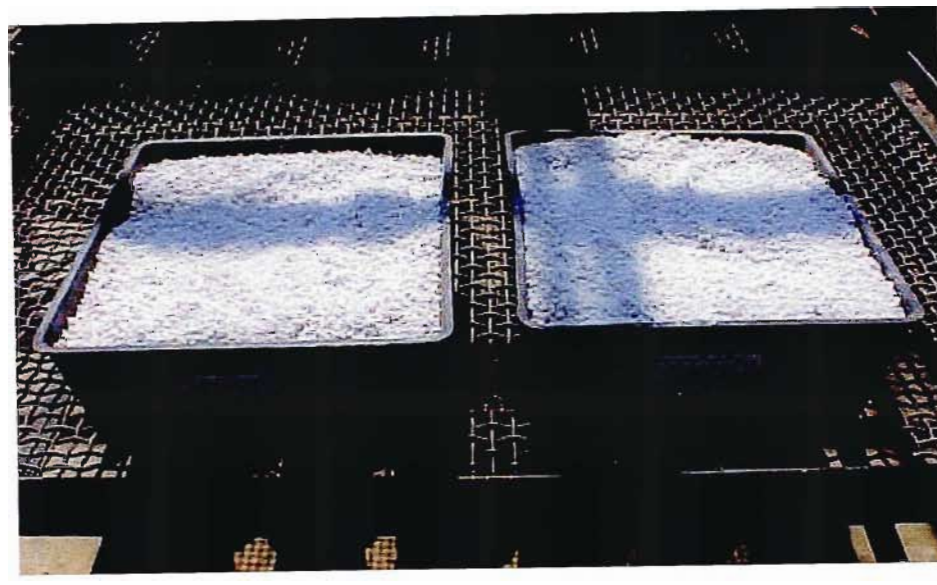
Storage temperature (°C)	Storage length (weeks)	Sprouting % [w]	Days to sprouting [x]	Leaf number [y]	Plant height (cm) [z]
5.5	6	60 b	12.89 b	4.2 b	48.1 b
5.5	8	100 a	10.20 c	7.8 a	78.0 a
2	6	100 a	11.90 bc	7.6 a	82.5 a
2	8	60 b	14.35 a	4.4 b	48.7 b

[w] LSD (5%) = 34.87 [x] LSD (5%) = 1.935

[y] LSD (5%) = 2.806 [z] LSD (5%) = 29.50

Letters a to c compare means in each column; n = 10

A



B

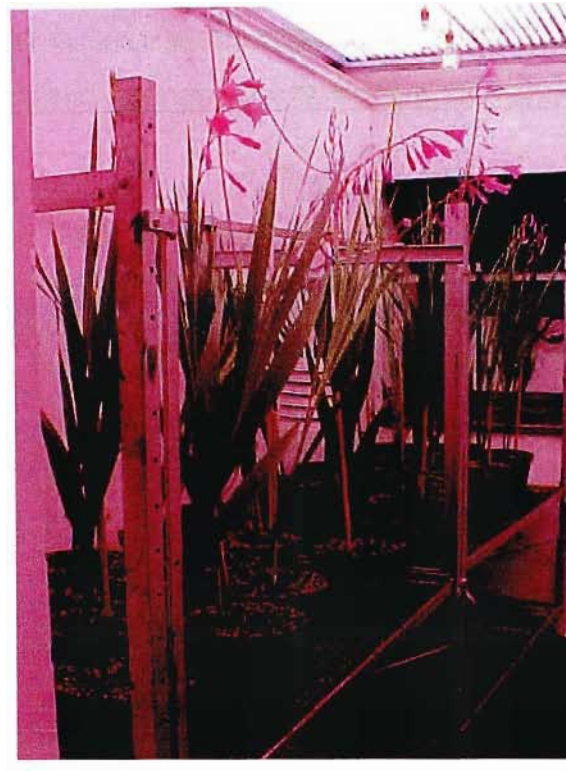


Plate 5.1 (A) The plastic containers filled with perlite in which the corms were buried during cold storage for forcing and (B) the forced plants in the controlled growth chamber in flower during September

5.1.4 Conclusions

The *G. scabridus* corms were successfully forced to flower out of season. Temperatures of 10, 5.5 and 2 °C for 6 to 8 weeks all show successful growth, however, the results indicated that corms need to be stored for longer at the warmer temperatures for successful corm growth. Thus it is suggested that the colder treatment of 2 °C for 6 weeks is the most suitable condition for the forcing of *G. scabridus* corms as this means a shorter time to flower production.

It is unclear at this stage as to the effect of external factors on the flowering of the corms and this provides scope for future work on the effect of external factors, such as light intensity, daylength and temperature, on improving the flowering percentage.

The wild *G. scabridus* corms show considerable potential to be manipulated in this way and this will enhance the promotion of commercial production as cut flowering spikes can be produced out of the normal flowering season, to satisfy potential consumer demand for this new product.

5.2 VASE LIFE, COLD STORAGE AND MARKET RESEARCH

5.2.1 Introduction

Cut flowers are an expensive commodity and considered a luxury item. As a result, the consumer expects to receive the best value for money (Hancke & Verryn, 1989a). The production value of cut flowers is increasing which also makes it imperative to achieve a good vase life (Merodio & de la Plaza, 1989). Hence, postharvest studies attempt to develop methods of managing the rate of floral senescence (Murali & Reddy, 1993).

From a commercial point of view, the *Gladiolus* is said to be 'the queen of bulbous flowers' and is rated as one of the most popular and marketable flowers in the world (Hamilton, 1976; Merodio & de la Plaza, 1989; Nunes, 1989). There are few flowers to surpass its beauty in the cut flower industry due to its long spikes and striking, showy colours (Anserwadekar & Patil, 1986; Serek *et al.*, 1994).

Cut *Gladiolus* flower spikes can remain fresh for a number of days (Anserwadekar & Patil, 1986). However, they often fail to open and to develop their florets to the extent that they do in the field (Mayak *et al.*, 1973). Like other flowers with spike inflorescences, gladioli are normally harvested with only a few open florets, and the life of the flower is a function of both the life of the individual florets as well as the postharvest expansion and opening of the buds remaining on the spike (Serek *et al.*, 1994). The use of preservatives to prolong the vase life of these cut spikes is essential to reduce the postharvest effect of cutting off their natural reserves (Ferreira, 1981).

Postharvest losses in cut gladioli can be as high as 50 % due to poor transport and storage (Nunes, 1989). Cool temperatures are critical in maintaining the quality of cut flowers after harvest, extending flower life by decreasing respiration rates and water loss, and by reducing the rate of ethylene production and flower sensitivity to ethylene (Nell, 1992; De Hertogh & Le Nard, 1993). Prolonged storage of flowers is important, as it enables marketing control and the possible export of flowers. After storage, *Gladiolus* spikes often remain with unopened florets and their longevity during vase life is significantly reduced (Meir *et al.*, 1995). Hence, the importance in determining the optimum conditions for cold storage.

G. scabridus flowers produce large, bright pink to mauve blooms (Scott-Shaw, 1999; Bryan, 2002) which could make it an attractive cut flower for commercial production. However, being a wild species, it is uncertain as to the response to postharvest treatments and market acceptance. Thus, appropriate methods were investigated for prolonging the postharvest life of *G. scabridus* flower spikes by determining the effect of floral preservatives and storage temperatures, on the longevity of the spikes as well as the response of the local horticultural industry and consumers to the species as a marketable product.

5.2.2 Materials and Methods

5.2.2.1 Vase life trials

Initial study

G. scabridus flower spikes were harvested from the KwaZulu-Natal Wildlife Biodiversity Research gardens, Queen Elizabeth Park, Pietermaritzburg. They were picked early in the morning in the tight bud stage with the lower 2 or 3 buds semi-opened and showing colour. The stems were cut at an angle with a sharp knife near the bases and placed in a bucket of warm (25 °C) distilled water for transport to the laboratory.

The spikes were further cut to a length of 80 cm with 2 or 3 leaves remaining. After recording the fresh mass, they were placed in 300 ml glass bottles containing 250 ml of one of the following floral preservatives: distilled water (control), Prolong® (contains a quaternary ammonium compound), Chrysal® (glucose, fructose and citric acid (I. Bertling, pers. commun., 2005)), 2 % (w/v) sucrose ($C_{12}H_{22}O_{11}$) and 2 % (v/v) ethanol, 1 % (w/v) sucrose, 2 % (w/v) sucrose, 4 % (w/v) sucrose, 1 % (w/v) sucrose and 0.5 % (v/v) JIK® (12 % (w/v) NaOCl), 2 % (w/v) sucrose and 0.5 % (v/v) JIK® and 4 % (w/v) sucrose and 0.5 % (v/v) JIK®. All solutions were made up with distilled water at room temperature at the recommended or required rates. Three spikes were placed into each bottle with 3 bottles per treatment. The tops of the bottles were sealed with Cling Wrap® to prevent any evaporative loss of solution.

The spikes were allowed to open at a constant room temperature of 22 °C and illumination of 17.9 µmol m^{-2} s^{-1}. Overall floret quality was scored on a daily basis using a range of 1 = poor to 5 = good (Appendix 4). Floret width and length were measured daily using digital

vernier calipers. The pH of each solution was measured every 3 days as well as the amount of solution absorbed by the cut spikes, through the topping up of the solution to 250 ml. The change in floral spike mass was also determined every 3 days.

The spikes were discarded when the lower 5 florets had senesced. Data was analysed statistically by means of ANOVA using Genstat (5th Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

Second study

The following flowering season, *G. scabridus* flower spikes were again harvested from the KwaZulu-Natal Wildlife Biodiversity Research Gardens, Queen Elizabeth Park, Pietermaritzburg. They were picked under the same conditions and prepared as previously authored.

The prepared spikes were placed in 300 ml glass bottles containing 250 ml of one of the following solutions: distilled water (control), 2 % (w/v) sucrose, 2 % (w/v) glucose ($C_6H_{12}O_6$) and 2 % (w/v) fructose ($C_6H_{12}O_6$) in distilled water. Two spikes were placed into each bottle with 3 bottles (6 spikes) per treatment for the vase life trial and another 3 bottles per treatment for sugar analysis. The tops of the bottles were sealed with Cling Wrap® to prevent the solution evaporating. The spikes were allowed to open at a constant room temperature of 22 °C and illumination of 17.9 µmol $m^{-2} s^{-1}$.

Overall floret quality was scored on a daily basis using a range of 1 = poor to 5 = good (Appendix 4). Floret width and length were measured daily using digital vernier calipers. The chlorophyll fluorescence of each spike was measured on a daily basis using a Plant Efficiency Analyser (PEA MK2 9613, Hansatech Instruments Ltd) for determination of Fv/Fm values (DeEll *et al.*, 1999). A plastic clip was placed on the leaf of each spike for 1 minute before a reading was taken (equilibration conditions were previously determined). The pH of each solution was measured every 3 days as well as the amount of solution absorbed by the cut spikes, through the topping up of the solution to 250 ml. The change in floral spike mass was also determined every 3 days.

The spikes were discarded when the lower 5 florets had senesced. Data was analysed statistically by means of ANOVA using Genstat (7th Edition, 7.2 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

Carbohydrate analysis

Six freshly harvested *G. scabridus* spikes were used for sugar analysis on the day of picking (Day 0). It was ensured that the lower 1 or 2 florets of these spikes were almost fully open. The leaves and the first 10 florets were removed from the spike, weighed, immediately frozen with liquid nitrogen (Duffus & Duffus, 1984; Gomez *et al.*, 2003) and placed on a freeze drier (Virtis). A further six spikes per treatment were sampled in the same way after 5 days in solutions (Day 5) of: distilled water (control), 2 % (w/v) sucrose, 2 % (w/v) glucose ($C_6H_{12}O_6$) and 2 % (w/v) fructose ($C_6H_{12}O_6$) in distilled water. A further 6 spikes per treatment were treated as before after 10 days in the same solutions (Day 10). Freeze dried material (Gomez *et al.*, 2003) was reweighed to determine dry mass and milled to a fine powder.

The Ferricyanide colorimetric reduction method (Ting, 1956; Smith 1972) was used to determine the concentration of fructose, glucose and sucrose within the plant tissue. A 0.05 g mass of freeze dried tissue was homogenised in 4 ml hot 95 % (v/v) ethanol and centrifuged at 1500 *g* for 20 min. This was filtered through Whatman No. 40 filter paper and the supernatant collected. The pellet was resuspended in 4 ml hot 95 % (v/v) ethanol and centrifuged again at 1500 *g* for 20 min. The process was repeated for a third time and the 3 supernatants combined (Cripps, 2001).

Reagents

The alkaline **ferricyanide solution** was made up by dissolving 160 g anhydrous sodium carbonate (Na_2CO_3) and 150 g disodium phosphate heptahydrate ($Na_2HPO_4.7H_20$) in 850 ml ultrapure water. Then 4 g potassium ferricyanide ($K_3Fe(CN)_6$) was added and the solution diluted to 1 L with ultrapure water.

The **arsenomolybdate solution** was made up by dissolving 25 g ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_20$) in 450 ml ultrapure water. Then 21 ml concentrated Sulfuric acid (H_2SO_4) was added followed by 3 g disodium arsenate ($Na_2HAsO_4.7H_20$) in 25 ml ultrapure water. This was heated at 55 °C in a water bath with constant stirring for 30 min.

Determination of Fructose

One millilitre of the supernatant was transferred to a glass boiling tube and 5 ml ferricyanide reagent was added. This was immersed in a water bath set at 55 °C for 30 min. After heating, the tube was quickly cooled in running water and 10 ml 2 N H_2SO_4 was added and vortexed thoroughly until no more gas evolved. Then 4 ml arsenomolybdate was added and the solution vortexed thoroughly again, after which it was transferred to a 100 ml volumetric flask and diluted to volume. The absorbance of the solution was measured at 745 nm and the readings compared to a fructose standard curve at known concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 g/100 ml (Appendix 5a).

Determination of Total Reducing Sugars

One millilitre of the supernatant was transferred to a glass boiling tube and 5 ml ferricyanide reagent was added. This was immersed in a boiling water bath (100 °C) for 10 min. After heating, the tube was quickly cooled in running water and 10 ml 2 N H_2SO_4 was added and vortexed thoroughly until no more gas evolved. Then 4 ml arsenomolybdate was added and the solution vortexed thoroughly again, after which it was transferred to a 100 ml volumetric flask and diluted to volume. The absorbance of the solution was measured using the same spectrophotometer (Beckman Coulter DU 800, software version 2.0, Build 67 2.9.012) at 745 nm and the readings compared to the linear portion of a standard curve containing glucose and fructose at known concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 g/100 ml (Appendix 5b).

Determination of Glucose

The concentration of glucose was estimated to be the value obtained by subtracting the concentration of fructose from the value obtained for total reducing sugars (Ting, 1956; Smith, 1981).

Determination of Total Sugars

Five millilitres of the supernatant was placed in a glass boiling tube and 1 ml HCl (1:1, v/v) was added. This was immersed in a boiling water bath (100 °C) for 15 min. After heating, the tube was cooled in running water. The volume was adjusted to pH 6 with 0.1 N NaOH, using

a pH meter. A 1 ml aliqout of this solution was pipetted into a 100 ml volumetric flask and diluted to volume and the procedure described for the determination of total reducing sugars was followed. Spectrophotometer readings were compared to a standard curve with a combination of sucrose, glucose and fructose each at known concentrations of 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 g/100 ml (Appendix 5c).

Determination of sucrose

The concentration of sucrose was estimated to be the value obtained by subtracting the concentration of total reducing sugars from the value obtained for total sugars (Ting, 1956; Smith, 1981).

Data was analysed statistically by means of ANOVA using Genstat (7th Edition, 7.2 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

5.2.2.2 Storage temperature

Initial study

G. scabridus floral spikes were harvested from the KwaZulu-Natal Wildlife Biodiversity Research Gardens, Queen Elizabeth Park, Pietermaritzburg and cut to a length of 80 cm with 2 or 3 leaves remaining on the spikes. These were placed into 10 L buckets with 2 L warm (25 °C) distilled water and left in a cold room at 4 °C with a constant lighting of 1.48 µmol m^{-2} s^{-1}. Three buckets, each with 6 floral spikes, were used and were placed in the cold room for 1, 2 and 3 weeks respectively (Plate 5.2). On removal from cold storage, spikes were placed in 300 ml glass bottles with 250 ml distilled water. Two spikes were placed into each bottle and treated as previously authored. Data was statistically analysed by means of a general analysis of variance (ANOVA) using Genstat (5th Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

Second study

Twenty-four freshly harvested *G. scabridus* spikes were cut to a length of 80 cm with 2 or 3 leaves intact and their fresh mass was recorded. Twelve of the spikes were sealed in microperforated, polypropylene bags (30 micron in thickness, 9 micron holes and with an

inner anti-mist coating) (Polylam Packaging©). The spikes were placed in cardboard cartons (85 x 28 cm) between layers of paper (Hancke & Verryn, 1989a) and stored upright (Wilfret, 1992) in a cold room set at 4 °C (Buschman, 1998b; Nell, 1992) with constant lighting (1.48 µmol m^{-2} s^{-1}). After 5 days in cold storage, 6 sealed (Plate 5.3 A) and 6 unsealed spikes (Plate 5.3 B) were removed, their weights recorded and 5 cm was cut off the bottom of each spike. They were reweighed and placed in solutions of 2 % (w/v) sucrose for shelf life tests. After 10 days in cold storage the remaining 6 sealed and 6 unsealed spikes were removed from cold storage and the same procedure was followed.

Spikes were placed under the same conditions a treated as previously authored. Floret width and length were measured daily using digital vernier calipers. The chlorophyll fluorescence of each spike was measured on a daily basis using a PEA as before. Data was analysed statistically as an ANOVA using Genstat (7th Edition, 7.2 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

5.2.2.3 Market research study

A market research study was carried out at the garden centres and florists in and around Pietermaritzburg and the south coast region of Kwazulu-Natal to determine the response and attitude towards this new species. Each outlet was presented with a vase containing 3 or 4 *G. scabridus* spikes along with a questionnaire (Appendix 6a) which they were asked to complete. The garden centres were Val-lea Vista Indigenous Nursery, McDonald's Garden Centre, Dunrobin Garden Pavilion and Green Finger's Nursery in Pietermaritzburg, Hilton Garden Centre and Our Secret Garden in Hilton, Celtiskloof Nursery in Howick and Southport Garden Centre, Ferns Wholesale Nursery, Joymac Nursery, Margate Decor Centre and Nursery, Froggy Pond Nursery, Paradise Plants, South Coast Garden Pavilion and The Hut Nursery, situated along the south coast of Kwazulu-Natal. Florists included Parklane Florist, Carters and Chelsea Florist, Midlands Flower Hub, Flowers on Victoria and Forget-Me-Not Florist in Pietermaritzburg.

A vase containing 6 *G. scabridus* spikes was placed at Our Secret Garden in Hilton and 58 customers were asked to complete a similar questionnaire (Appendix 6b).

All participants were asked to answer using numeric values (on a given scale of 1 to 5) along with their comments. Numeric data was encoded using the descriptive frequency analysis option in the SPSS-PC 11.5 data editor computer program for Windows.

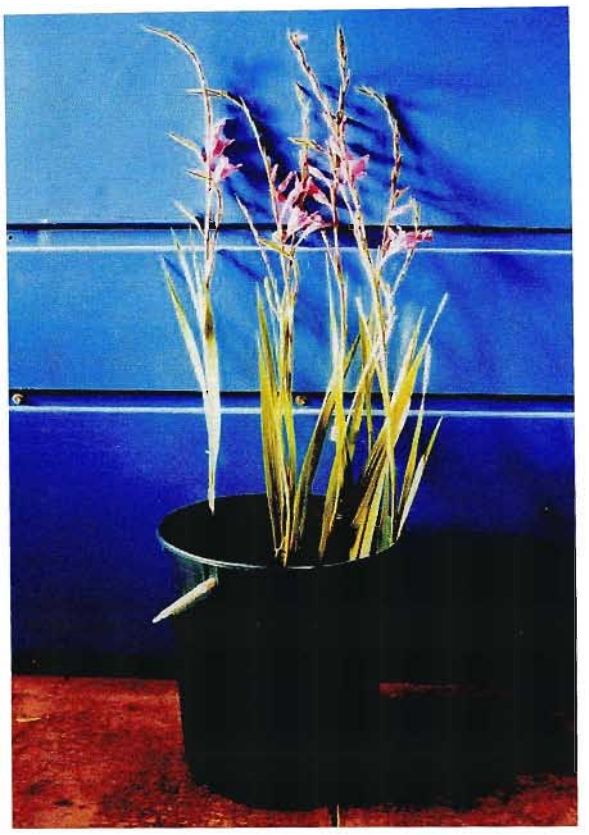
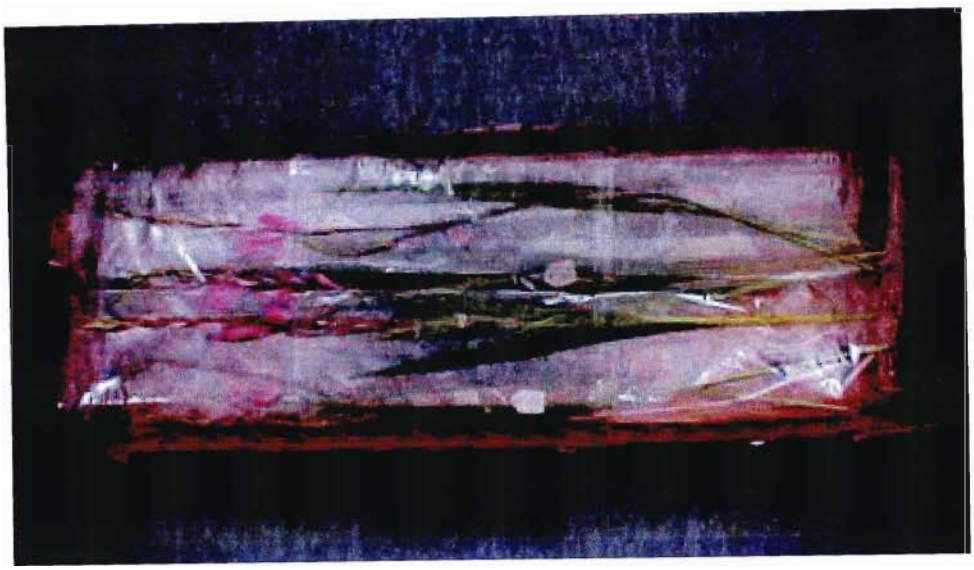


Plate 5.2 Cut *G. scabridus* spikes just after picking and prior to cold storage at 4 °C in the tight bud stage with the lower florets showing colour and just starting to open

A



B

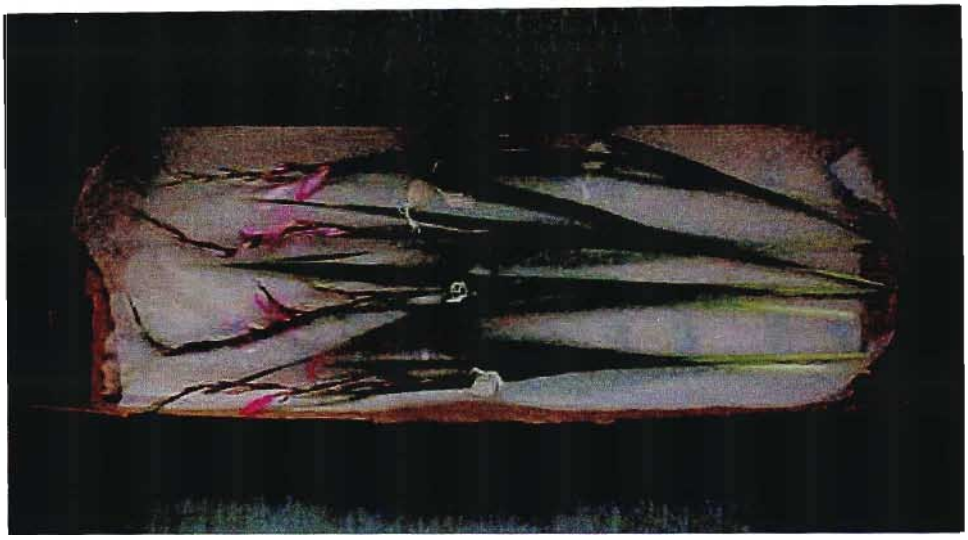


Plate 5.3 *G. scabridus* spikes (A) sealed in polypropylene sleeves and layered in boxes and (B) unsealed and layered in boxes prior to cold storage at 4 °C for 5 and 10 days.

5.2.3 Results and Discussion

5.2.3.1 Vase life trials

Initial study

Vase life

G. scabridus vase life was longest with 2 % sucrose, the spikes lasting for an average of 12.5 days (Table 5.2) This was, however, not significantly different to the control, in which spikes lasted for 11.3 days implying that both these solutions are suitable for prolonging *G. scabridus* vase life. *Gladiolus* spikes held in sugar solutions have previously been found to develop better and have a longer vase life (Marousky, 1971; 1972; Mayak *et al.*, 1973; Bravdo *et al.*, 1974; Kofranek & Halevy, 1976; van der Merwe *et al.*, 1986; Meir *et al.*, 1995; Roychowdhury & Sarkar, 1995; Rekha *et al.*, 2001; Singh *et al.*, 2004). Both 2 % sucrose and the control resulted in a significantly longer vase life ($p<0.001$) than all the other treatments used. Spikes in 1 and 4 % sucrose lasted for 7.1 and 8.3 days respectively. Thus 1 % sucrose was too low for a significant effect and 4 % sucrose was too high. Previous authors have noted that the effect of sucrose is due to reduced vascular blockage, increased water uptake and lowered water loss through transpiration and the supplementation of sugars which are rapidly combusted by the flowers (Marousky, 1968; van der Merwe *et al.*, 1986; Hancke & Verryn, 1989a; Gowda & Gowda, 1990). However, higher levels of sucrose may result in a negative osmotic gradient thus reducing *G. scabridus* vase life.

The significantly shortest vase life of 5.0 days was obtained when using 2 % sucrose with 2 % ethanol, and sucrose in combination with 0.5 % Jik®. The vase life of cut spikes of *G. gandavensis* cv. Spic and Span was extended following a single treatment of 7 % ethanol (Hwang & Kim, 1995). However, ethanol does not appear to be a beneficial floral preservative in combination with sucrose for the extension of *G. scabridus* vase life. The same may be true for JIK®. NaOCl in a vase solution, effectively controls bacterial growth thus improving vase life, but Singh *et al.* (2000) found that it did not appreciably increase the vase life of gladioli. Its efficacy only increased when used in combination with 2 % sucrose which did not occur in the *G. scabridus* postharvest studies. *G. scabridus* may be sensitive to chloride ions, hence the presence thereof in a solution of JIK® may be the cause of the reduction in vase life of these spikes.

The commercially available solutions, Chrysal® and Prolong® produced disappointing results with a vase life of 6.0 and 8.0 days respectively (Table 5.2). Bakker (1979) found that the vase life of *Gladiolus* cv. Pussicat, was markedly extended with the use of Chrysal®, however, unsatisfactory results have been obtained when using Chrysal® to extend the vase life of cut freesias and lilies (Barendse, 1975) and cut *Kniphofia* (Hettiarachchi & Balas, 2005). This product is used extensively in the cut flower industry with what would seem to be conflicting results. Chrysal® contains glucose and fructose both derivatives from the breakdown of sucrose (Smith, 1993). Lukaszewska (1978) and Dumitras *et al.* (2002) found that glucose improved the vase life of *Gladiolus* hybrids. Similar results have been achieved using fructose to prolong the vase life of cut roses (Bhattacharjee, 1998; 1999). Chrysal® also contains citric acid which was previously used by Reist (1977) to lower *Gladiolus* vase life pH without any apparent negative effects to the spikes. However, Chrysal® did not improve the vase life of *G. scabridus* spikes.

Prolong® is also a well-known floral preservative used in the floricultural industry. However, the presence of a quaternary ammonium compound in this solution does not seem to have a positive effect on *G. scabridus* vase life. Farhoomand *et al.* (1980), used Physan 20, which also contains a quaternary ammonium compound, to increase the vase life of gladioli with poor results.

The concentration of the chemicals that make up these commercial compounds is unknown which makes it very difficult to speculate as to why they do not have a positive effect on *G. scabridus* vase life. The erratic results that they produce commercially may indicate that insufficient experimental work has been done to determine the most effective concentrations for cut flowers. When used in conjunction with *G. scabridus* spikes, the concentrations may be too low to have any positive effect or may be too high and thus cause cell damage within the stems. There may, however, be other compounds present in these commercial products which have a negative effect on vase life. Chrysal® and Prolong® are not food products so legally the manufacturer is not obliged to indicate all the active ingredients present in these compounds (I. Bertling, pers. commun., 2005). However, the results indicate that use of these commercial products should be avoided for the extension of the vase life of *G. scabridus*.

Spike quality

Bravdo *et al.* (1974) and Kofranek and Halevy (1976) found that sucrose slightly increased *Gladiolus* spike quality. *G. scabridus* spikes placed in sucrose, remained healthy with large, attractive florets. The leaves remained green until the onset of senescence. However, florets in 2 % sucrose were visually slightly better in appearance (Plate 5.4) and bright mauve to pink in colour hence they we given a quality rating of 4.9 (Table 5.2), which was significantly better than all the other treatments used ($p<0.001$). Florets in 1 % sucrose tended to be lighter pink in colour with an overall quality rating of 3.1, however, significantly poorer ($p<0.001$) in quality compared to those spikes in 4 % sucrose which were slightly darker pink with a rating of 4.0.

Most of the florets on *G. scabridus* spikes in the sucrose solutions opened, except for a few at the tip of the spike. Ferreira *et al.* (1986) showed that the basal florets seem to contain sufficient carbohydrate reserves to ensure full development in contrast to the less developed terminal florets which rarely develop completely. However, van der Merwe *et al.* (1986) observed that the application of [U-14C]sucrose absorbed from the vase medium, resulted in a higher accumulation of radioactivity in the less developed terminal *Gladiolus* florets compared with the more developed basal ones, thus promoting the development of the terminal florets.

Nunes (1989) found distilled water to be very effective in maintaining *G. communis* floret quality and longevity. *G. scabridus* spikes in distilled water were also given a quality rating of 4.0. These florets were generally healthy in appearance, however, some were slightly pale pink in colour. The leaves remained green and healthy until senescence. Although maintaining as good a vase life as 2 % sucrose, distilled water does not have as significant an effect on floret quality.

Florets on spikes held in Chrysal®, developed white blotches on some of the petals, hence they were given a rating of 3.4, and there was also slight chlorosis along the leaf edges. Hanke and Reimherr (1986) found that high concentrations of Chrysal® impaired flower attractiveness of Anemone cv Mona Lisa. Blue flowers tended to become a poor violet and red flowers showed some marginal discolouration and distortion. The floret rating using Prolong®, was not significantly different to Chrysal® with a value of 3.0 due to the presence of small, brown blotches on the petals as well as chlorosis of the leaves. These results

confirm yet again that these commercial solutions are unsuitable for postharvest use in *G. scabridus* and would thus not be recommended.

The treatment with 2 % sucrose and 2 % ethanol, gave rise to spikes and florets of significantly poorer quality with a rating of 1.1. Many of the florets failed to open and became dry on the spike with the development of brown marks on the petals of those that did open. Leaves became dry and brown in appearance. Ethanol is thus unsuitable for use as a floral preservative for *G. scabridus* spikes. Parmar *et al.* (2002) noted that the use of 10.0, 1.0 and 0.1% ethanol resulted in a lighter colour intensity of the petals of *Gladiolus* florets.

Solutions with sucrose and Jik® also resulted in very poor quality spikes with a rating from 0.8 to 1.9. The presence of Jik® seems to prevent the florets from fully developing as they became dry, crinkled and deformed, many failing to open before the spike senesced. The spike and leaves became black in appearance which would be unsuitable for the floricultural market (Plate 5.2 B). This appears to be a phytotoxic reaction to the JIK® solutions possibly due to the presence of chloride ions.

***Table* 5.2 *G. scabridus* spike longevity and floret quality as influenced by the floral preservatives**

Solution	Vase life (days) [x]	Floret quality [y]
distilled water [z]	1.3 a	4.0 b
Chrysal®	8.0 b	3.4 bc
Prolong®	6.0 cd	3.0 c
2 % sucrose & 2 % ethanol	5.1 d	1.1 e
1 % sucrose	7.1 bc	3.1 c
2 % sucrose	12.5 a	4.9 a
4 % sucrose	8.3 b	4.0 b
1 % sucrose & 0.5 % JIK®	6.1 cd	1.9 d
2 % sucrose & 0.5 % JIK®	5.0 d	1.3 de
4 % sucrose & 0.5 % JIK®	5.0 d	0.8 e

[x] days from placement in solution until the lower 5 florets had senesced and the spike was discarded (LSD (5%) = 1.3080)

[y] overall floret quality rating from 1 = poor to 5 = good (LSD (5%) = 0.6161)

[z] control

Letters a to e compare mean values in a column; n = 3 x 3 spikes

Plate 5.4 ***G. scabridus*** **spikes after holding for 8 days in 2 % sucrose (left) and 1 % sucrose and 0.5 % JIK® (right) at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}**

Floret width

The effect of the vase life solutions on floret width was highly significant ($p<0.001$). The significantly widest florets were produced with solutions of 4 % sucrose (55.61 mm) and 2 % sucrose (53.15 mm) respectively (Figure 5.1). This was followed by the control (45.39 mm) and then 1 % sucrose (44.31 mm). These results are confirmed by Mayak *et al.* (1973) and Murali (1990) who found that sucrose increased the diameter of *Gladiolus* florets. Florets have been found to be the most active organs for sucrose accumulation (Bravdo *et al.*, 1974). Sugars play an important role in flower development and opening either as energy sources for respiration or as osmotically active substances which aid in maintaining turgidity of the expanding corollas, therefore improving the water uptake and balance (Siegelman *et al.*, 1958; Mayak *et al.*, 1973; Bravdo *et al.*, 1974; Kofranek & Halevy, 1976; Hancke & Verryn, 1989a; Rekha *et al.*, 2001).

Two percent sucrose with 2 % ethanol resulted in florets of moderate width of 40.64 mm. The commercially available additives, Prolong® and Chrysal®, were not significantly different to this solution with floret widths of 39.76 and 38.57 mm respectively. Those treatments containing 0.5 % Jik® resulted in the significantly smallest florets (Figure 5.1). These treatments with poor floret opening therefore reduce the water uptake of the spikes and are thus unsuitable for postharvest quality improvement of *G. scabridus* cut spikes.

Floret length

Floret length was also significantly affected by the various treatments ($p<0.001$), with a similar trend to the effect on floret width (Figure 5.2). Yet again 4 and 2 % sucrose resulted in the significantly largest and more open florets of 53.47 and 52.40 mm lengths, followed by distilled water (42.43 mm) and then 1 % sucrose (41.78 mm). This shows yet again that sucrose is a more beneficial vase life solution than distilled water. Waithaka *et al.* (2001) clearly demonstrated that the opening of *Gladiolus* florets requires the import of carbohydrate which appears to be very important for *G. scabridus* floret opening. Two percent sucrose with 2 % ethanol once again produced florets of moderate length (39.56 mm). The commercial solutions gave rise to significantly poorer floret development with no significant difference between the floret lengths of Prolong® (37.07 mm) and Chrysal® (36.69 mm). Solutions with 1 and 2 % sucrose and 0.5 % Jik® resulted in the smallest florets of 30.50 and 35.92 mm respectively, whereas 4 % sucrose and 0.5 % Jik® resulted in florets of 37.72 mm in length (Figure 5.2).

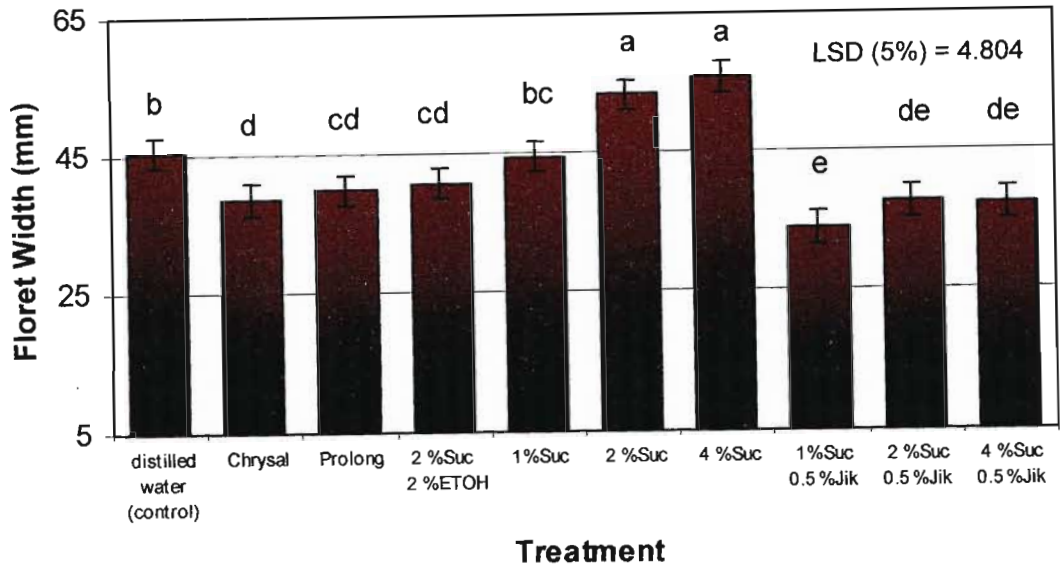


Figure 5.1 Mean *G. scabridus* floret width as affected by floral preservatives held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

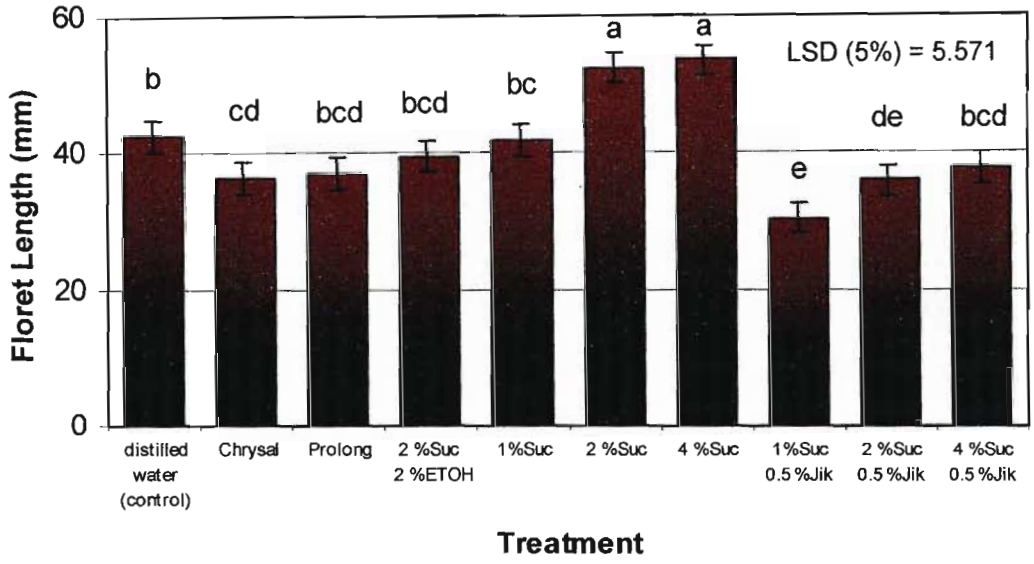


Figure 5.2 Mean *G. scabridus* floret length as affected by floral preservatives held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

Floral spike mass

Vase life treatment and the change in floral spike mass over time both had a highly significant effect ($p<0.001$) on the amount of solution taken up by the spikes. There was a non-significant interaction between these main effects ($p=1.000$). The ageing of flowers is usually reflected in a decrease in their fresh mass (Ferreira, 1981). Overall *G. scabridus* mean spike mass was initially 27.93 g which increased after 3 days in solution to 28.26 g and then showed a gradual decrease to 15.02 g, below the initial floral spike mass (Figure 5.3). This is due to the initial uptake of solution followed by the gradual senescence of the spike. Yamane *et al.* (1995) speculate that the export of water and dry matters may be responsible for *Gladiolus* spike mass loss as indicated by a high level of sucrose in the petals which later decreases.

Chrysal® gave rise to the significantly highest spike mass of 26.89 g with the other commercially available product, Prolong®, resulting in one of the lowest spikes masses of 20.92 g (Figure 5.4). Treatments with sucrose resulted in spikes of moderate mass all of which were higher than the control. This is confirmed by Bravdo *et al.* (1974) who found that spikes in solutions of sucrose showed a higher mass than the water control. Sugars have a beneficial effect on maintaining the fresh mass in cut flowering shoots by inducing stomatal closure in leaves and thus reducing water loss (Bravdo *et al.*, 1974; Rekha *et al.*, 2001) as well as replacing depleted natural carbohydrate (Anserwadekar & Patil, 1986).

Vase life solution uptake

Vase life treatment and the duration of the trial both had a significant effect ($p=0.024$ and $p<0.001$ respectively) on the amount of solution taken up by the spikes. There was a non-significant interaction between these main effects ($p=0.874$). Overall mean uptake was initially very high at 65.1 ml after 3 days in solution. This then decreased significantly to 13.7 ml just before the termination of the trial (Figure 5.5).

Prolong® showed the highest mean solution uptake of all the treatments (41.8 ml) followed by distilled water (35.0 ml), 2 % sucrose and 2 % ethanol (32.4 ml) and then Chrysal® (31.3 ml), with no significant difference between any of these solutions (Figure 5.6). There was also no significant difference between treatments with sucrose alone and sucrose with Jik®, which all showed lower levels of solution uptake, 4 % sucrose being the lowest at 20.2 ml. Aarts

(1957) observed that higher sugar concentrations decreased transpiration and thus decreased water uptake. Gorini and Arrigo (1974) and Anserwadekar and Patil (1986) found that gladioli held in sucrose absorbed less water and showed reduced wilting, yet the spikes attained a higher fresh mass than gladioli held in water alone, indicating that cut flower longevity of gladioli, and *G. scabridus* in particular, is associated with the maintenance of fresh mass as indicated by the sucrose solutions.

Solution pH

There was a highly significant interaction between treatment and time ($p<0.001$) on the change in solution pH. At the start of the trial, most solutions were acidic, the commercial solutions, Chrysal® and Prolong®, especially so at 2.92 and 3.89 respectively. Solutions with Jik® were, however, extremely basic (Figure 5.8). Even with the continual addition of solution to the bottles, the general trend of each treatment pH was to decrease over time, although the distilled water and Prolong® increased slightly from 4.18 to 5.28 and from 2.93 to 3.54 respectively. The pH of the Jik® solutions decreased considerably over time from about 11.50 ending at a neutral of about 7.00. Jik® acts as a bactericide in the vase life solution (Salunke *et al.*, 1990) killing any unwanted micro-organisms and perhaps the effect thereof is a significant decrease in pH.

G. scabridus spikes thus cause the vase life solution pH to decrease over time as the solution is taken up (Figure 5.7). Flowers persist longer in acidic water than in basic pH water as water that is acidic is taken up more rapidly and deters the growth of numerous microorganisms (Gowda & Gowda, 1990; Armitage, 1993). The JIK® solutions were basic which could have contributed to their poor effect on vase life and floret quality.

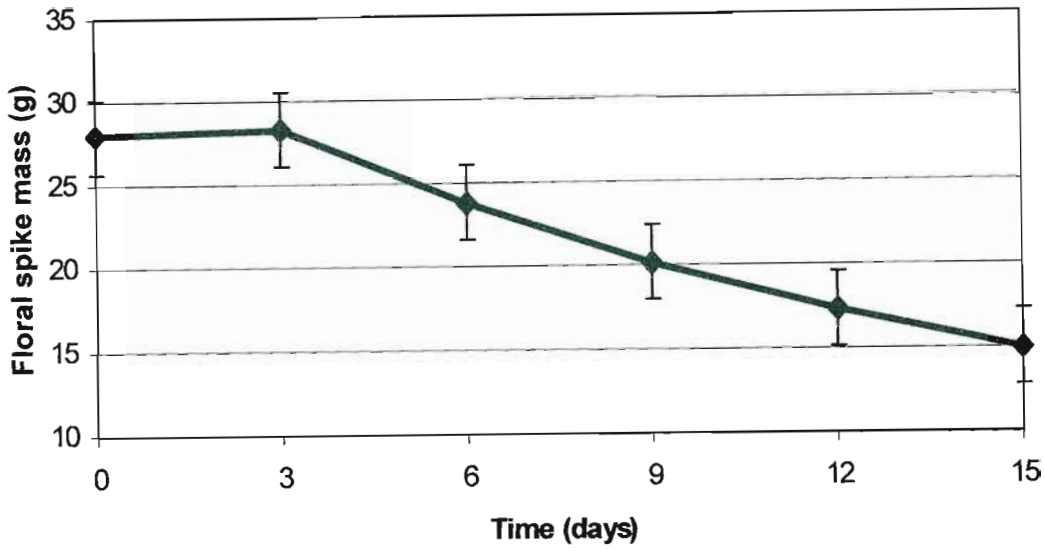


Figure 5.3 Mean change in *G. scabridus* spike mass as affected by floral preservatives at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

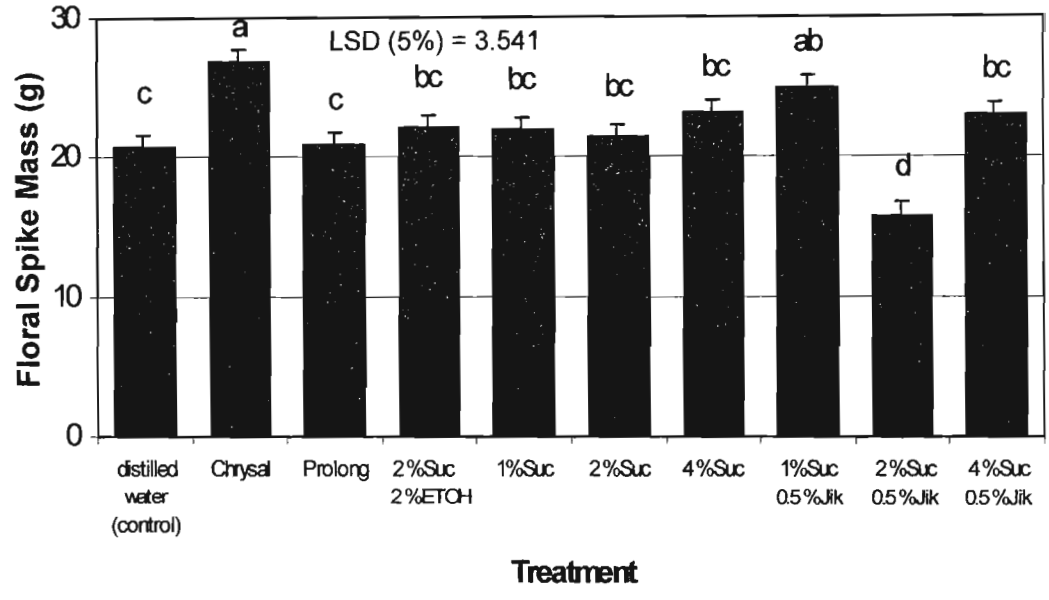


Figure 5.4 Effect of floral preservatives on overall mean *G. scabridus* spike mass held for 15 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

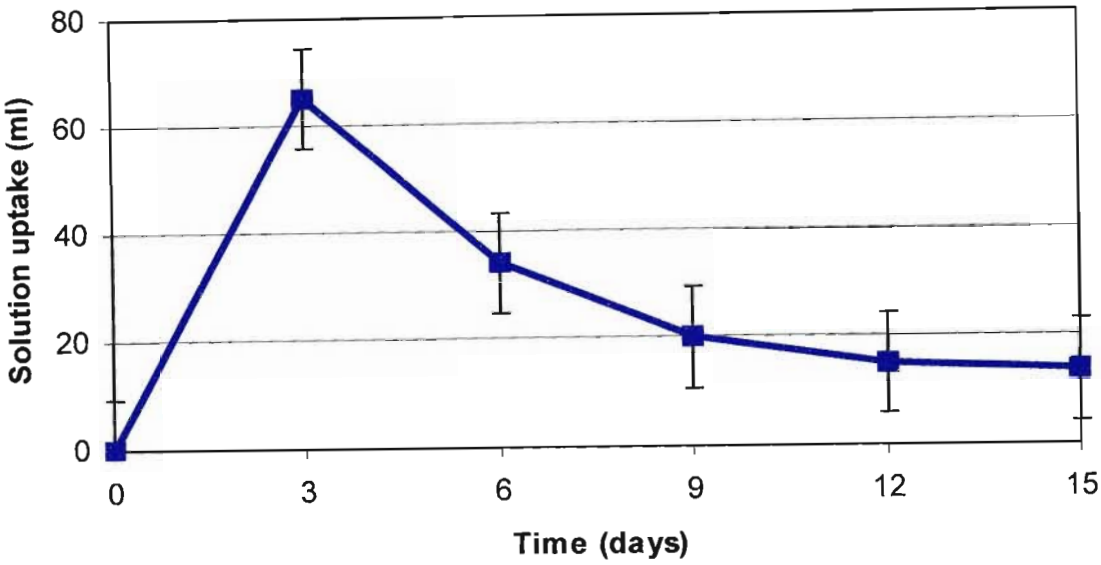


Figure 5.5 Mean change in floral preservative uptake by *G. scabridus* spikes at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

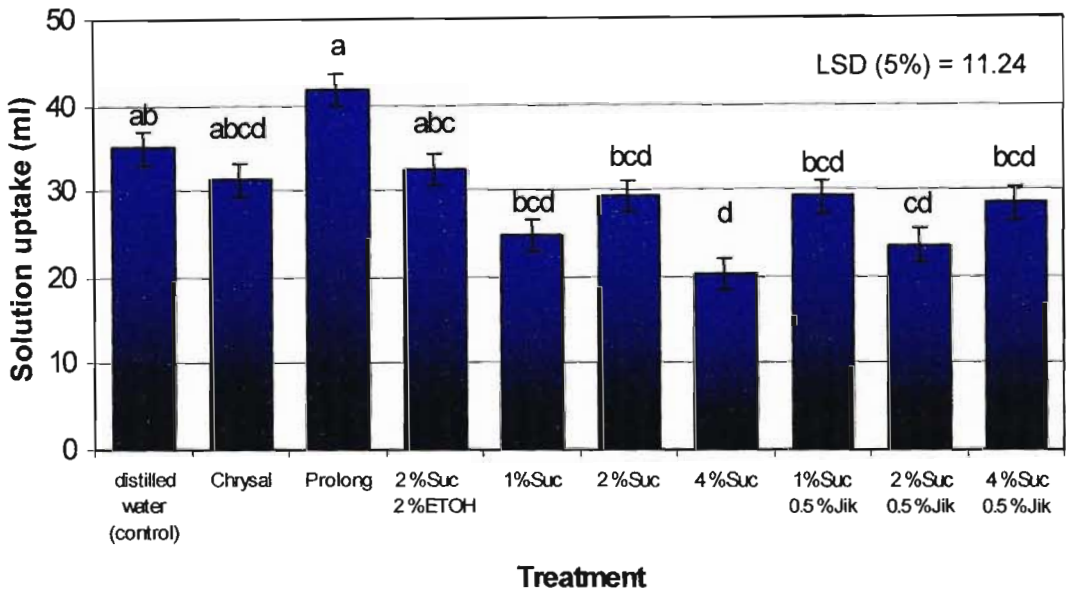


Figure 5.6 Mean uptake of solution by *G. scabridus* spikes as affected by floral preservatives held for 15 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

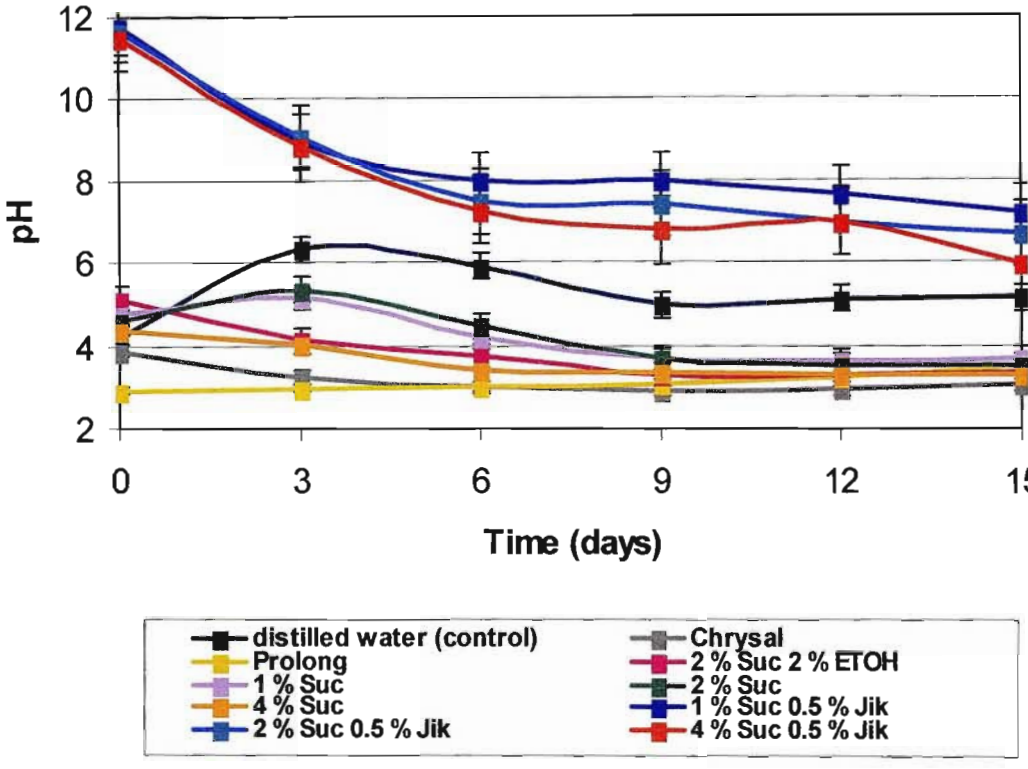


Figure 5.7 Change in floral preservative pH over time whilst holding *G. scabridus* cut spikes for vase life determination at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

Second study

Vase life

The only significant difference (p=0.019) in *G. scabridus* vase life treatments during this trial was between 2% sucrose and distilled water (Table 5.3). Two percent sucrose resulted in the longest vase life of 13.0 days and distilled water resulted in the lowest vase life of 11.1 days. Neither of these treatments were significantly different to 2 % fructose (12.3 days) or 2 % glucose (12.0 days). These results differ from those obtained in the initial trial which showed no significant difference between 2 % sucrose and distilled water in improving vase life. However, results from this season confirm the importance of a carbohydrate source for improving the postharvest vase life of cut *G. scabridus* spikes. This is also confirmed by Anju and Santosh (2004) who found that sucrose gave the longest vase life in cut spikes of *Gladiolus* cv. 'Pink Friendship'.

Spike quality

G.scabridus spike quality was significantly affected by the 4 floral preservatives (p=0.016). As in the initial trial, 2 % sucrose showed the significantly highest mean spike quality (4.8). This was not significantly different to that of fructose (4.1) but was significantly different to glucose (3.3) and distilled water (3.9) (Table 5.3). Spikes held in 2 % sucrose and 2 % fructose remained healthy until the onset of senescence. The large florets were bright pink to mauve in colour and most of them opened with no visible abnormalities. Leaves remained green and healthy which also improved the postharvest quality and visual appeal of the spikes (Plate 5.5). These solutions could thus be recommended as suitable postharvest treatments for *G.scabridus* spikes, but as 2% sucrose also improves vase life, this would be the most beneficial solution to use, confirming results from the initial trial.

Spikes held in 2 % glucose were given the lowest quality rating. Some of the florets became dry and crinkled in appearance with dark purple tips. Previous studies have led to the speculation that when the mitochondria within the plant cells are unable to utilize a substrate efficiently, this results in a decline in respiration which enhances petal senescence (Kaltaler & Steponkus, 1976). A number of the leaves also developed blotches on their surfaces.

Floret quality of spikes in distilled water was good but they were slightly pale in colour and the leaves started yellowing early on which detracted from the otherwise healthy appearance of the spikes. This would indicate that a carbohydrate source prolongs the health of cut *G. scabridus* spikes. The lack of carbohydrate results in a lack of reserves and energy supply to the leaves hence their early degradation.

***Table* 5.3 *G. scabridus* spike longevity and floret quality as influenced by the floral preservatives**

Solution	Vase life (days) [x]	Floret quality [y]
distilled water [z]	11.1 b	3.9 b
2 % sucrose	13.0 a	4.8 a
2 % fructose	12.3 ab	4.1 ab
2 % glucose	12.0 ab	3.3 b

[x] days from placement in solution until the lower 5 florets had senesced and the spike was discarded (LSD (5%) = 1.483)

[y] overall floret quality rating from 1 = poor to 5 = good (LSD (5%) = 0.862)

[z] control

Letters a to b compare mean values in a column; n = 3 x 2 spikes

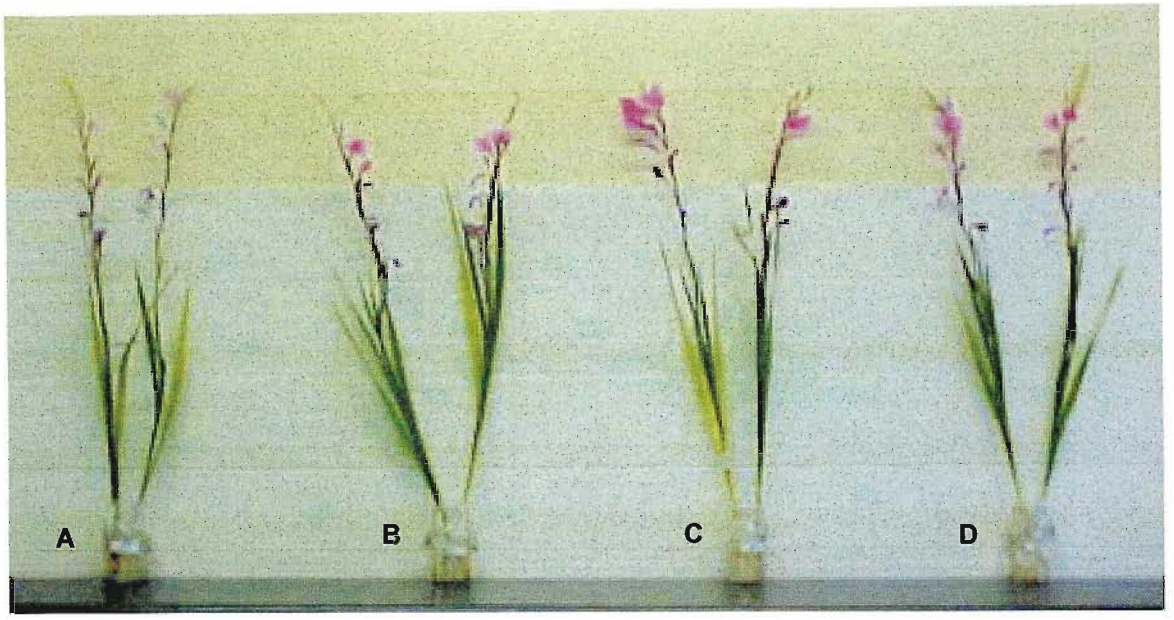


Plate 5.5 Cut *G. scabridus* spikes after 8 days in solutions of (A) distilled water (control), (B) 2 % glucose, (C) 2 % fructose and (D) 2 % sucrose, held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

Floret width

There was a highly significant effect of vase life solution on the width of the *G. scabridus* florets ($p<0.001$). Spikes in 2 % sucrose produced the widest florets of 55.29 mm (Figure 5.8). This was significantly different to all the other treatments. There was no significant difference in floret width on spikes held in 2 % fructose (49.55 mm) and 2 % glucose (48.50 mm). The control spikes had the lowest floret width of 42.80 mm which was significantly different to all the carbohydrate treatments. This again confirms previous results that sugars play an important role in the opening of florets on *G. scabridus* spikes.

Floret length

The floral preservatives also had a highly significant effect on floret length ($p<0.001$). As with floret width the spikes in 2 % sucrose produced the biggest florets (52.90 mm) (Figure 5.9). This was, however, significantly different to all the other treatments except 2 % fructose (49.75 mm). Florets in 2 % glucose opened to a mean value of 45.85 mm whereas those in distilled water showed the lowest values of 43.50 mm.

Floral spike mass

The effect of vase life treatment was highly significant on mean floral spike mass ($p=0.005$) as was the change in mass over time ($p<0.001$), however, the interaction of these two factors was non-significant ($p=0.998$). Mean spike mass was initially 23.84 g, which increased after 3 days in solution to 25.72 g due to the initial uptake of solution and this was followed by a gradual decrease in mass as the spikes senesced to a final value of 13.66 g, below the original mass (Figure 5.10).There was no significant difference in the mean spike mass between 2 % sucrose (22.35 g), 2 % fructose (22.23 g) and 2 % glucose (21.31 g), however, all were significantly higher than the control (18.56 g) (Figure 5.11). The carbohydrate treatments allow for a constant supply of sugar and thus maintain cell integrity and delay senescence in these spikes (Salunke *et al.*, 1990). It is suggested that spikes in distilled water have no exogenous source of carbohydrate thus will have their reserves depleted far quicker resulting in a lower spike mass.

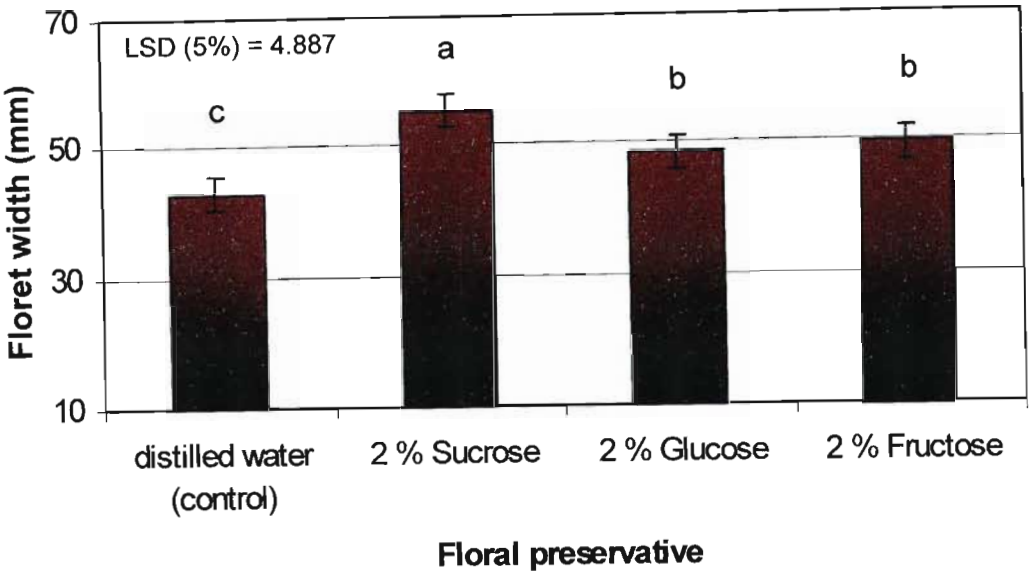


Figure 5.8 Mean *G. scabridus* floret width as affected by floral preservatives held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

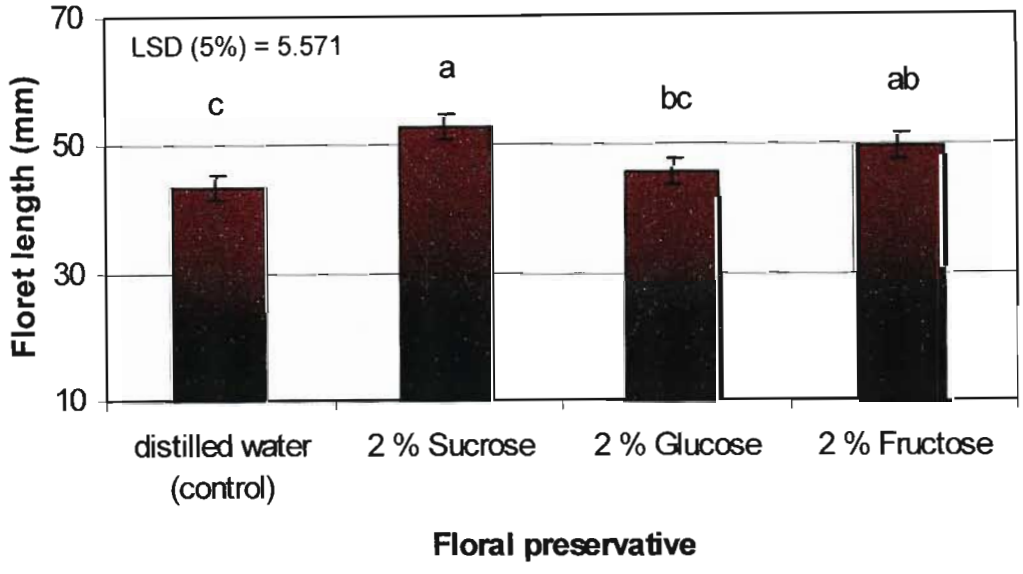


Figure 5.9 Mean *G. scabridus* floret length as affected by floral preservatives held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

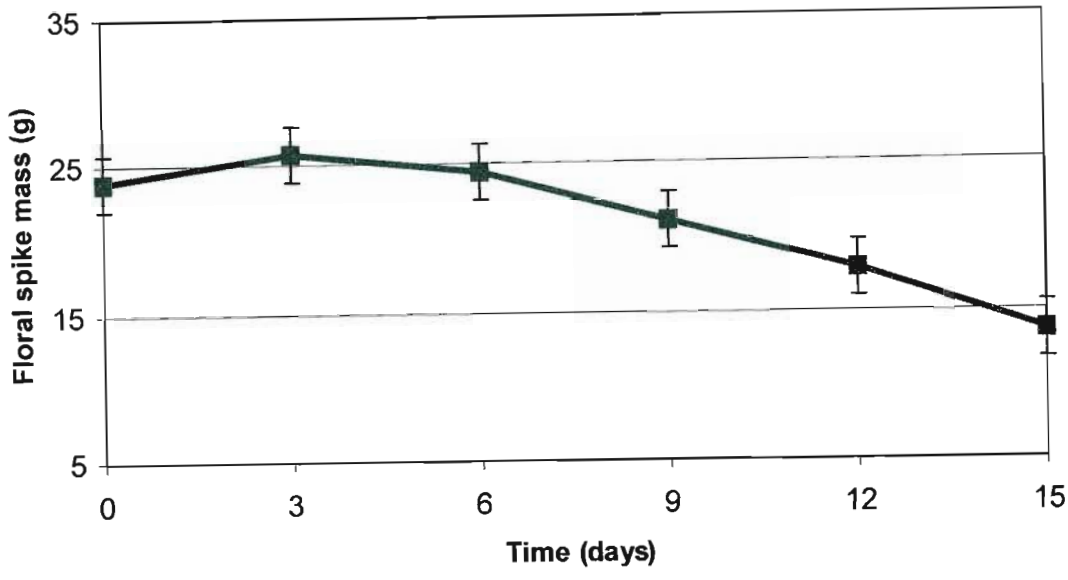


Figure 5.10 Mean change in *G. scabridus* spike mass as affected by floral preservatives at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

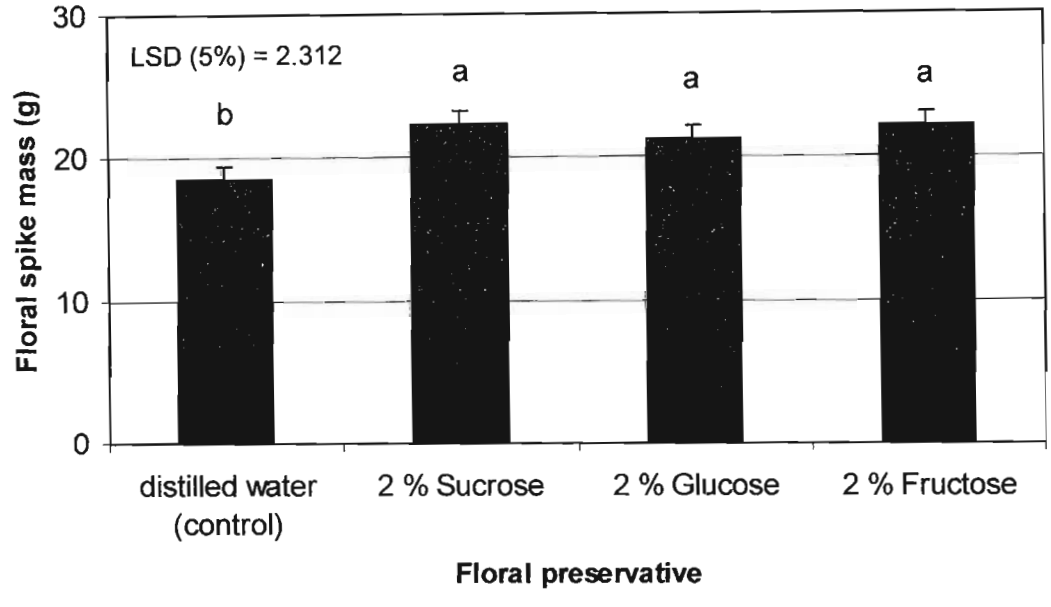


Figure 5.11 Effect of floral preservatives on overall mean *G. scabridus* spike mass held for 15 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

Vase life solution uptake

Vase life treatment and the duration of the trial both had a highly significant effect ($p<0.001$) on the amount of solution taken up by the spikes during the second trial. There was a non-significant interaction between these main effects ($p=0.839$). Overall initial uptake after 3 days in solution was 41.21 ml which decreased to a value of 16.75 ml just before the trial was terminated (Figure 5.12). Spikes in 2% fructose and 2 % glucose absorbed the significantly highest mean volumes of 30.50 ml and 28.30 ml respectively (Figure 5.13). These results were significantly different to those obtained for distilled water (22.33 ml) and 2 % sucrose (21.07 ml). This confirms results from the initial trial in which distilled water and 2 % sucrose absorbed lower amounts of solution. Marousky (1968) suggested that sucrose decreases solution absorption due to stomatal closure, presumably as an osmotic effect.

Solution pH

There was a highly significant interaction between vase life treatment and the duration of the trial ($p<0.001$) (Figure 5.14). All solutions remained acidic throughout the trial. The carbohydrate solutions decreased slightly over time whereas distilled water showed an initial decrease followed by an increase till the termination of the trial. The preservation of cut gladioli spikes is enhanced and water uptake increased in an acidic vase life solution (Reist, 1977). Therefore the pH of all the solutions tested, never had a negative effect on the vase life of *G. scabridus* spikes.

Chlorophyll fluorescence

Relative chlorophyll fluorescence is a an extremely sensitive, non-destructive, non-invasive measurement technique that determines the photosynthetic efficiency of plant tissues containing chloroplasts (van Kooten *et al.*, 1991; Deell *et al.*, 1999; Pompodakis *et al.*, 2005), such as *G. scabridus* leaves. Vase life treatment and the duration of the trial both had a highly significant effect ($p<0.001$) on the chlorophyll fluorescence readings of *G. scabridus* spikes. There was a non-significant interaction between these main effects ($p=0.561$). Fully expanded leaves usually show the maximum photosynthetic activity (therefore higher Fv/Fm ratios) which decreases slowly as they senesce, the leaves eventually becoming yellow and thus unable to photosynthesize because of chlorophyll breakdown and loss of functional chloroplasts (Salisbury & Ross, 1992; Deell *et al.*, 1999), resulting in lower Fv/Fm ratios. The

initial overall mean Fv/Fm ratio for freshly cut, healthy *G. scabridus* spikes was 0.8276. This then decreased gradually over time correlating with a gradual decrease in the photosynthetic state of *G. scabridus* spikes and then dropped suddenly, from about day 10, to a final mean ratio of 0.5178 for senescing spikes (Figure 5.15).

The vase life preservatives had a highly significant effect on the fluorescence ratios ($p<0.001$). The control treatment resulted in an overall mean Fv/Fm ratio of 0.6975 which was significantly lower than all the sugar solutions. There was no significant difference between 2 % fructose (0.7493 Fv/Fm), 2 % glucose (0.7414 Fv/Fm) and 2 % sucrose (0.7370 Fv/Fm) (Figure 5.16). There was no significant difference in the vase life between distilled water, 2 % fructose and 2 % glucose during this trial thus the Fv/Fm ratios clearly indicate the importance of a carbohydrate in the vase life solution to maintain the internal physiological health of the spikes by improving photosynthetic activity.

The fluorescence parameter Fv/Fm proved to be a useful indicator as to which vase life solutions maintain the internal health of *G. scabridus* cut spikes. However, its use as a means of predicting vase life is somewhat difficult as the ratios decline slowly and follow the pattern of senescence, suddenly decreasing as the spike nears the end of its viable postharvest life. One would prefer an earlier indication of when final senescence is to take place. It does, however, give an indication of the change in spike health over time but this can be observed visually. Similarly, Schroder *et al.* (1998) found that the chlorophyll fluorescence test was unsuitable for determining life expectancy in cut roses.

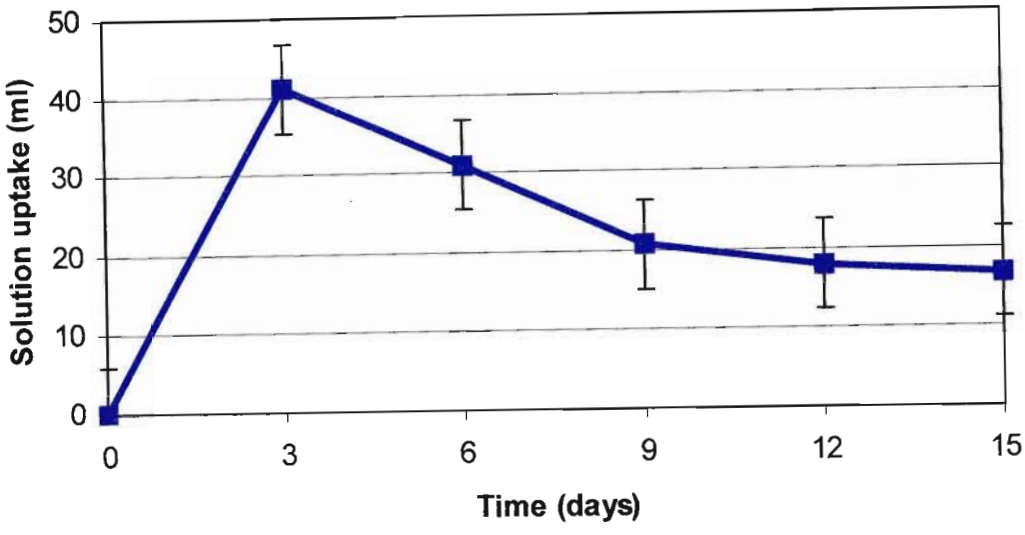


Figure 5.12 **Mean change in floral preservative uptake by *G. scabridus* spikes whilst holding in floral preservatives at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}**

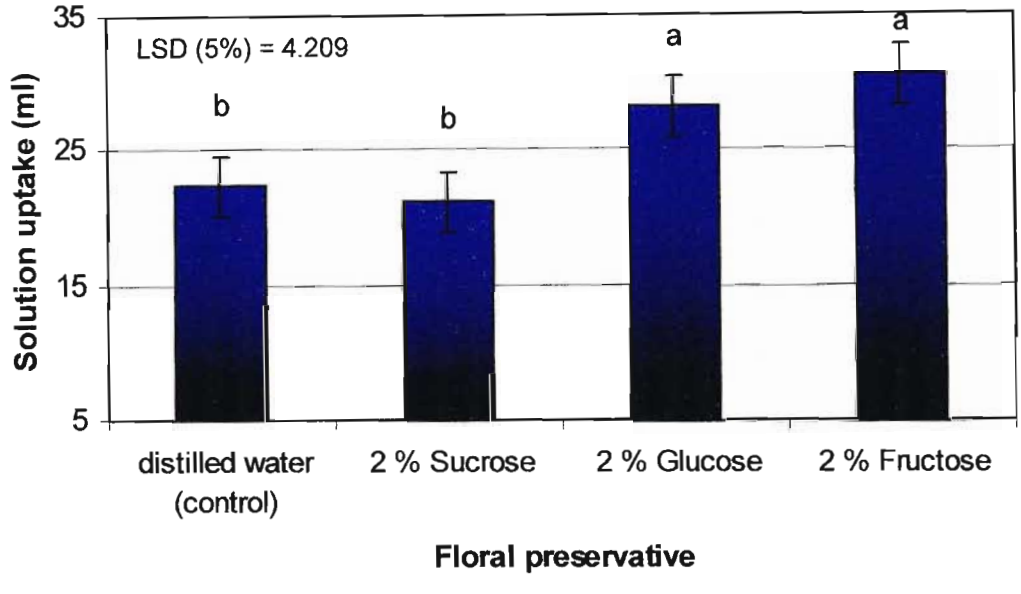


Figure 5.13 **Mean uptake of solution by *G. scabridus* spikes as affected by floral preservatives for 15 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)**

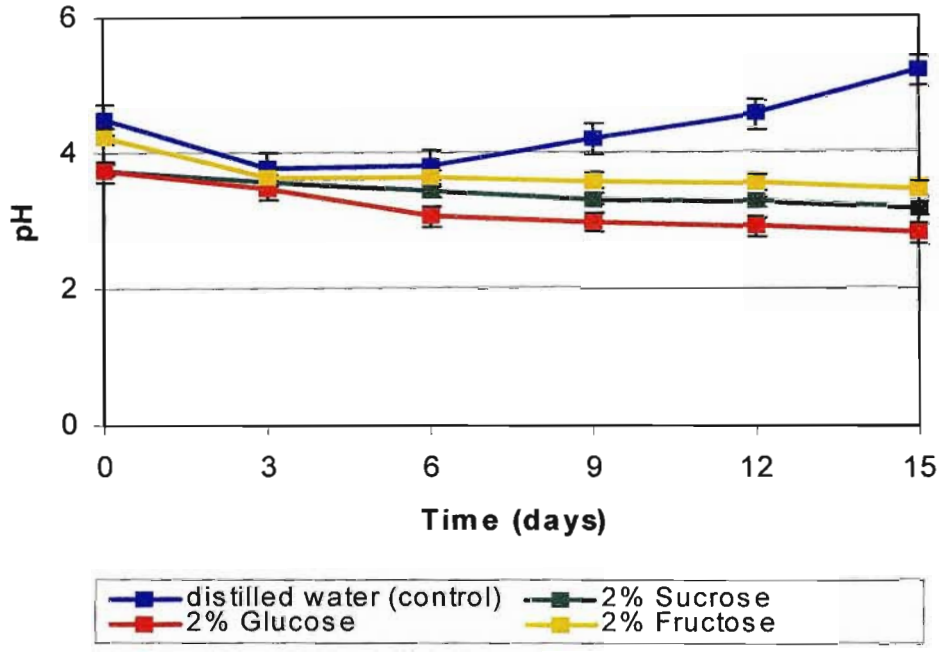


Figure 5.14 Change in floral preservative pH over time whilst holding *G. scabridus* cut spikes for vase life determination at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}.

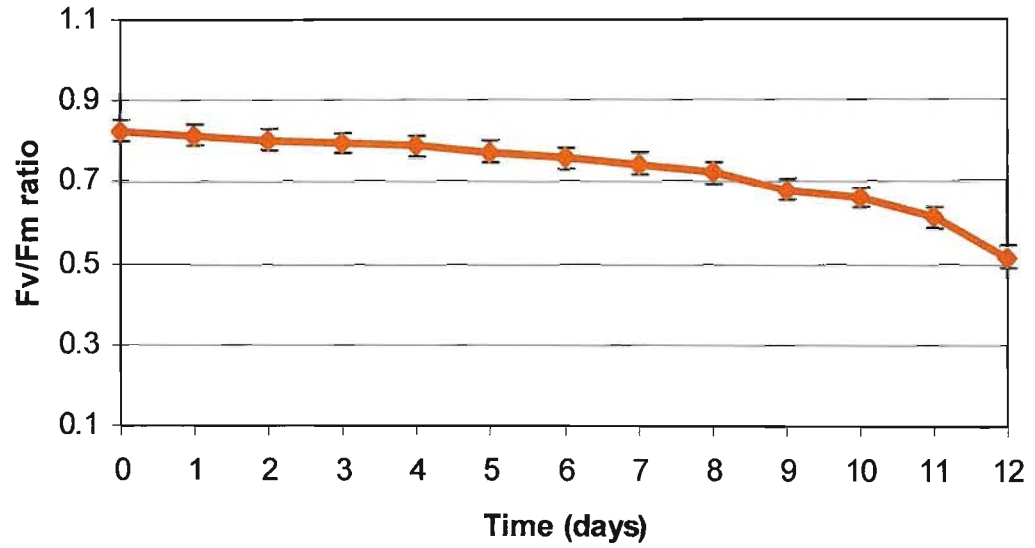


Figure 5.15 **Mean change in *G. scabridus* spike chlorophyll fluorescence over time as affected by floral preservative solutions at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}.**

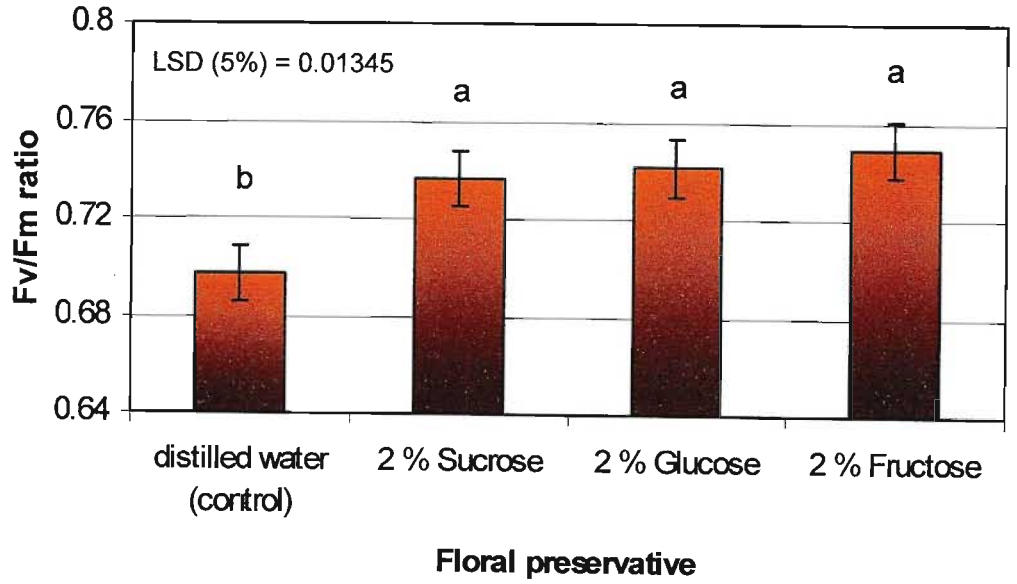


Figure 5.16 **Mean chlorophyll fluorescence ratios of *G. scabridus* spikes held in floral preservative at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)**

Carbohydrate analysis

Fresh mass of G. scabridus florets

There was a highly significant interaction ($p<0.001$) between sampling date and floret position on the fresh mass of *G. scabridus* florets. There were no significant differences between treatments on day 5 and 10. The fresh mass of the newly-opened florets decreased progressively with each sampling date. The fresh mass of florets on freshly harvested *G. scabridus* spikes was highest in the open florets at the base of spike and decreased towards the upper most florets (Figure 5.17). After 5 days in solution, the fresh mass of the basal (oldest) florets had decreased (with senescence) and that of the middle florets had increased substantially with bud opening. The fresh mass of the florets on spikes which had been in solution for 10 days was highest on the open florets near the tip of the spike and that of the senescing middle and basal florets had decreased.

Dry mass of G. scabridus florets

There was a highly significant interaction ($p<0.001$) between sampling date and floret position on the dry mass of *G. scabridus* florets. There were no significant differences between treatments on day 5 and 10. Collier (1997) found that dry mass increases in tulip and alstroemeria florets, were proportional to the fresh mass increases during all stages of floret development. The distribution of dry mass of *G. scabridus* florets up the spikes followed a similar pattern to that of the fresh mass (Figure 5.18). The dry mass of the newly-opened florets decreased progressively with each sampling date. The maximum dry mass in freshly harvested spikes was in the lower, open florets and decreased gradually up the spike. After 5 days in solution, the dry mass of the lower senescing florets had fallen and the dry mass of the middle open florets had increased. Day 10 revealed that the upper florets in position 7 to 8 had the highest dry mass compared to the florets below it on the spikes.

Fresh and dry mass of G. scabridus leaves

The fresh and dry mass of *G. scabridus* leaves was significantly higher ($p<0.001$) on day 0 (3.021 and 0.978 g respectively). There were no significant changes in mass between day 0 and day 5 but both fresh and dry masses were significantly lower on day 10 compared to day 0. There was no significant differences in fresh and dry masses between treatments on each sampling date (Figure 5.19).

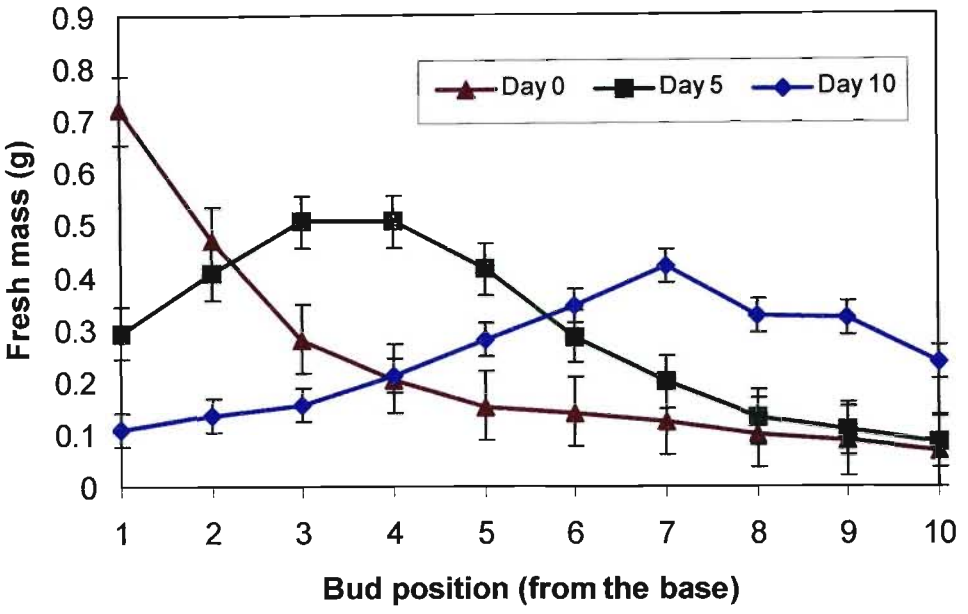


Figure 5.17 The mean fresh mass *of G. scabridus* florets from freshly harvested spikes and spikes held in vase life solutions of distilled water, 2 % (w/v) sucrose, 2 % (w/v) glucose and 2 % (w/v) fructose for 5 days and 10 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}.

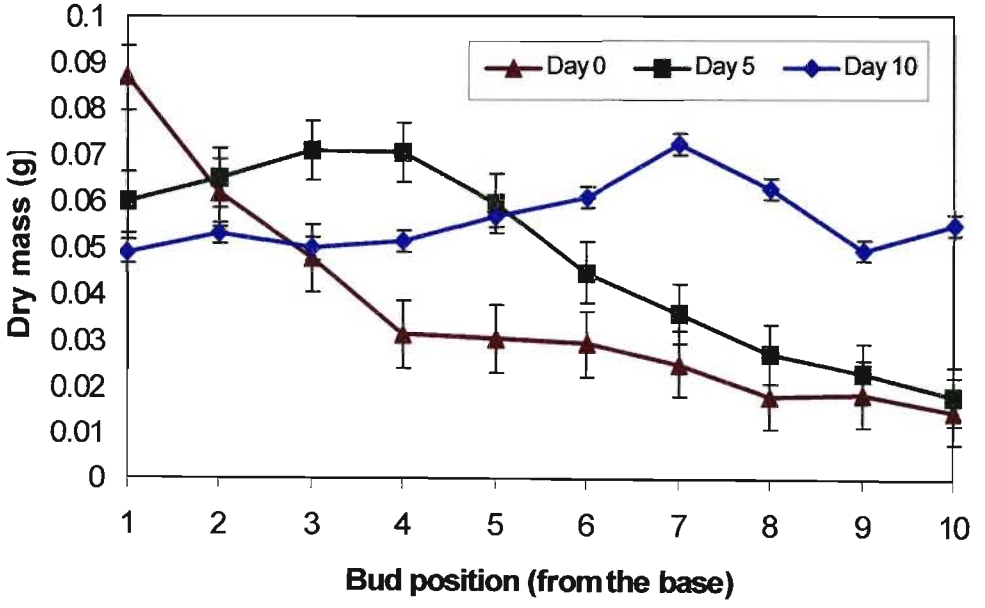


Figure 5.18 The mean dry mass *of G. scabridus* florets from freshly harvested spikes and spikes held in vase life solutions of distilled water, 2 % (w/v) sucrose, 2 % (w/v) glucose and 2 % (w/v) fructose for 5 days and 10 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}.

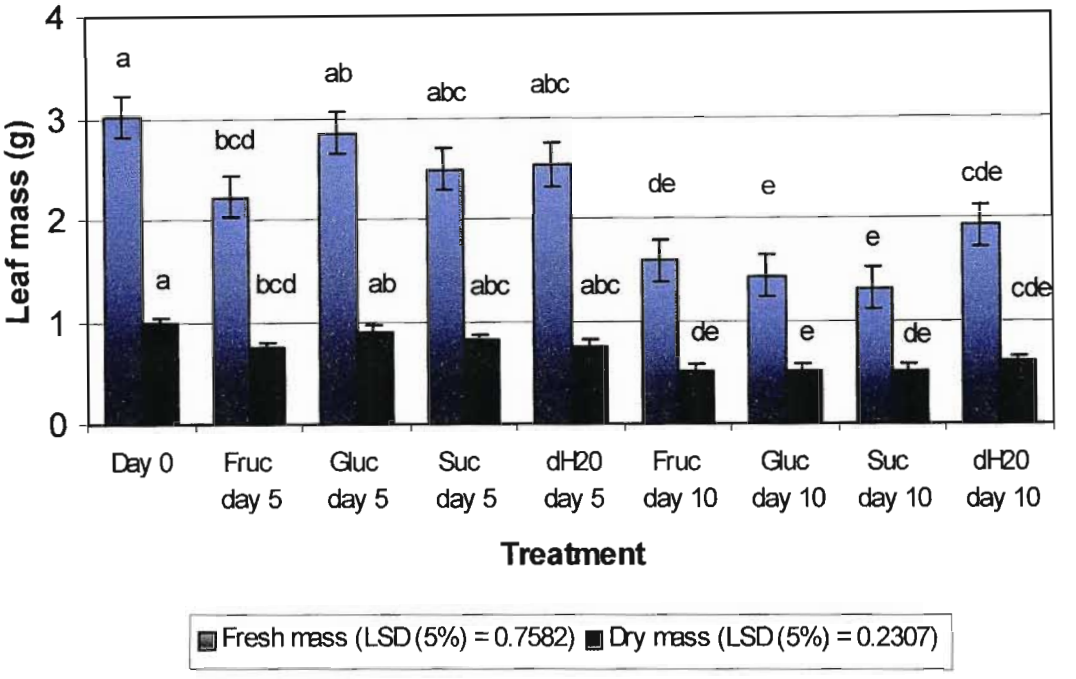


Figure 5.19 Fresh and dry mass of leaves of *G. scabridus* from freshly harvested spikes and spikes held in vase solution for 5 days and 10 days at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

Sugar content

Florets

The sugar content of the *G. scabridus* florets changed markedly during the postharvest life of the spikes and mirrored the changes in floret fresh and dry masses, with the highest concentration of total sugars being evident in florets that were open: the basal florets on day 0, the middle florets on day 5 and the uppermost florets on day 10 (Figures 5.20 and 5.21). Unopened and senescing florets always had lower sugar concentrations than these open florets as found by Hussain *et al.* (2001) in gladioli hybrids. It appears that there is a remobilization of carbohydrates from senescing petals into developing *Gladiolus* buds for their expansion (Yamane *et al.*, 1995) and into the ovary (Ho & Nichols, 1975; Mor *et al.*, 1980) thus accounting for the change in these sugar levels. It has been suggested that during senescence, the invertase inhibitor prevents sucrose being hydrolysed to glucose and fructose and thus enables carbohydrates to transfer from wilting petals to neighbouring organs (Halaba & Rudnicki, 1985). Waithaka *et al.* (2001) found that the total sugar content of open flowers on freshly harvested gladioli spikes, was about half that of open florets on spikes that had been in solution for 5 days. However, the total sugar content of open *G. scabridus* florets remained consistent throughout the trial at a concentration of ± 3.5 g / 100 ml for each harvest date with no significant differences between treatments.

Glucose, fructose and sucrose make up the soluble sugar pool in plants (Buysse & Merckx, 1993). *G. scabridus* florets at all stages of development contained varying concentrations of these 3 important sugars. There was, however, a significantly higher concentration of sucrose present in the *G. scabridus* florets in all the vase life solutions, with a smaller quantity of fructose and an even smaller amount of glucose (Figures 5.20 and 5.21). Waithaka *et al.* (2001) found that fructose was the principle soluble carbohydrate in gladioli florets whereas Collier (1997) noted that glucose was the dominant carbohydrate in tulip tepals. In cut roses, Kaltaler and Steponkus (1974) found both fructose and glucose to be the predominant sugars in the petal tissues whereas Lukaszewska *et al.* (1991) observed that sucrose content was the highest. The results confirm that sucrose plays an important role in the opening of *G. scabridus* florets and hence the postharvest life of these spikes. Sucrose is the form in which most sugar is transported in plants (Raven *et al.*, 1992; Proctor *et al.*, 1996). It is possible that stored sucrose in the relatively thick stem tissue of *G. scabridus* spikes could contribute to the higher levels of sucrose in *G. scabridus* florets,

especially when placed in solutions of other sugars. In a previous study to determine the fate of a glucose preservative in cut roses, it was found that once in the stem, the glucose was immediately converted to sucrose which migrated to the flower where it was rapidly hydrolysed (Paulin, 1980), so perhaps the higher concentration of sucrose in all treatments can be accounted for in the same way.

The vase life solutions also had a significant effect ($p<0.001$) on the overall levels of fructose, glucose and sucrose within the florets (Figure 5.22). Spikes evaluated on day 0 and spikes placed in distilled water had significantly lower levels of fructose and glucose compared to spikes in the sugar solutions. Spikes in distilled water harvested on both day 5 and day 10 also showed higher levels of sucrose within the florets compared to those spikes in sugar solutions, which may yet again be evidence for the storage of sucrose in the stems of *G. scabridus* spikes which becomes mobile at this stage. There were no other significant difference between sugar levels in the spikes.

Leaves

There were no significant differences between the levels of fructose ($p=0.069$) and glucose ($p=0.443$) in the *G. scabridus* leaves as a result of the various treatments and sampling dates, although the levels in leaves sampled at day 0 were consistently higher than those sampled on day 5 and 10. However, there was a significant ($p=0.038$) increase in the mean levels of sucrose in the leaves from day 0 (2.066 g / 100 ml) to a mean level of 2.337 g / 100 ml on day 5 and 2.361 g / 100 ml on day 10. There were no significant differences between the treatments on day 5 or 10. The increase in sucrose clearly confirms the results obtained with the florets that sucrose is the predominant sugar in *G. scabridus* spikes and has the greatest mobility within these spikes.

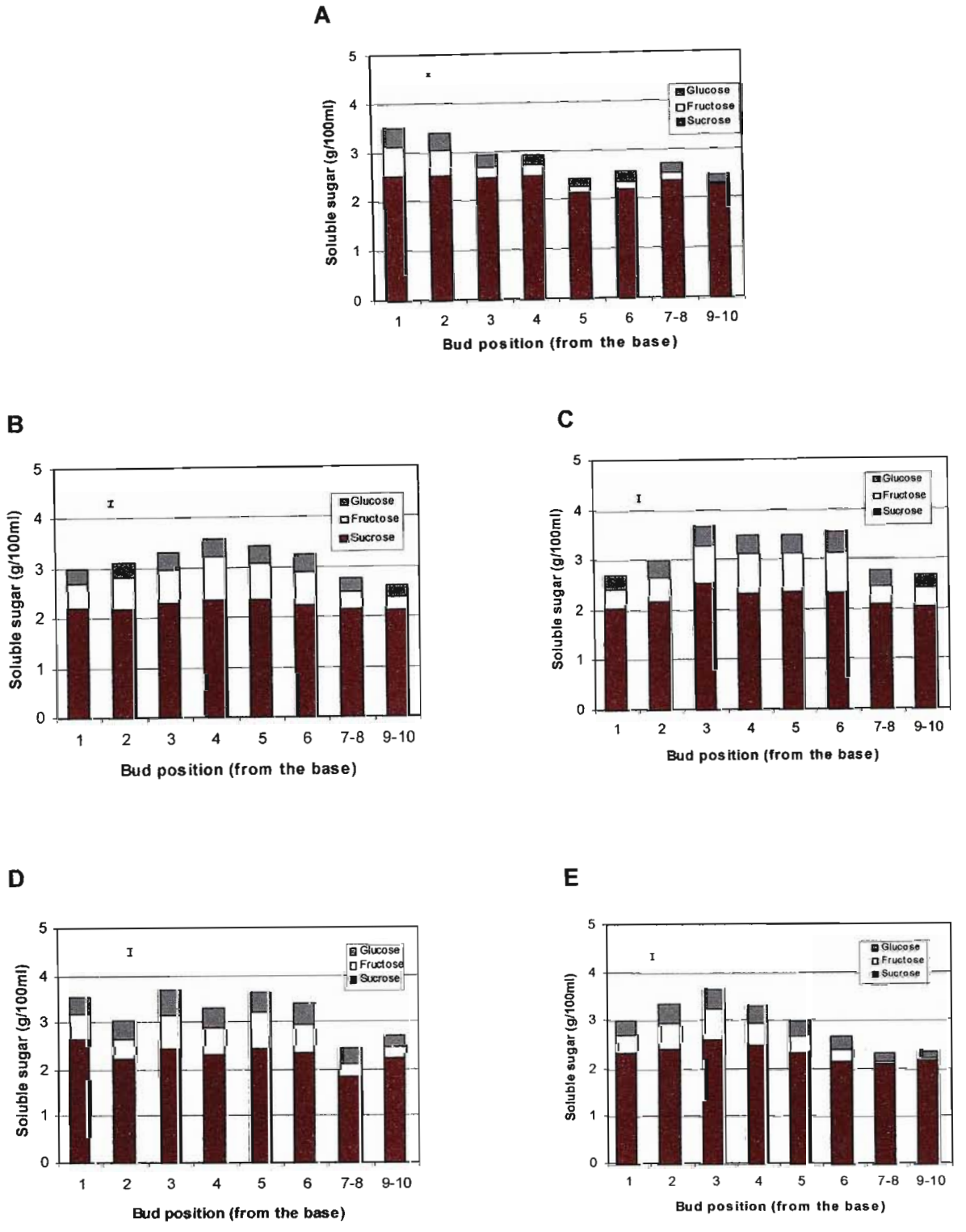


Figure 5.20 Soluble sugar concentration in florets of *G. scabridus* spikes at day 0 (A) and after 5 days in 2 % (w/v) fructose (B), 2 % (w/v) glucose (C), 2 % (w/v) sucrose (D) and distilled water (E) at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}.

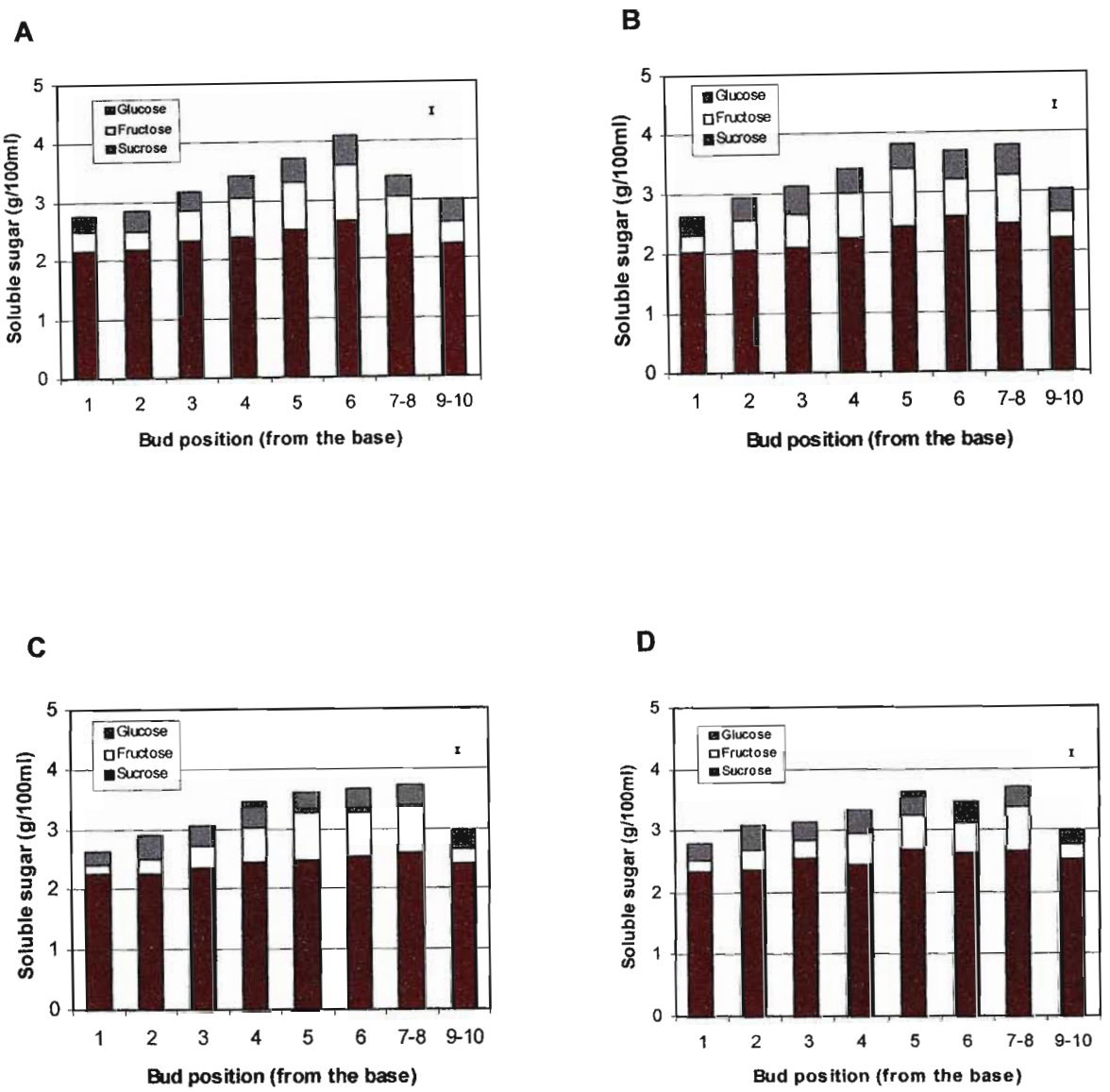


Figure 5.21 Soluble sugar concentration in florets of *G. scabridus* spikes after 10 days in 2 % (w/v) fructose (A), 2 % (w/v) glucose (B), 2 % (w/v) sucrose (C) and distilled water (D) at 22 °C and a constant illumination of 17.9 μmol m^{-2} s^{-1}.

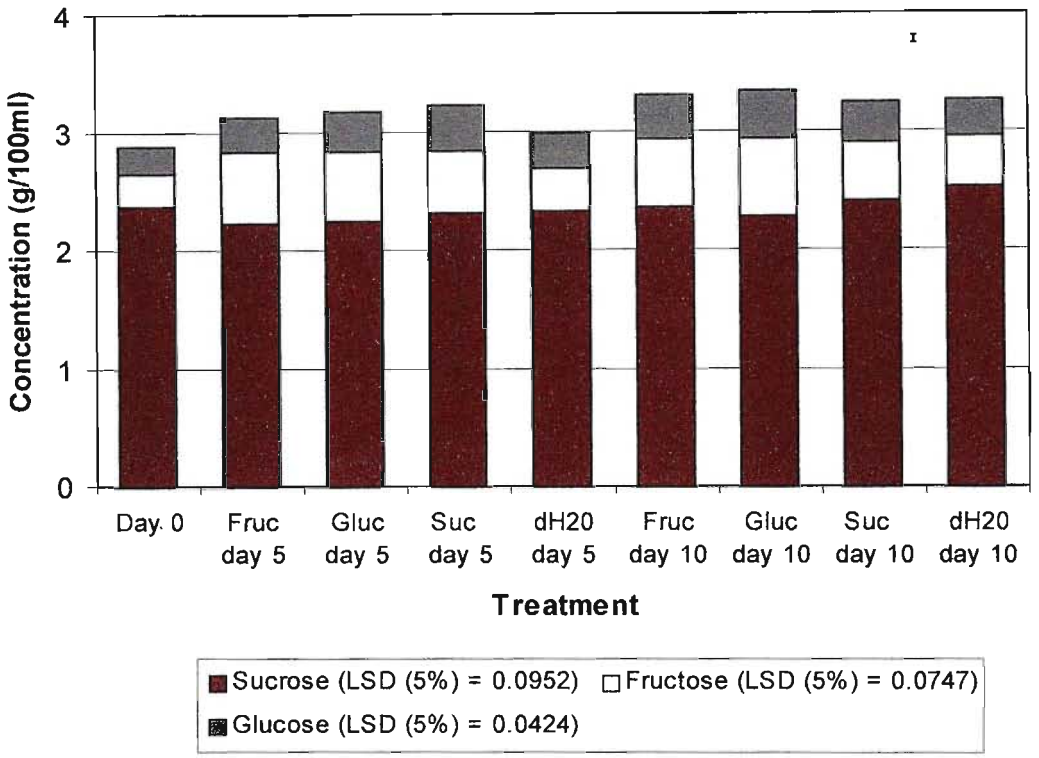


Figure 5.22 Mean overall soluble sugar concentrations in florets of *G. scabridus* spikes as affected by vase life solution held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

5.2.3.2 Storage temperature

Initial study

Low temperature is the most important factor in preserving the freshness of cut flowers (Nowak & Rudnicki, 1990; Nell, 1992). There was a highly significant difference in vase life ($p<0.001$) and spike quality ($p<0.001$) between *G. scabridus* spikes stored at 1, 2 and 3 weeks at 4 °C (Table 5.4). Spikes stored for 1 week, had a mean vase life of 10.3 days. Overall spike and floret quality had a mean rating of 4.2 (Plate 5.6). Spikes looked healthy, floret colour was good and the florets opened normally. Singh *et al.* (2002) also found that gladioli spikes could be wet-stored for 7 days with minimum loss of vase life.

Jiang *et al.* (1989) noted that the quality of *Gladiolus* flowers stored for 2 weeks at 0 °C with a sucrose treatment was as high as that of fresh flowers. *G. scabridus* spikes stored for 2 weeks resulted in visually acceptable florets with a good pink colour (mean rating of 3.5), however, a few of the upper buds failed to open. Vase life was a mean value of 8.0 days. This implies that cold storage of *G. scabridus* spikes at 4 °C for 2 weeks is too long as it results in poorer quality spikes compared to those stored for 1 week.

Anserwadekar and Patil (1986) and Song *et al.* (1992) found that prolonged cold storage resulted in the failure of buds of some *Gladiolus* species to open. A 3 week cold storage period of *G. scabridus* spikes resulted in poor bud opening, with some florets senescing on the spikes before opening. They were given a mean quality rating of 1.8. Floret colour was poor, many becoming dark pink and even purple in colour making them commercially unviable. Mean spike vase life was 4.8 days. The leaves started yellowing soon after cold storage, an indication that the flowers were stored too long (Nell, 1992). Three weeks is thus an unsuitable length of time for the cold storage of *G. scabridus* spikes. This again confirms that 1 week of cold storage at 4 °C is recommended for better post storage *G. scabridus* spike development.

Gladioli display a phenomenon known as gravitropism (Wilfret, 1992) which is a bending response to gravity, that mainly occurs during horizontal storage and transport (Halevy & Mayak, 1981; Philosoph-Hadas *et al.*, 1995). *G. scabridus* spikes that were cold stored at a slight angle in the bucket, began to bend vertically upwards just below the tip of the spike (Plate 5.6), an important factor which will need careful consideration with regards to the postharvest care and quality of these cut spikes.

Plate 5.6 A *G. scabridus* spike after 1 week in cold storage at 4 °C showing good floret development with gravitropical bending of the spike tip

***Table* 5.4 *G. scabridus* spike longevity and floret quality as influenced by length of cold storage at 4 °C**

Storage time (weeks)	Vase life (days) [y]	Floret quality [z]
1	10.3 a	4.2 a
2	8.0 b	3.5 b
3	4.8 c	1.8 c

[y] days from placement in solution after cold storage until the lower 5 florets had senesced and the spike was discarded (LSD (5%) = 1.093)

[z] overall floret quality rating from 1 = poor to 5 = good (LSD (5%) = 0.994)

Letters a to b compare mean values in a column; n = 3 x 2 spikes

Second study

Spike moisture loss

Cut flowers contain considerable amounts of water in their tissues (Nowak & Rudnicki, 1990). The percentage moisture loss of *G. scabridus* spikes stored at 4°C was significantly affected by the interaction between the number of days in cold storage and the presence or absence of the polypropylene covers (p=0.002). Spikes that were stored for 10 days and left unsealed showed the significantly greatest moisture loss of 35.68 % compared to all the treatments (Table 5.5). Spikes kept in the polypropylene covers for both 5 and 10 days lost the least amount of moisture (1.30 and 5.01 % respectively). Singh *et al.* (2003) also found that polypropylene plastic sleeves reduce postharvest moisture loss. Halevy and Mayak (1981) note that the loss of fresh weight of flower tissue is a symptom of senescence and indicates a loss of membrane integrity and consequently increased permeability and leakage of the cells. The results show that the polypropylene covers prevent moisture loss from cut *G. scabridus* spikes thus promoting the postharvest life of these spikes.

***Table* 5.5 Percentage moisture loss of *G. scabridus* spikes as influenced by packaging and length of cold storage at 4 °C**

Treatment	Days in cold storage	
	5	10
Polypropylene bags	1.30 c	5.01 c
Control	21.82 b	35.68 a

[z] LSD (5%) = 4.022

Letters a to b compare mean percentage values; n = 3 x 2 spikes

Vase life

The vase life of *G. scabridus* spikes after cold storage at 4 °C was significantly affected by the number of days in cold storage ($p<0.001$) as well as the presence or absence of the polypropylene covers ($p=0.017$). The interaction of these effects was non-significant ($p=0.310$). Spikes cold stored for 5 days had an average vase life of 10.0 days in solution. This was significantly better than the vase life of 6.0 days achieved with spikes that had been stored for 10 days (Table 5.5). Although cold storage delays the senescence of *G. scabridus* spikes, the shorter the storage period the better the vase life. Thus 5 days would be the recommended cold period for dry storage of these spikes resulting in a better post storage vase life.

Cut *G. scabridus* spikes that were sealed in polypropylene bags had a significantly better vase life of 9.0 days than those that were left unsealed (7.0 days) (Table 5.6). Nowak and Rudnicki (1984), and Rekha and Shankaraiah (2002) also improved the vase life of cut *Gladiolus* spikes by using polyproylene packaging material. Similar results have been achieved with cut roses (Bredmose, 1979), *Nerine bowdenii* (Nijlunsing & Barendse, 1976), and *Nephrolepsis exaltata* fronds (Singh *et al.*, 2003), where the plastic sleeves were shown to reduce moisture loss from the cut spikes thereby maintaining cell integrity and spike health

thus improving subsequent vase life. This modified atmosphere packaging material successfully improves the storage performance of *G. scabridus* spikes.

Pulsing *G. scabridus* spikes prior to storage may aid in extending the post harvest life of these cut spikes even further (Kofranek & Halevy, 1976; Hancke & Verryn, 1989a; Nowak & Rudnicki, 1990; Singh & Sharma, 2003).

Spike quality

The quality of *G. scabridus* spikes after cold storage was significantly affected by the number of days in cold storage ($p=0.009$) as well as the presence or absence of the polypropylene covers ($p<0.001$). The interaction of these main effects was non-significant ($p=0.644$). After 5 days at 4 °C, spikes had maintained their colour and looked very healthy whereas after 10 days of cold storage they became slightly brown in appearance. The florets and leaves of spikes stored for 5 days maintained their health and colour for longer after storage than those stored for 10 days. Cut *G. scabridus* spikes cold stored for 5 days had mean quality rating of 3.7 which was significantly better than spikes stored for 10 days which had a rating of 2.9 (Table 5.6). These results suggest that a cold storage period of 5 days at 4 °C results in the best postharvest spike quality of *G. scabridus* spikes.

Packing is a key factor in preserving flower quality (Kroll, 1995). Sealed *G. scabridus* spikes had a significantly higher quality rating of 4.0 than those that were not sealed (2.7) (Table 5.7) (Plate 5.7 A). As mentioned, the prevention of moisture loss maintains cell integrity and thus improves *G. scabridus* spike quality. Cut gladioli are very sensitive to grey mould during storage (Nowak and Rudnicki, 1990). *G. scabridus* spikes that had been sealed for 10 days did show signs of grey mould development. *G. scabridus* flower spikes secrete droplets of a sticky, nectar-like substance and these droplets enhanced the formation of the mould thus making these spikes commercially undesirable (Plate 5.7 B). Spraying or dipping the spikes in a fungicide solution prior to storage has been shown to reduce this problem (Salunke *et al.*, 1990). The inner anti-mist layer of the polypropylene sleeves would have prevented any further fungal infections due to the prevention of moisture droplets forming on the inside of the covering which could enhance the presence of disease.

***Table* 5.6 *G. scabridus* spike longevity and floret quality as influenced by length of cold storage at 4 °C**

Storage time (days)	Vase life (days) [y]	Floret quality [z]
5	10.0 a	3.7 a
10	6.0 b	2.9 b

[y] days from placement in solution after cold storage till the lower 5 florets had senesced and the spike was discarded (LSD (5%) = 1.520)

[z] overall floret quality rating from 1 = poor to 5 = good (LSD (5%) = 0.565)

Letters a to b compare mean values; n = 3 x 2 spikes

***Table* 5.7 *G. scabridus* spike longevity and floret quality after storage at 4 °C as influenced by packaging**

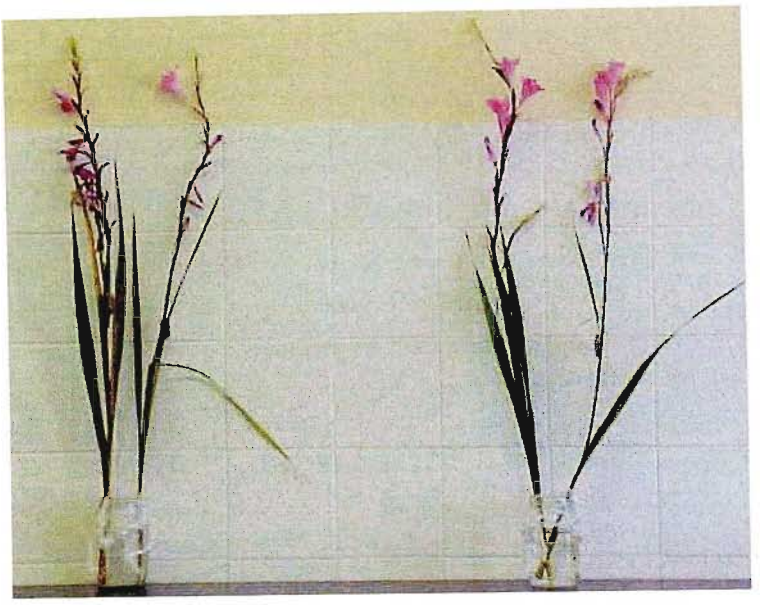
Treatment	Vase life (days) [y]	Floret quality [z]
Polypropylene bags	9.0 a	4.0 a
Control	7.0 b	2.7 b

[y] days from placement in solution after cold storage till the lower 5 florets had senesced and the spike was discarded (LSD (5%) = 1.520)

[z] overall floret quality rating from 1 = poor to 5 = good (LSD (5%) = 0.565)

Letters a to b compare mean values; n = 3 x 2 spikes

A



B

Plate 5.7 ***G. scabridus*** **spikes after cold storage at 4 °C (A) for 10 days without polyproylene (left) and 5 days in polypropylene sleeves (right) and (B) after 10 days with the development of grey mould (a)**

Floret width

G. scabridus floret width was significantly affected by the number of days in cold storage ($p=0.041$). Spikes stored for 5 days at 4 °C opened to an average width of 43.2 mm which was significantly wider than florets on spikes stored for 10 days which had a width of 38.5 mm (Figure 5.23). Significantly wider florets ($p<0.001$) developed on spikes that had been sealed (46.1 mm) whilst those on spikes that were unsealed opened to an average width of 35.7 mm (Figure 5.24). There was no significant interaction between days in storage and the sealing of the spikes ($p=0.112$).

Floret length

G. scabridus floret length was significantly greater ($p=0.023$) on spikes stored for 5 days (41.6 mm) than on those stored for 10 days (36.2 mm) (Figure 5.23). Sealed spikes resulted in larger florets of 44.0 mm in length whilst unsealed spikes produced florets with an average length of 33.7 mm (Figure 5.24). There was no significant interaction between days in storage and the sealing of the spikes.

Thus cold storage at 4 °C for 5 days results in better *G. scabridus* floret size which can also be improved if used in conjunction with polypropylene covers.

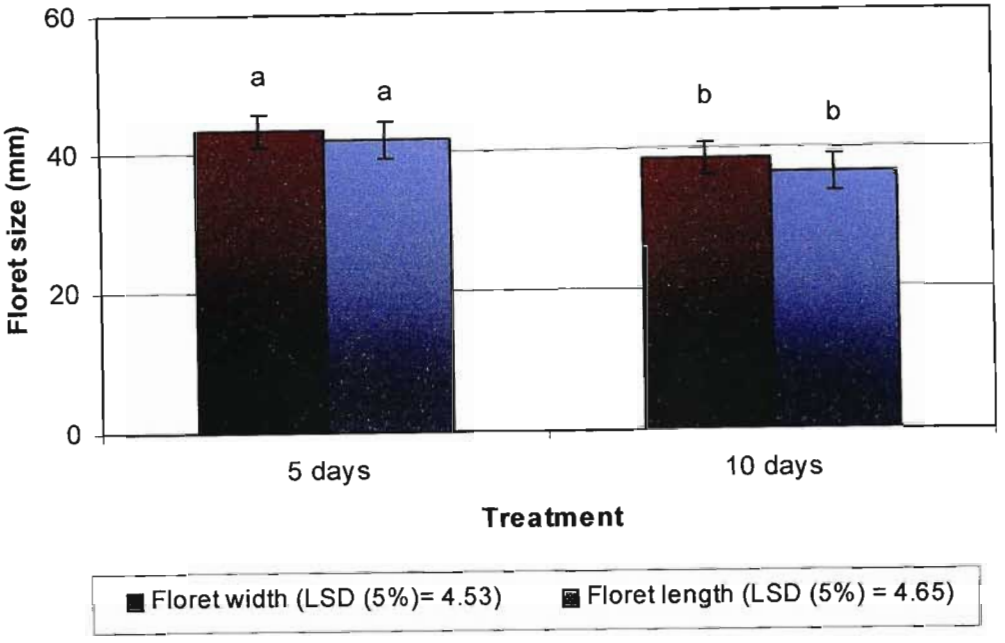


Figure 5.23 Mean *G. scabridus* floret size as affected by 2 % sucrose at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1} after cold storage for 5 or 10 days at 4 °C. (Different letters indicate significant differences between treatments according to the LSD value.)

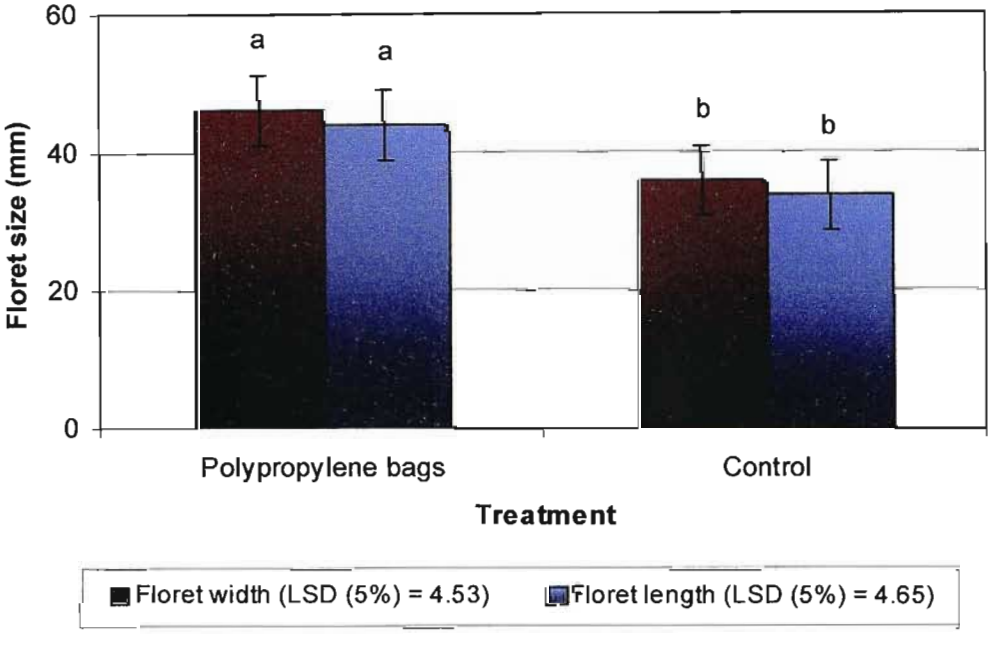


Figure 5.24 Mean *G. scabridus* floret size as affected by 2 % sucrose at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1} after cold storage at 4 °C with or without polypropylene bags. (Different letters indicate significant differences between treatments according to the LSD value.)

Floral spike mass

After cold storage, overall floral spike mass in 2 % sucrose increased significantly ($p<0.001$) over the first 3 days from 16.16 to 18.22 g, and then decreased over time to a final mean mass of 11.68 g which is consistent with the results for solution uptake. There was no significant interactions any of the treatments and the length of time in the vase life solution. However, there was a highly significant interaction between the number of days in cold storage and the presence or absence of the polypropylene covers ($p<0.001$) on *G. scabridus* spike mass (Table 5.8). Spikes stored for both 5 and 10 days showed a higher spike mass when using polypropylene covers. Both sealed and unsealed spikes show a significantly higher spike mass when stored for 5 days. Thus cold storage at 4 °C for 5 days in polypropylene sleeves is the better treatment for maintaining *G. scabridus* floral spike mass in solution.

***Table* 5.8 *G. scabridus* spike mass (g) as influenced by packaging and length of cold storage at 4 °C**

Treatment	Days in cold storage [z]	
	5	10
Polypropylene bags	24.73 a	12.60 b
Control	13.01 b	10.29 c

[z] LSD (5%) = 1.039

Letters a to b compare mean percentage values; n = 3 x 2 spikes

Vase life solution uptake

The uptake of 2 % sucrose by *G. scabridus* spikes was significantly affected by an interaction between the number of days in cold storage and the duration of the vase life trial ($p<0.001$) as well as between the number of days in cold storage and the presence or absence of the polypropylene covers ($p<0.001$) . Solution uptake followed the same pattern as in the other vase life trials with a highly significant ($p<0.001$) and rapid initial overall uptake of solution (37.42 ml) followed by a gradual decrease to 16.92 ml (Figure 5.25). Spikes that had been stored for 5 days initially absorbed a significantly greater amount of solution than those at 10 days.

Sealed spikes stored for 5 days at 4 °C, showed the highest mean solution uptake of 30.33 ml. This was significantly higher than unsealed spikes left in cold storage for 5 days (25.38 ml) (Table 5.9). Thus, storage in polypropylene sleeves promotes post storage solution uptake. However, both sealed and unsealed spikes showed the significantly lowest uptake of solution after 10 days in cold storage (16.92 and 16.75 ml respectively) thus the shorter storage time of 5 days is recommended.

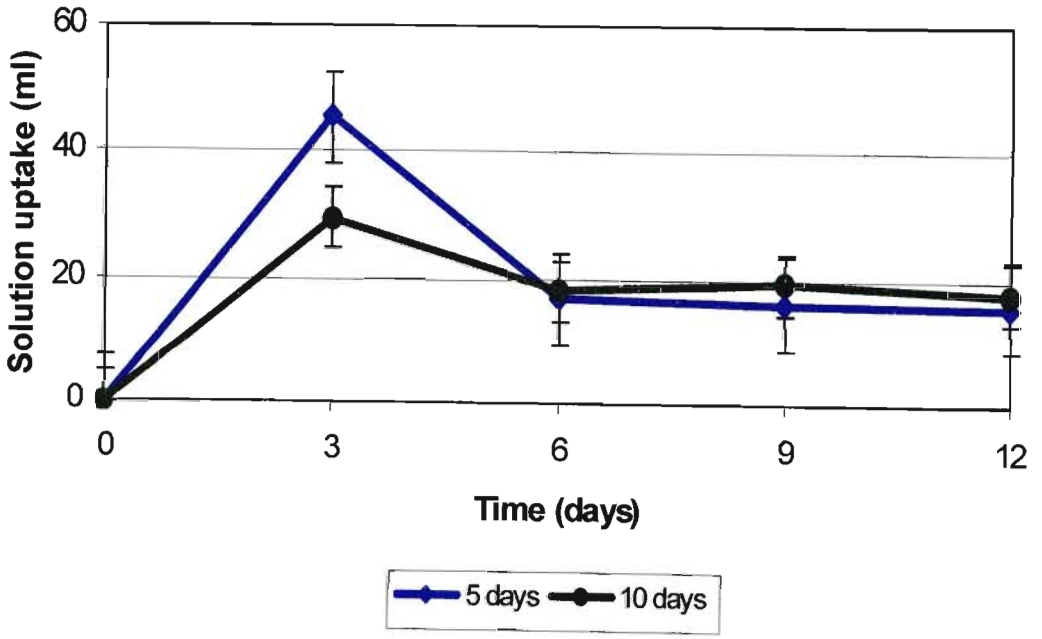


Figure 5.25 Mean change in solution uptake of 2 % sucrose after cold storage at 4 °C by *G. scabridus* spikes held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

***Table* 5.9 Solution uptake (ml) of *G. scabridus* spikes as influenced by packaging and length of cold storage at 4 °C**

Treatment	Days in cold storage [z]	
	5	10
Polypropylene bags	30.33 a	16.92 c
Control	25.38 b	16.75 c

[z] LSD (5%) = 4.840

Letters a to b compare mean percentage values; n = 3 x 2 spikes

Chlorophyll fluorescence

Sealed *G. scabridus* spikes showed a significantly higher ($p<0.001$) mean fluorescence Fv/Fm ratio of 0.575 compared to unsealed spikes of 0.364 (Table 5.10) thus showing a better internal quality. The number of days in cold storage also affected these readings ($p=0.009$). Spikes stored for 5 days gave an average ratio of 0.507 which was significantly better than those stored for 10 days (0.451) (Table 5.10). Overall, spikes gave an initial mean average ratio of 0.756 which significantly decreased ($p<0.001$) over time to 0.110 at spike senescence. The first microscopic changes in leaf senescence occur in the chloroplasts where they lose their characteristic starch deposits and the mitochondria become distorted (Thimann, 1980) thus resulting in lower fluorescence values. A plant with a good internal quality (a good performing photosynthetic system), is able to produce sufficient energy for uniform and long lasting flowering (Lootens & Heursel, 2001). Therefore a shorter storage period of 5 days and using the polypropylene sleeves for cut *G. scabridus* spikes helps maintain mitochondrial integrity, thereby reducing senescence and resulting in healthier spikes for a longer period after storage. This confirms previous results that these conditions are the most suitable for *G. scabridus* cold storage.

In this situation the use of chlorophyll fluorescence is a useful tool to determine which treatment resulted in the most significant stress to the cut spikes.

***Table* 5.10 Mean Fv/Fm ratios of *G. scabridus* spikes after storage at 4 °C as influenced by packaging and length of cold storage**

Treatment	Fv/Fm ratio [z]
Polypropylene bags	0.575 a
Control	0.364 b
5 days in cold storage	0.507 a
10 days in cold storage	0.431 b

[z] LSD (5%) = 0.0564
Letters a to b compare mean compare mean values in a column; n = 3 x 2 spikes

5.2.3.3 Market research study

Within the South African horticultural industry there appears to be a growing awareness of indigenous plants and their potential as both as cut flowers and ornamental garden subjects.

Question 1 - Do you sell / buy many indigenous wild flowers?

The highest percentage (40 %) of retail outlets responded with a 3 (sometimes) to this question (Figure 5.26). This indicates that the selling of indigenous plants, and in particular wild flowers, is not a high priority. Each nursery differs in this approach, some selling mostly indigenous plants whereas others were more balanced selling both indigenous and exotic. However, indigenous plants are becoming more popular. It was stated that 'indigenous flowers only really sell well when in flower'. The colour and appeal of a flowering plant tends to create a demand for these plants. Indigenous bedding plants are more popular during drought conditions. Currently some of the more popular indigenous genera include *Scadoxus*, *Eucomis*, *Dietes* and *Watsonia*. Ball Straathof© seed company has recently introduced the 'Kirstenbosch Seed Collection' which come as impressively packaged indigenous seeds of wild flowering plants. These include nemesias, gazanias and Felicia daisies and are proving to be very popular.

Eighty percent of the florists indicated that consumers often require specific cut flowers and the demand is mainly for exotics, purely from a lack of knowledge and awareness of the indigenous potential. However, when in season, indigenous cut flowers make lovely additions to country bunches and if striking enough, can be sold in bunches on their own but cater more for the 'impulse buy' market in this regard. All the florists felt that the indigenous market is definitely one worth expanding on.

The highest consumer rating of 27.6 % was given to a 2 (seldom). About 86 % of the consumers tend towards buying both indigenous and exotics instead of one or the other. More than 60 % said they preferred indigenous flowers because they attracted birds, bees, chameleons and insects to their garden. Cut flowers for at least half the consumers appear to be a luxury item and thus only bought on special occasions. About 42 % said that when they did buy cut flowers, there was no thought as to whether they were indigenous or not, as long as the flowers were 'healthy', 'bright' and 'pretty'. The cost is a very important consideration for consumers.

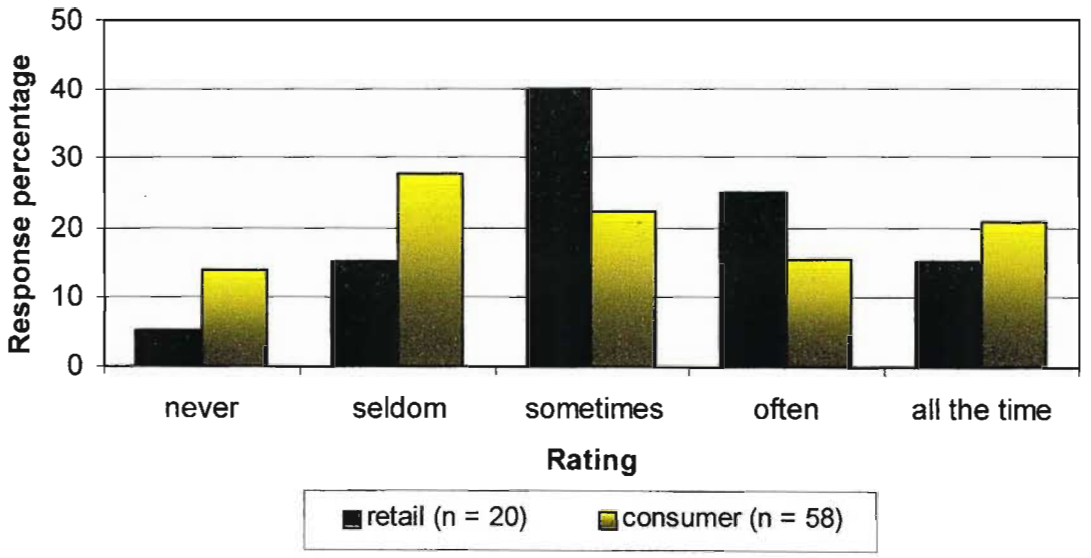


Figure 5.26 Retail and consumer response to Question 1 of the *G. scabridus* market research questionnaire: do you sell / buy many indigenous wild flowers?

Question 2 - Do you sell / buy many hybrid gladioli (when in season)?

The greatest number of retail outlets (30 %) responded with a 2 (seldom) to this question (Figure 5.27). The main supplier of gladioli bulbs to the retail nurseries and garden centres in South Africa is Hadeco©. A big concern for at least 90 % of these outlets is that although gladioli bulbs do sell when available in Spring, they are overpriced which greatly affects the sale thereof. The feeling is that more could be sold if the price was reduced. Gladioli bulbs are not as popular as the exotic winter bulbs such as daffodils and tulips. Forty percent of the outlets were coastal in position. These outlets mentioned that the hybrid gladioli do not do as well in the coastal regions which affects the sale thereof. Many of the florists indicated that gladioli are popular due to the wide variety of colours that are available, however, their cost hinders higher sales.

The highest customer response was 34.5 % for customers that said they never (1) purchase gladioli because they are too expensive.

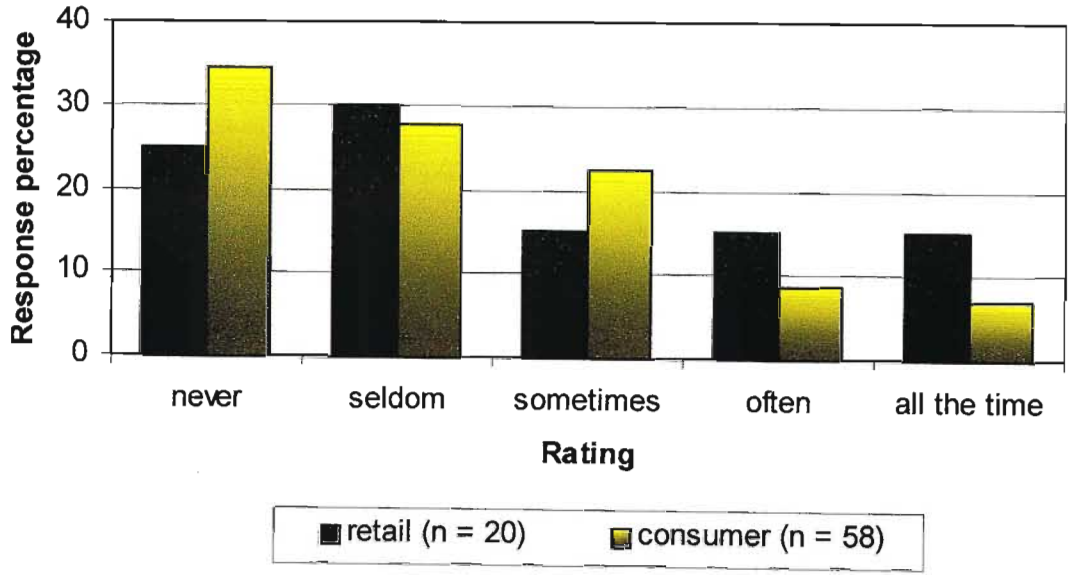


Figure 5.27 Retail and consumer response to Question 2 of the *G. scabridus* market research questionnaire: Do you sell / buy many hybrid gladioli (when in season)?

Question 3 - What are your initial Impressions of G. scabridus?

There was a very positive response to *G. scabridus* from both retailers and customers alike. Nursery and florist responses only fell into 2 categories: 20 % felt it was quite impressive (4) whilst the other 80 % thought it was very impressive (5) (Figure 5.28). Comments included 'lovely', 'magnificent', 'unusual' and 'stunning'. They thought it will be well received by indigenous enthusiasts and although different from the usual hybrids, will sell well when in full flower and if long-lasting. There were a few queries as to its vase life as this seems to be a big concern. The florist 'Flowers on Victoria' took it upon themselves to put some spikes in wet oasis to observe the vase life and were very surprised and impressed with the results.

Customer response variates showed ratings from impressive (3) to very impressive (5). Comments included 'pretty', 'beautiful', 'delicate', 'stunning', 'delightful' and 'attractive especially when viewed close up'. About 85 % felt it would look very attractive in flower arrangements and bouquets. Others said they would love to plant them in their garden and they would be stunning *en masse* in such a setting.

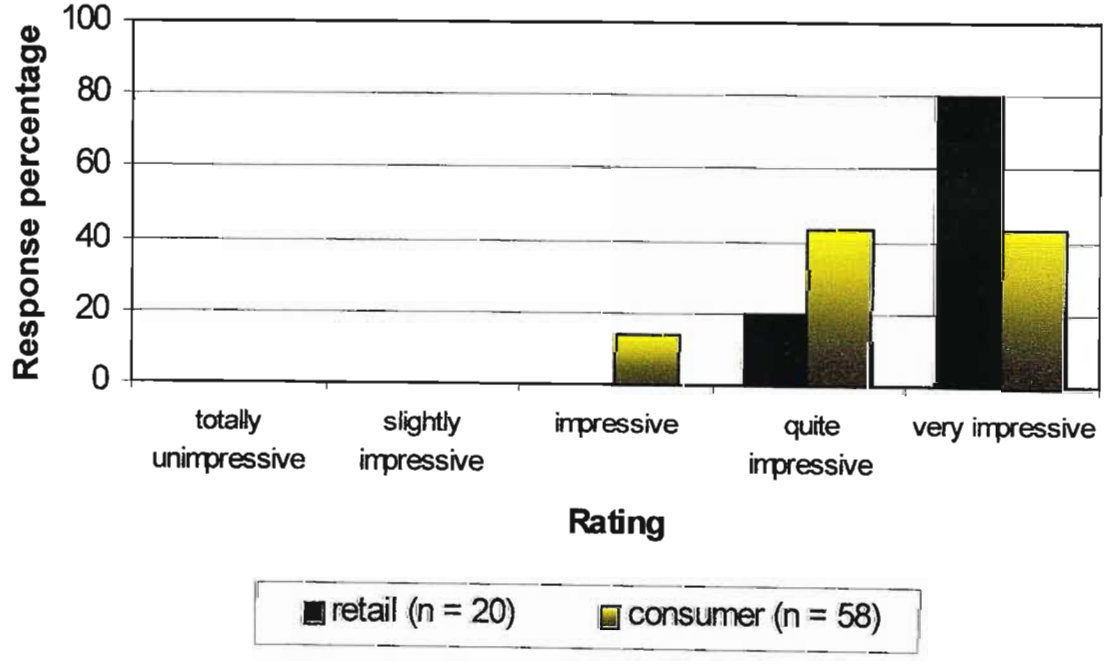


Figure 5.28 Retail and consumer response to Question 3 of the *G. scabridus* market research questionnaire: what are your initial impressions of *G. scabridus*?

Question 4 - What characteristics of the species would help promote the sale thereof?

There was a great deal of overlap with comments in this regards. It was said that it would not be difficult to sell this species due to the 'beautiful colour', 'very attractive markings' and 'impressive petal design' and the long, graceful stems which would make it ideal for arrangements. The nurseries said that non-flowering younger plants would be easier to sell if accompanied with spikes in flower or photos thereof.

Question 5 - Any negative comments?

There were no negative comments.

Question 6 - Is there is a market for this species to be sold as:

(i) a cut flower?

The majority of nurserymen (65 %) and consumers (65.5 %) felt that the species has excellent potential (5) to be grown as a cut flower (Figure 5.29).

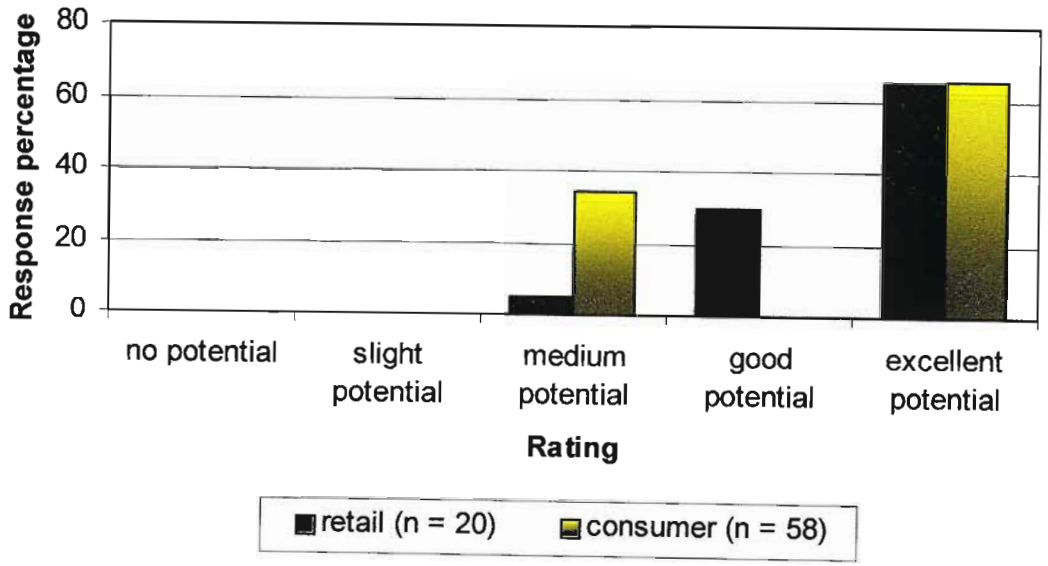


Figure 5.29 Retail and consumer response to Question 6a of the *G. scabridus* market research questionnaire: is there is a market for this species to be sold as a cut flower?

(ii) a flowering staked plant in a bag?

Yet again the majority of nurserymen (80 %) and consumers (65.5 %) felt that the species has excellent potential (5) to be sold in this way (Figure 5.30).

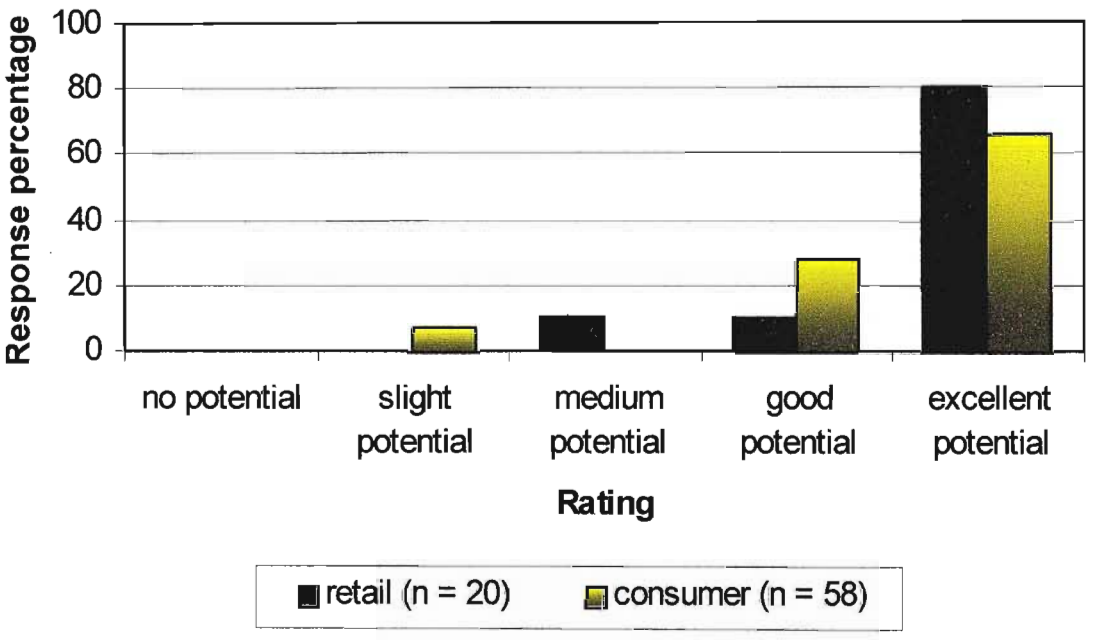


Figure 5.30 Retail and consumer response to Question 6b of the *G. scabridus* market research questionnaire: is there is a market for this species to be sold as a flowering staked plant in a bag?

(iii) seed?

There were a variety of responses with regards to the potential for *G. scabridus* to be sold as seed (Figure 5.31). The highest rating for retailers was 30 % for medium potential (3) whereas that for consumers was 50 % for excellent potential (5). Perhaps the retailers were more hesitant because they feel that it would sell better when in full flower, providing instant colour for the garden and vase! The consumers seemed more willing to consider this as a viable option. *G. scabridus* seeds would have to be marketed and packaged correctly if they are to be sold in this way.

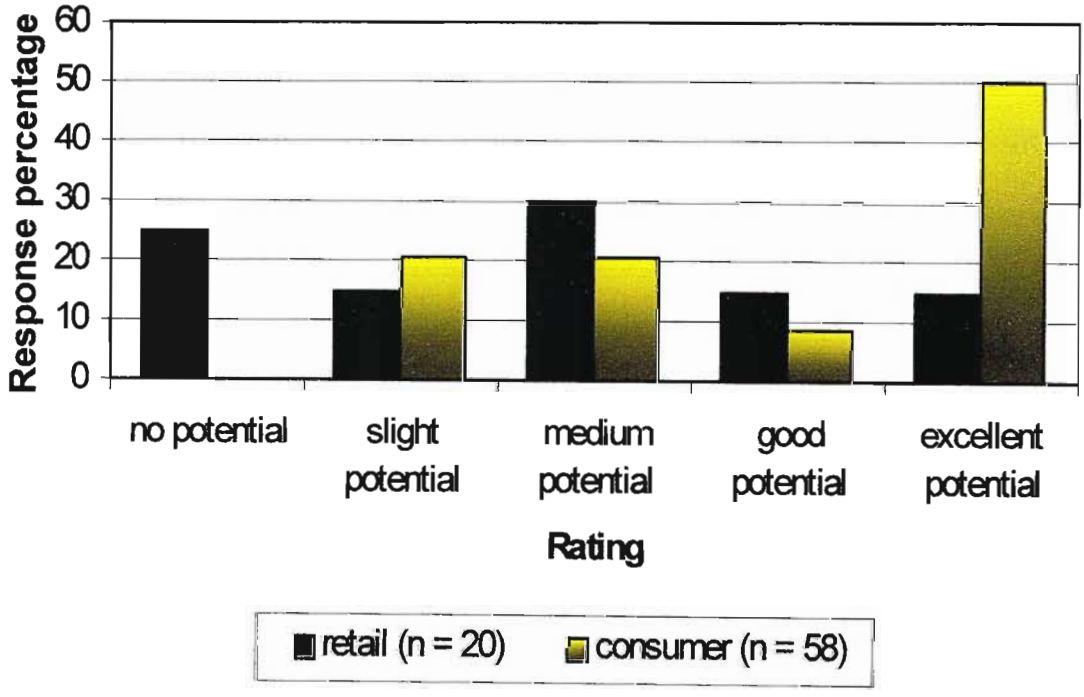


Figure 5.31 Retail and consumer response to Question 6c of the *G. scabridus* market research questionnaire: is there is a market for this species to be sold as seed?

Question 7 - Any other comments?

It was generally stated that an important consideration when selling this product will be the price. This will greatly affect the demand and supply. Another comment was with regards to its resistance to the common *Gladiolus* pest thrips and how this would affect marketability of the species. Most nurseries wanted to know when the species would become available as they will definitely want to sell it.

5.3 ETHYLENE AND CARBON DIOXIDE EVOLUTION

5.3.1 Introduction

Ethylene is a naturally-occurring plant growth regulator and colourless gas produced in small quantities by floral crops as they age (Mayers *et al.*, 1997). Although an activator for the normal physiological processes of ageing, it shortens the longevity of many flowers and plants by altering the metabolic and hormonal function of flower cells and permeability of cell walls, leading to premature flower death, petal fading and wilting, leaf, bud and flower drop and other undesirable characteristics (Salunke *et al.*, 1990; Nell, 1994).

The role of ethylene in the senescence of *Gladiolus* petals and the life of the cut spikes is, however, unclear with conflicting reports in the literature. Rudnick *et al.* (1986) found that ethylene was involved in *Gladiolus* flower senescence and Serek *et al.* (1994) notes that this is variety dependant. However, Nell (1994) found that gladioli were not sensitive to ethylene found in the air surrounding the florets and suggests that gladioli are ethylene-insensitive geophytes.

An awareness of the harmful effects of ethylene has increased in the last few years. Ethylene damage occurs as a result of the production by the floriculture crop in question or exposure to other ethylene producing products. Therefore, it is important to determine whether a crop produces ethylene during its postharvest phase to allow for suitable management of this process.

The development and opening of cut flowers is an active growth process (Bravdo *et al.*, 1974). The respiration of a flower is an index of the rate at which the flower consumes its reserves (mainly starch and sugar) and other metabolites. The high respiration rate of cut flowers is indicative of their rate of growth and senescence (Reid, 1992). Carbon dioxide measurements will thus be indicative of the rate of senescence of a crop and aid the grower in the postharvest management thereof. Delaying respiratory peaks has been reported to increase vase life of cut flowers (Coorts, 1973; Salunke *et al.*, 1990).

With the knowledge that ethylene and carbon dioxide can play significant roles in the postharvest life of cut flowers, an attempt was made to determine the presence of these gases as produced by cut *G. scabridus* spikes.

5.3.2 Materials and Methods

5.3.2.1 Ethylene production

Individual florets were excised from *G. scabridus* spikes at a stage where they were just starting to open and they were placed into 20 ml glass vials containing 18 ml of one of the following solutions: distilled water (control), Prolong®, Chrysal®, 2 % (w/v) sucrose ($C_{12}H_{22}O_{11}$) and 2 % (v/v) ethanol, 1 % (w/v) sucrose, 2 % (w/v) sucrose, 4 % (w/v) sucrose, 1 % (w/v) sucrose and 0.5 (v/v) % JIK® (12 % (w/v) NaOCl), 2 % (w/v) sucrose and 0.5 % JIK® (v/v) and 4 % (v/v) sucrose and 0.5 % (v/v) JIK® at the recommended rates. There were 4 replications per treatment. The flowers were allowed to open at a constant room temperature of 22 °C and illumination of 17.9 µmol m^{-2} s^{-1}.

Ethylene production of each floret was measured on a daily basis until senescence. Each floret, in its glass vial, was placed inside a 1 litre cylindrical glass jar which was sealed with a glass lid and held for 1 hour at 22 °C (Plate 5.8 A). A Varian Star 3400 Gas Chromatograph (GC) was used, fitted with a non-polar 100 % Dimethyl polysiloxane capillary column (SGE) set at 60 °C and a flame ionising detector set at 150 °C. The temperature of the injector was 120 °C. Each day, 100 µl of pure ethylene gas (Afrox) was injected into the GC to confirm as calibration. A 100 µl aliqout of the air in the jar was then withdrawn and injected into the GC using a microsyringe to determine the ethylene production of *G. scabridus* florets.

5.3.2.2 Carbon dioxide evolution

Florets used for ethylene analysis were also used for the measurement of carbon dioxide (CO_2) evolution, as an indicator of respiratory activity. This was measured on a daily basis over a period of 6 days with a PP systems environmental gas monitor at a room temperature of 22 °C. Each floret, in its vial, was placed in a 900 ml cylindrical glass jar of which the initial CO_2 concentration (ppm) had been determined. The jar was sealed for 20 minutes after which the new head space CO_2 concentration (ppm) was determined (Plate 5.8 B). Carbon dioxide evolution was calculated as µl l^{-1} g^{-1} hr^{-1} and the results were analysed statistically as a randomised block design using Genstat (5th Edition, 4.21 Release, Lawes Agricultural Trust, Rothamsted Experimental Station).

A

B

Plate 5.8 ***G. scabridus*** **florets (A) in a sealed glass jar from which 100 µl of air was drawn for ethylene analysis in the GC and (B) in sealed glass jars for CO_2 evolution measurement.**

5.3.3 Results and Discussion

5.3.3.1 Ethylene production

Hwang *et al.* (1995) suggest that ethylene is involved in the senescence of *Gladiolus* flowers and Serek *et al.* (1995) note that the ethylene production of florets detached from the spikes of *Gladiolus* flowers varies between cultivars. However, ethylene may be difficult to detect and can be active at very low concentrations (Mayers *et al.*, 1997).

Standard ethylene gas injections indicated that the retention time of ethylene was close to 5.15 minutes (Figure 5.32). None of the *G. scabridus* florets in any of the treatments or at any stage of floral development produced ethylene at a measurable concentration (Figure 5.33). Murali and Reddy (1993) and Serek *et al.* (1994) report that some *Gladiolus* cultivars do exhibit a climacteric rise in ethylene production before the onset of petal wilting which declines substantially with petal wilting. However, studies on *Gladiolus* petal senescence by Bravdo *et al.* (1974) indicated a gradual progression in senescence without the accelerated wilting associated with climacteric ethylene production. The results obtained suggest that *G. scabridus* is similar, or that ethylene evolution is extremely low.

5.3.3.2 Carbon dioxide evolution

There was no significant interaction of the CO_2 evolution values between florets in the different floral preservatives or the time over which the experiment was conducted ($p=0.840$). However, the main effects of treatment had a significant effect on CO_2 evolution ($p=0.030$) and that of time was highly significant ($p<0.001$).

Florets in distilled water, Chrysal® and Prolong® resulted in the highest evolution of CO_2 and these were significantly different from florets in 1 % sucrose and JIK®, 1 % sucrose and 4 % sucrose which produced the lowest levels of CO_2 (Figure 5.34).

CO_2 evolution was significantly higher as the florets started to open (186.4 $\mu l\ l^{-1}\ g^{-1}\ hr^{-1}$), and then decreased substantially from day 3 and remained fairly constant at around 85 $\mu l\ l^{-1}\ g^{-1}\ hr^{-1}$ as the florets reached their maximum size and started to senesce (Figure 5.35). This is similar to work by Serek *et al.* (1994; 1995) who found that the respiration of *Gladiolus* florets

tended to be low in tight buds and in senescent florets with a broad 'climacteric-like' peak during opening and development. However, in *Gladiolus* cv. Captain Busch, the pattern was found to be non-climacteric. Burzo *et al.* (1989) found that the CO_2 evolution of *Gladiolus* florets decreased after 3 days postharvest with the respiration intensity of open florets lower than that of the buds. This evolution pattern of CO_2 from *G. scabridus* florets needs to be considered with regard to postharvest storage of the spikes. As CO_2 evolution is normally reduced at lower temperatures (Ferreira, 1981), a managed cold chain is clearly required for postharvest storage of *G. scabridus*.

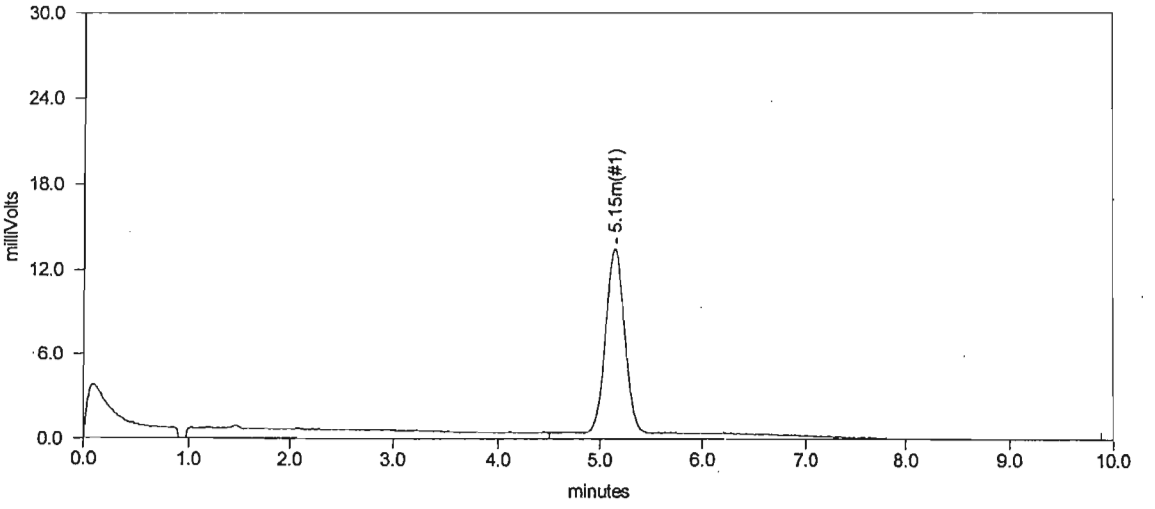


Figure 5.32 Resulting chromatograph after injecting 100 µl ethylene gas into the GC

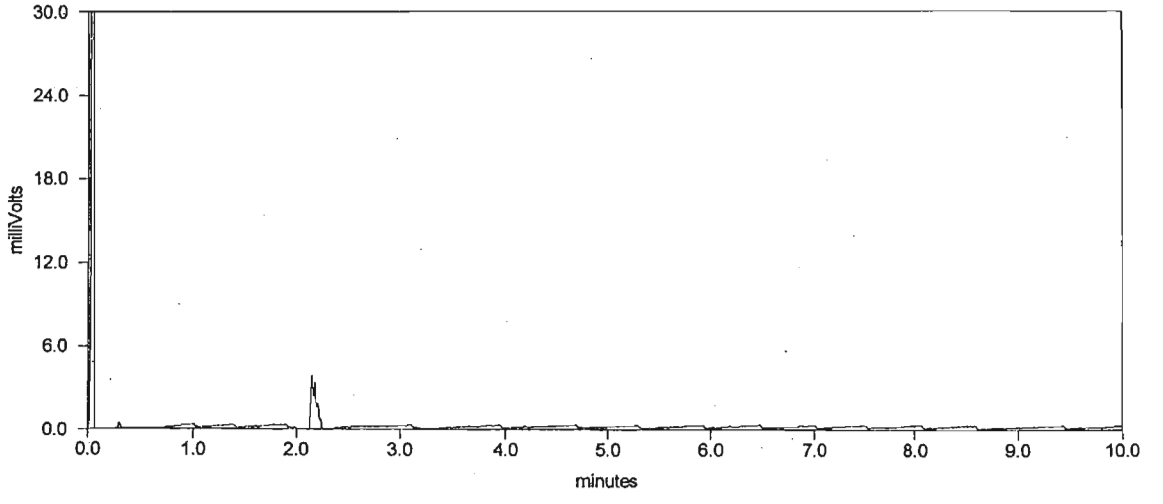


Figure 5.33 Resulting chromatograph after injection of 100 µl of air from inside a sealed glass jar in which a *G. scabridus* floret had been held for 1 hour

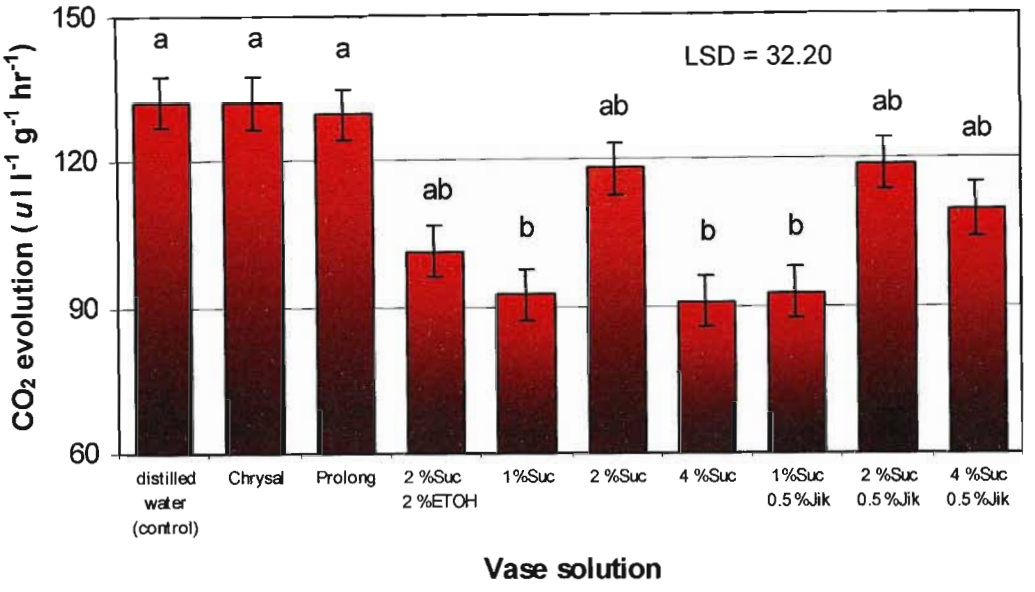


Figure 5.34 The mean CO_2 evolution of *G. scabridus* spikes as affected by floral preservatives held at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}. (Different letters indicate significant differences between treatments according to the LSD value.)

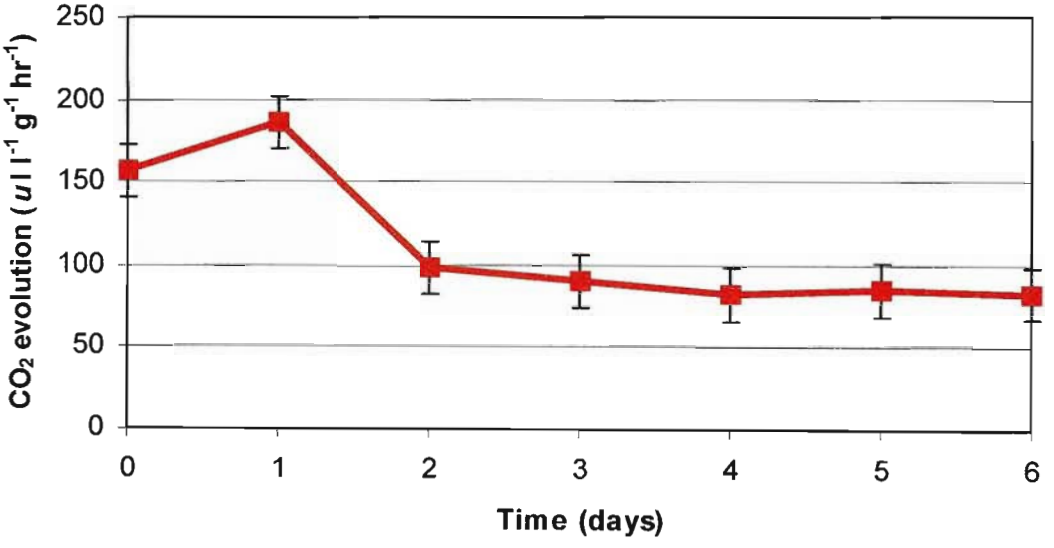


Figure 5.35 Mean *G. scabridus* floret CO_2 evolution over 6 days whilst holding in floral preservatives at 22 °C and a constant illumination of 17.9 µmol m^{-2} s^{-1}

5.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SUGAR ANALYSIS

5.4.1 Introduction

In flowering plants, nectar is often secreted away from the flower itself and is then called extrafloral nectar, the function of which is still unclear, although nectar in the broadest sense is not always associated with the flower and the process of pollination. The composition of nectar varies widely among different species but it is basically made up of about 40 % sugar-water, with other organic substances present in relatively small amounts (Real, 1983; Barth, 1985; Percival, 1965).

It was observed that *G. scabridus* flower spikes secrete droplets of a sticky, viscous, nectar-like substance at various places along the spike from the moment it appears in the tight bud stage, until the onset of floret senescence (Plate 5.9). These secretions occur at various times throughout the day and night. The secretion was sweet to the taste and it was noted that there were often insects, ants in particular, feeding off it. Ants are social insects and great lovers of nectar and regularly collect it from flowers (Proctor *et al.*, 1996) which is a strong indication that this secretion could be a form of extrafloral nectar.

Any organ of the flower may be modified into a nectary or bear nectariferous tissue (Percival, 1965). Extra-floral nectaries occur on the stipules at the base of the leaves of some vetches (*Vicia* spp) and near the base of the leaf blades in cherry laurel (*Prunus laurocerasus*) (Proctor *et al.*, 1996). Some irises and orchids, which have inferior ovaries, produce large beads of nectar on the outside of the flower although nectar is also secreted at the top of the ovary (Percival, 1965). However, the literature does not make mention of such a phenomenon in other *Gladiolus* species. It was therefore decided to investigate the sugar composition of this secretion through high performance liquid chromatography (HPLC) analysis to validate the assumption that it is extrafloral nectar.

5.4.2 Materials and Methods

Fresh exudate samples were randomly collected from various positions on *G. scabridus* floral spikes grown in a glasshouse at 24 °C (day) and 18 °C (night). These were diluted with ultrapure water and stored at -18 °C in 5 ml Eppendorf vials until analysis.

Ten *G. scabridus* samples, replicated 3 times, were filtered through millipore filters (0.45 µm) and 100 µL aliquots of each filtered sample was injected into an HPLC system (Model ERC-7515A, ERC Inc., Tokyo, Japan) and allowed to run for 45 minutes using the following specifications: column: Rezex 8 u 8 % Ca Monos packed column (Phenomex); mobile phase: water (filtered ultrapure); flow rate: 0.3 ml min^{-1}; temperature: 75 °C; detector: refractive index at 40 °C. Sugar quantification was achieved by comparing sample peaks at the retention times of previously prepared standards of sucrose, glucose, fructose, ribose, galactose, xylose, mannitol and sorbitol at various concentrations.

5.4.3 Results and Discussion

HPLC analysis indicated that the only recognisable carbohydrates present in the samples were sucrose, fructose and glucose, the most common sugars found in nectar (Baker & Baker, 1983; Percival, 1965; Proctor *et al.*, 1996). Sucrose is a disaccharide which can be broken down into the hexose sugars glucose and fructose in the presence of the enzyme invertase (Raven *et al.*, 1992; Proctor *et al.*, 1996). Thus the presence of glucose and fructose in the samples indicates that some sucrose had been broken down into its component parts.

There are 4 classes of nectar: hexose-dominant, hexose-rich, sucrose-rich and sucrose-dominant (Baker & Baker, 1983). Goldblatt and Manning (1999), Goldblatt *et al.* (2001) and Manning *et al.* (2002), note that gladioli produce ample quantities of sucrose-rich to sucrose-dominant nectar of moderate sugar concentration from their septal nectaries. The *G. scabridus* samples showed a greater concentration of sucrose (21.752 µg) than glucose (1.600 µg) or fructose (2.851 µg) (Table 5.11) indicating that this is a sucrose-rich or sucrose-dominant secretion. Nectar is derived from the phloem solution (de la Barrera & Nobel, 2004). It has already been shown that sucrose is the main soluble sugar in *G. scabridus* spikes which could account for the high concentration of sugar in these secretions. It is

therefore assumed that due to the high sugar content of the analysed samples, that this secretion is a form of extrafloral nectar. It is unclear as to whether the species also secretes nectar at or in any other part of the *G. scabridus* floret. Goldblatt & Manning (1999) indicate that gladioli generally produce nectar at the base of the perianth tube, at the top of the ovary.

Extra-floral nectaries may play an important role in insect-plant relationships although this is not always to the advantage of the plant. In *Paeonia*, the sepals secrete nectar which ants exploit even before the nectarless flower opens, therefore reducing the chance of pollination (Percival, 1965). However, in many tropical plants, nectar is specially provided outside the flowers to attract ants. The ants, being powerfully equipped for biting and stinging, protect the plant from various kinds of attack, including nectar-robbery by corolla piercing insects (Proctor *et al.*, 1996). In *Thunbergia grandiflora*, the calyx is a ring of nectar secreting tissue, and 'ant-guards' are usually present on the calyx thus detering Xylocopa bees from robbing the flower by piercing the base of the corolla tube (Percival, 1965).

The pollination strategies of *Gladiolus* are unusually diverse and involve various bee species, foraging either for nectar or pollen, or moths, large butterflies, beetles, passerine birds and long-proboscid flies foraging for nectar (Goldblatt & Manning, 1999; Goldblatt *et al.*, 2001; Goldblatt & Manning, 2002). However, gladioli with slender, elongate perianth tubes, white to cream or pink perianths, usually marked with pink or red nectar guides on the lower tepals, that lack floral odour, and produce sucrose-rich nectar, are usually pollinated by one or two species of long-proboscid flies of the families Tabanidae or Nemestrinidae (Goldblatt & Manning, 1999; Goldblatt *et al.*, 2001). Goldblatt & Manning (1999) confirmed that *G. scabridus* falls into this pollinator category.

The extra floral nectar on *G. scabridus* could be a method of attracting these long-proboscid flies to the florets. The flowers lack odour so they are not attracted by this means. However, the nectar or honey guides on the lower tepals highlight the form and architecture of the floret as the visitor approaches thus attracting it to the floret and drawing it to the nectar inside. Thus the provision of additional nectaries away from the floret, possibly acts as a decoy to keep the ants away from nectar in the floret and from attacking the flies, or protecting the florets from unwanted, foraging insects by means of the ant-guard (Percival, 1965).

***Table* 5.11 Mean concentration of carbohydrates in extra floral nectar samples of *G. scabridus* as a result of HPLC analysis**

Carbohydrate	Mean concentration (µg)
Sucrose	21.752
Glucose	1.600
Fructose	2.851

A

B

C

Plate 5.9 Extra floral nectar secretions from a *G. scabridus* spike (A) as it emerges from the leaves, (B) as it matures and (C) with fully open florets

5.5 CONCLUSIONS

Extending the postharvest life of freshly cut flowers is the key to increasing floral sales for the growth and development of the floricultural industry. It is important that this type of research be continuously explored especially with the introduction of new species. The better the quality and the longer the life of the floral spikes of these new species, the greater will be the demand for them and the more successful their introduction to the cut flower industry. *G. scabridus* has shown to have potential in this area.

Of all the floral preservatives tested, 2 % sucrose resulted in the longest vase life and improved the size and quality of the florets the best, therefore it is recommended that this solution be used for the extension of the postharvest life of *G. scabridus* cut spikes. It has been shown that carbohydrates plays a vital role in the postharvest life of these spikes: during the development of the infloresence it appears that there is a transfer of carbohydrates from the senescing lower florets to those developing acropetally. Although the other important soluble sugars had beneficial postharvest effects, sucrose has been found to be the main soluble sugar within the spikes and thus the focus should remain on this carbohydrate.

Solutions with JIK® and ethanol in combination with sucrose should be avoided as this produced poor postharvest results. Use of commercially available solutions should be used with caution. Solutions that are freely available for use in the commercial sector, may not necessarily be advantageous for wild species.

Temperature appears to play an important role in the longevity of *G. scabridus* cut flower spikes. The most suitable storage period for wet spikes is a week and 5 days for spikes stored dry. The storage of these flowers thus makes it possible to adjust to market demands as they occur. Cold storage and the use of polypropylene sleeves has been shown to delay senescence of the spikes which will aid in postharvest storage and transport over long distances. Used in conjunction with 2 % sucrose after storage, proved beneficial in this regard. Further studies could test the effect of pulsing the spikes in combination with these results to determine whether their postharvest life could be improved even further.

The florets do not appear to produce ethylene which will mean that the storage of this product with ethylene sensitive crops will not affect them in any negative way. The use of

polypropylene covers during cold storage is thus also a viable option as there will not be a build up of ethylene within the sleeves to enhance senescence. The lack of ethylene is, however, accompanied by an apparent climacteric production of CO_2 which decreases as senescence progresses. This process is important in the postharvest life of the florets, and confirms the need for a managed postharvest cold chain.

The response of both the local horticultural industry and consumers alike has been very positive and has shown that there is certainly a receptive market for the species thus creating unlimited potential for the species to be used as both a cut flower and as a bedding garden plant. The most critical factor affecting both its supply and demand will be the cost. If enough stock material becomes available, the corms and cut spikes of this indigenous species could be sold at a reasonable price.

The basic postharvest requirements for *G. scabridus* have been determined and can be used to promote its production as a cut flower with the enhancement of its quality after harvest, to satisfy consumer standards and requirements.

CHAPTER 6

DISCUSSION AND FINAL CONCLUSIONS

Southern Africa has one of the highest known concentrations of endangered plants in the world (Hilton-Taylor, 1996). A substantial number of indigenous geophytes are at risk of extinction and it is essential that conservation measures be implemented to save these species. The commercial propagation and production of such plants is a way of ensuring the continued availability of plant material and also provides an enormous and valuable source of material that could be utilized in the horticultural industry. Knowledge and information are the keys to successfully introducing a new species. This does not only include knowledge of conservation practices in the wild but also cultivation requirements and growth responses.

G. scabridus has proved to be extremely responsive to the various cultural practises and propagation methods that have been investigated. With relatively little initially known about the species, a vast amount of information has been gathered on its growth and development, and response to the various cultural practises that cut flowers are exposed to commercially. It has been found to compare favourably to industry benchmarks.

G. scabridus has been observed to be a prolific producer of seeds. This is therefore an important and large source of plant material and genetic diversity which must not be overlooked. Optimum seed germination of gladioli is important to gladioli hybridizers, plant breeders and propagators. Under the right conditions *G. scabridus* seeds have been found to germinate easily and rapidly. The results indicate that they prefer mild temperatures and preferably removal of the oily wing around the small seed for adequate germination with the presence of light for suitable radical elongation and seedling development after emergence. Unfortunately, plants grown from seed take much longer to flower than those produced from corms. However, it has been shown that in a nutrient rich environment, the time to flowering can be reduced. Fresh seed contains higher levels of moisture and has a much higher viability and vigour than seed that has been stored, due to a lower fungal infection. The development of long term seed storage recommendations, which includes factors like storage temperatures and relative humidity, is important for the preservation of endangered species as practically it is not always suitable to use fresh seed for germination. Thus future work should develop methods to overcome pathogenic problems to improve the storability of *G. scabridus* seed.

An important advantage of growing seeds is to obtain new varieties through breeding or selection (Pickell, 1972). Numerous gladioli are available in South Africa alone, which provide a needed infusion of new characteristics into the modern hybrids (Dole & Wilkins, 1999). *G. scabridus* has interesting characteristics to offer in this regard. In breeding, plants are chosen for their good qualities: *G. scabridus* has tall spikes, plenty of flowers, an attractive colour, prolific cormel and seed production, all of which have potential for further development.

The National Botanical Gardens in South Africa, together with local indigenous seed companies, promote conservation through cultivation by making seeds available to growers and gardeners. Similarly, once sufficient stocks are available, *G. scabridus* seed can be distributed in this way. The basic conditions for germination have been outlined, hence, seed propagation can play an important role in conservation through mass cultivation by growers and gardeners alike. However, the distribution of endangered species through such channels should be done in accordance with the rules set out by the Convention on Biological Diversity (CBD). The CBD is a global, comprehensive agreement addressing all aspects of biological diversity, genetic resources, species and eco-systems, and serves to safeguard natural endangered resources (McGough, 1997; McSecretariat of the convention on Biological Diversity, 2005). It emphasises the legal and ethical aspects arising from the movement and selling of endangered plant material, thus incorporating important principles such as sustainable use and benefit-sharing, and the economic implications thereof.

The conditions for cultivation of *G. scabridus* by tissue culture have been demonstrated, and it has been shown to be possible to produce large numbers of plants from a small plant population in a short space of time. This is highly advantageous with an endangered species, as plant material can be bulked up for the continued production and distribution thereof. This serves as a continued source of new material for further research techniques, and material can also be made available to collectors and growers. Both *G. scabridus* axillary bud and cormel halve explants provide successful regenerate material for such a process. It may also be beneficial to investigate micropropagation methods using other plant parts of the species, such as inflorescence stalks, shoot tips, meristems, and stolon tips, which have all shown to have regenerative potential in other *Gladiolus* species. The tissue culture of an endangered species is an important way of ensuring germplasm preservation and the removal of the pressure on a species from its natural habitat. This can ultimately lead to commercialization, hence, its crucial role in the conservation of *G. scabridus*.

Transplanting *in vitro* cultured *Gladiolus* plantlets into the soil can be a problematic process as was found with *G. scabridus*. However, the production of cormlets allows for the avoidance of this step and the convenient storage and handling of this material, thus avoiding this difficult hardening off stage.

The forcing ability of a bulbous plant is an important commercial consideration. Producing cut spikes out of season is advantageous in that the producer can expect a much higher price for his product out of the normal flowering season. Forcing also means a higher availability of the spikes to meet the demands for an attractive product like *G. scabridus*. A higher availability would also increase an awareness of the species and also hopefully improve sales. *G. scabridus* corms show a tolerance to cold storage and can be successfully forced out of the normal flowering season. However, future work should focus on developing a clearer understanding of the effect of external factors such as light intensity, daylength and temperature on the flowering of forced corms, to improve the flowering percentage and promote uniform flowering.

The growth of *G. scabridus* can be improved in a controlled environment. The species is adapted to stressful, harsh conditions in the wild, and grows naturally in soils that are low in nutrients as indicated by the studies at Bivane Dam. However, the species appears to thrive under fertile conditions, with the enhancement of growth and a reduction in time to flowering, which is commercially significant. Leaf analysis data from Bivane Dam, as well as from plants growing in the glasshouse, showed that the species has adapted to lower nutrient levels to allow for sufficient growth and development to take place. Optimum levels of these elements in *G. scabridus*, as indicated by leaf analysis, are clearly not comparable to the norms used for hybrid gladioli. This is advantageous in that, although a nutrient rich environment improves growth and reduces the time to flowering, application levels will not need to be as high as those used for commercially propagating hybrid gladioli in order to attain optimum growth responses such as corm development and flowering.

Although each nutrient plays an important role in plant growth, the nutritional studies showed that nitrogen and potassium play a vital role in *G. scabridus* plant development, but this only really becomes significant during the second season of growth. Higher levels of nitrogen appear to affect vegetative growth, thus improving plant height and leaf number as well as affecting flowering, whereas higher levels of K affect cormel development. This improvement of the above ground growth thus improves the formation of the underground storage organ,

which supplies nutrients for next season's growth. This is an important consideration for a grower, as this means that the fertilizer applications will need to be altered during the growing season according to the plant growth response and time of application. Thus the N to K ratio will need to be altered with higher N at the beginning of the season to enhance vegetative growth and flowering, which will in turn improve daughter corm development, and then higher K near the end of the season to enhance cormlet formation. The application of micronutrients is also important.

The production of cormels must not be overlooked, as this is also a very important source of plant material. *G. scabridus* corms have been found to produce numerous cormels around the perimeter of the corms. It is advised that these should always be removed at the end of the growing season to be planted out again in the new season for the production of more plants, that is, bulking up stock plants, for the continued propagation of the species, or for use in tissue culture propagation. For maximum cormel development from the corms, it is advised to plant the cormels out as early as possible in the season and remove them as late as possible (Pickell, 1972).

Once flowering size corms have been attained at the end of the final growing season, they can be planted yearly from then on and will result in the constant seasonal production of seeds, new daughter corms and cormels. Thus within a short space of time a large population and source of plant material can be built up as one daughter corm can provide an abundance of plant material.

Although the process of enhancing the growth cycle from seed to flowering is very advantageous, it is important that each stage of this cycle be carefully managed, and in order to get the best production from the plant, germplasm must be kept under strict control to ensure a constant supply of material. Before planting seeds, cormels or corms, it is essential to ensure that the media is sterilized to reduce the chance of disease. Plants must be constantly monitored for pests, diseases and any abnormalities that occur as well as resistance to chemicals used. *G. scabridus* appears to be tolerant to the many pests and diseases associated with gladioli. No significant disease problems were observed which may be as a result of growing the species in a protected environment. However, further work will need to be conducted to confirm this. It has been established that wild gladioli do not behave or respond as the hybrids do. One cannot predict what wild species will do and assume that the response will be the same. These plants have adapted to a different environment and hence have different needs and requirements.

Future work on nutrition and cultivation could involve the use of foliar fertilizer applications and their effects on plant growth, as well as the mulching of the plants. Soil samples from Bivane Dam indicated that phosphorus and zinc tend to be higher in these soils, so perhaps studies focussing specifically on these elements need to be conducted to determine the exact role that they play in the growth of *G. scabridus*.

Postharvest studies play a vital role in cut flower production as this is the stage which ensures that the product gets to the consumer in a suitable condition or at the correct marketing stage. Flowers must last as long as possible in order for the consumer and the producer to get the best value for their money. In addition to florists' requirements for good size and colour, other criteria include marketable spikes per corm with up to 15 florets per spike, good vase life and bud opening qualities if cool stored (Salinger, 1985). *G. scabridus* has been shown to meet all these criteria. The spikes produce magnificent blooms, a sight which would certainly attract any prospective cut flower grower or breeder (Littlejohn & van der Walt, 1992). Pink gladioli are commercially very popular (Salinger, 1985). The striking, large, bright pink and mauve flowers and good bud presentation of *G. scabridus* would make it an ideal cut flower to satisfy ever increasing consumer demands for new and interesting products, on both a local and international scale. A very positive aspect of the postharvest work was the market research studies. The ultimate goal of flower production is to supply consumers with a product that they find attractive and hence will buy. The studies showed a wonderful appreciation for this new product and confirms that *G. scabridus* would be a viable and sort after product when introduced to the horticultural market both as a cut flower and a bedding plant. An important consideration in this regards will, however, be the cost affecting both the supply and demand of the corms and spikes.

Gladiolus cut flowers can well stand transportation over long distances (Anserwadekar & Patil, 1986) and it has been shown that *G. scabridus* spikes can be successfully cold stored in conjunction with polypropylene packaging. This is an important requirement for cut flower production which improves the commercial marketability of the spikes and creates flexibility for both local and export sales. Further physiological work relating to pulsing could be useful. The production of grey mould on spikes that have been stored for longer periods of time due to the presence of extra-floral nectar, is unfortunately not commercially acceptable so if longer storage periods are desired, the use of fungicide sprays or dips needs to be investigated.

Knowledge of the positive postharvest aspects of *G. scabridus* may encourage wild harvesting of the species. This would have to be monitored very carefully especially with regards to plant trafficking in protected areas. Unfortunately it is impossible to monitor every protected area but conservationists and staff members of groups such as KwaZulu-Natal Wildlife will need to be extremely vigilant. Programmes also need to be undertaken to inform members of the public of the detrimental effects of illegal wild flower harvesting.

Another way to ensure the survival of indigenous flora is to focus attention on the replacement of plants removed from the wild. In Europe, over exploitation of certain bulb species from the wild led to an initiative between Turkey and the Netherlands to control the harvesting of wild species and promote conservation. This involved labelling all bulbs sold by Dutch companies as to their origin ('wild' or 'cultivated stock') thereby monitoring the movement and sales of indigenous species, establishing a propagation project to replace bulbs collected 'from the wild' by growing bulbs from 'cultivated stock' and banning the collection and sale of certain species. Permits and quotas are also issued to exporters to control the sale of these bulbs (Bogers, 1992; Ekim *et al.*, 1997). Projects like these can only serve to promote an awareness and appreciation of local indigenous flora and control the sale and production thereof. Currently, The Threatened Plant Conservation Unit at KwaZulu-Natal Wildlife is involved in the *ex-situ* production of threatened plants for restoration projects as well as determining suitable methods of propagation and growth requirements, the relevant research of threatened plants and the establishment of live genebanks of threatened plants (Church, 2001) including *G. scabridus*. Due to low manpower, attention cannot be given to every threatened species. Hence, researchers play an important role in assisting this process. The need for research on *G. scabridus* has been met and propagation methods and growth requirements have been determined. KwaZulu-Natal Wildlife mainly propagate *G. scabridus* from seed, however, it is recommended that the focus on plant establishment also be emphasised elsewhere to enhance the establishment of larger amounts of plant material in a shorter space of time. At the end of the growing season, corms should be dug up and as many cormels as possible removed and propagated separately. This will create a much larger source of plant material.

To conserve plants one needs information on what the threats to their survival are and where the plants still occur so that their localities may be protected (Synge, 1980). Ecological studies at Bivane Dam not only provided useful and important information on the soil conditions in which *G. scabridus* grows but also on its growth in the wild and perhaps why

it may be threatened. It appears that nutrition does not play a significant role in the low distribution of *G. scabridus*, as they have adapted their growth accordingly. They are only prevalent in rocky, quartzite outcrops where they are safe from predation due to their inaccessibility, by becoming wedged between the rocky crevices. This may be an important reason for the low distribution of the species. Scott-Shaw (1999) notes that future ecological needs with regards to *G. scabridus* include the recording of locations of sub-populations outside protected areas and the securing of stocks of plants in cultivation. By locating other quartzite areas in northern KwaZulu-Natal, one is likely to find other populations of the species. Studies in other *G. scabridus* areas will hopefully reveal more ecological information and confirm that already revealed. The bulking of material by KwaZulu-Natal Wildlife as suggested, will ensure a rapid and important means of creating large sources of plant material *ex situ*. Future ecological research could also look at fire and its role in decreased plant populations, as this may play a role in this process (Goldblatt & Manning, 1998). Investigations into the pollination strategies may also provide further evidence as to why the species is threatened (Goldblatt *et al.*, 2001).

There is a sentiment among the conservation movements, that wild species should not be cultivated or kept in private hands. However, it is in private collections where much of the germplasm of rare species and varieties is maintained, and we will probably have cause to be grateful for such genetic treasures in the future. The Indigenous Bulb Growers Society (Littlejohn & van der Walt, 1992) is one such group who provides material to private collectors, where much of the germplasm of rare varieties and species is maintained, thereby aiding in the survival of genetic resources. Examples of such species which have been saved from the brink of extinction by being brought into cultivation in this way are *G. aureus*, *G. watermeyerii, G. citrinus, Moreae loubseri* and *Ixia maculata* (Doutt, 1997). The CBD recognizes that species and genes must be used for the benefit of humans, however, the availability of rare plant material through such channels should be carefully considered in conjunction with the rules laid out by the convention to ensure the sustainable use and lack of exploitation of indigenous resources.

Thus the philosophical question arises as to whether the use and commercialisation of indigenous plants is an acceptable means of conservation? This of course could be considered from many different angles. However, it must be remembered that the horticultural trade has the financial backing, outlets and other means of multiplying and distributing these plants (Winter & Botha, 1994). Revenue generated from sales of these

plants and plant material, can be used for development and further conservation practices. If this process is conducted in a controlled manner in accordance with the CBD, it can be a successful way of ensuring the maintenance and distribution of endangered species.

Commercialisation can only have a positive and beneficial impact on conservation efforts provided this is done in a sytematic and careful way. *G. scabridus* has shown to have considerable potential to be utilized in this way and can be considered a valuable addition to the cultivar range. The studies conducted on propagation and nutrition, have indicated that *G. scabridus* can be successfully moved from a wild plant to a commercially viable one. It is

hoped that this will lead the way for the survival process of this plant as well as many other threatened species in the near future. In this way South African plants can continue to be a source of pleasure and profit to amateur and professional alike the world over. Through the commercialisation of endangered, indigenous plants, such as *G. scabridus*, it is hoped that their conservation status will be improved, a process vital to their survival, and that people will benefit from the beauty of these plants for years to come.

APPENDICES

Appendix 1 Leaf analysis norms for *Gladiolus* (Tandon, 1993; Cresswell & Weir, 1997)

Element	Low [x]	Sufficient [y]	High [z]
Nitrogen (%)	2.50 - 2.99	3.00 - 5.5	> 5.5
Phosphorus (%)	0.23 - 0.24	0.25 - 1.0	> 1.0
Potassium (%)	2.00 - 2.49	2.50 - 4.0	> 4.0
Calcium (%)	0.40 - 0.49	0.50 - 1.5	> 1.5
Magnesium (%)	0.12 - 0.14	0.15 - 0.3	> 0.3
Sodium (%)	-	-	>0.5
Manganese (ppm)	25 - 49	50 - 200	> 200
Zinc (ppm)	11 - 19	20 - 200	> 200
Copper (ppm)	5 - 7	8 - 20	>20
Iron (ppm)	40 - 49	50 - 200	>200

[x] no symptoms but nutrient is too low for optimum crop performance

[y] no symptoms but level is adequate

[z] no symptoms but level is higher than necessary which may cause an imbalance or loss of quality

Appendix 2a Grass species found growing at *G. scabridus* sites at Bivane Dam

Plant name	Habitat characteristics
Diheteropogon amplectens (Broad-leaved Bluestem)	Commonly occurs in high-lying grassland and in bushveld. Grows on various soils but prefers stony soil and is often found on rocky hills (a).
Heteropogon contortus (Spear Grass)	Widespread in open grassland and bushveld areas. Common on well drained, sandy soils. Generally found on stony slopes and disturbed soils such as roadsides where it can form thick stands (a).
Eragrostis curvula (Weeping Love Grass)	Usually grows in disturbed places such as old cultivated lands and roadsides, mostly in well-drained fertile soil (a).
Themeda triandra (Red Grass)	Occurs in all veld types in southern Africa, but is especially common in undisturbed climax grassland. Grows on all soil types (a).
Panicum maximum (Guinea Grass)	Prefers damp places with fertile soil, such as in the shade of trees and shrubs and along rivers but also adapts well to a variety of other soils and conditions (a).
Tristachya leucothrix (Hairy Trident Grass)	Commonly in high-rainfall sourveld, where it prefers under utilized veld and veld that is rarely burnt. Often also found on stony slopes and in marshy areas. Prefers sandy soils (a).
Panicum natalense (Natal Panicum)	Common in open sour and mountainous grassland in cool high-rainfall areas. Grows on well-drained sand or sandy loam and on stony soils, often on stony slopes or veld that is regularly burnt (a).
Cymbopogon excavatus (Broad-leaved Turpentine Grass)	Occurs on most soils but prefers stony, sandy soils in disturbed as well as undisturbed sour grassveld (a).
Melinis repens (Natal Red Top)	Generally in disturbed areas such as cultivated lands and roadsides. Grows on all soil types. Sometimes also found in open grassland and stony ridges (a).
Trachypogon spicatus (Giant Spear Grass)	Common in open, undisturbed sour grassland but also occurs in bushveld. Grows on most kinds of soil with a preference for sandy soils (a).
Melinis nerviglumis (Bristle-leaved Red Top)	Generally on shallow stony soils in undisturbed, open grassland or bushveld and is usually found on stony slopes (a).

(a) van Oudtshoorn (1999)

Appendix 2b Tree and shrub species found growing at *G. scabridus* sites at Bivane Dam

Plant name	Habitat characteristics
Faurea saligna (Willow Beechwood)	Occurs in lower to medium altitude open woodland and on stony hillsides, sometimes along river banks (a).
Pterocarpus angolensis (Kiaat / Wild Teak)	Occurs in woodland and wooded grassland and on mountain tops. Regarded as an indicator of well-drained soils (b). Common on rocky hillsides in northern KZN (c).
Englerophtyum magalismontanum (Stem-fruit)	Characteristic of rocky outcrops and hills. Also occurs in forest, wooded ravines and along river banks, especially among rocks in sandy soil (a).
Ekebergia capensis (Cape Ash)	Occurs in a variety of habitats including evergreen woodland, riverine, coastal sandveld or montane forests from sea level to about 1500m ASL Always in well-drained soil (a; b).
Dalbergia armata (Thorny-rope)	Common in woody ravines, in evergreen forest and in riverine fringe forest (a).
Zanthoxylem capense (Small Knobwood)	Mostly in dry to evergreen woodland and on hill slopes, but adapted to a wide range of ecological niches (b). Often in rocky places and also at higher altitudes amongst rocks and even into the mist belt (a).
Combretum zeyheri (Large-fruited Bushwillow)	Occurs at medium to low altitudes, in open woodland, on rocky hillsides and sometimes along rivers, tolerating a wide range of soils including those that are fairly heavily mineralised (a).
Combretum erythrophyllum (River Bushwillow)	Mostly along rivers and streams. Sometimes away from rivers in wooded grassland where the water table is high enough. Not restricted to any specific type of soil (b).
Rhoicissus tridentata (Bushman's Grape)	Occurs in a wide range of habitats, often associated with boulders on rocky knoppies or stony hillsides. Also found in bush clumps in open grassy woodland and at the margins of evergreen forest in high-rainfall areas as well as coastal dunes and coastal forest (a).
Acacia ataxacantha (Flame Thorn)	Occurs among sand dunes, in open bush and jesse bush, in wooded grassland and on rocky hillsides (a).

(a) Coates Palgrave (2002)
(b) Venter and Venter (1996)
(c) Jacana (1997)

Appendix 2b continued

Plant name	Habitat characteristics
Dombeya rotundifolia (Wild Pear / White Dombeya)	Found in woodland, dry wooded grassland and on rocky mountain slopes (b) at a wide range of altitudes (a).
Pavetta edentula (Gland-leaf Brides-bush)	Occurs in bushveld and grassland on steep rocky hillsides (a).
Euclea divinorum (Magic Guarri)	Occurs in bushveld, thicket, thorn scrub, on hillsides, along river banks and in woodland (a).
Ficus abutilifolia (Large-leaved Rock Fig)	On rocky outcrops, hills and ridges. Commonly found growing on cliffs (b).
Dichrostachys cinerea (Sickle-bush)	Occupies a diverse range of habitats, including woodland at high and low altitudes, forest margins, scrubs and grassland. It grows on all soil types (b). More common at lower altitudes (a).
Euphorbia cooperi (Bushveld Candelabra Euphorbia)	Found on rocky hills and outcrops in wooded grassland or scrub (c).
Solanum giganteum (Healing-leaf Tree)	Occurs in woodland, on mountain slopes, often in deep shade in forest clearings and at the margin of thicket and disturbed areas (a).
Sclerocarya birrea (Marula)	Grows on various types of woodland and bush on sand to sandy loam soil (b). Occurs in medium to low altitudes (a).
Ficus glumosa / stuhlmannii (Hairy Rock Fig)	Occurs on rocky outcrops when it becomes a rock splitter, along dry watercourses or in open country frequently in valleys where it reaches its greatest size (a).
Ochna natalitia (Showy Ochna)	Occurs in open bushveld, wooded grassland, frequently in shallow soil among rocks when it seldom reaches 2m, or forming part of the under-storey in forested kloofs and at the margins of evergreen forests (a).
Erythroxylum delagoense (Small-leaved Cocoa-tree)	Occurs in dry woodland or scrub, coastal grassland and sometimes on forest margins, usually on dry sandy soils and often among rocks (a).

(a) Coates Palgrave (2002)
(b) Venter and Venter (1996)
(c) Manning (2001)

Appendix 2c Flower species found growing at *G. scabridus* sites at Bivane Dam

Plant name	Habitat characteristics
Wahlenbergia grandiflora (Giant Bell Flower)	A perennial that grows in colonies in grassland (a).
Tephrosia macropoda (Creeping Tephrosia)	Straggling perennial that occurs in open and rocky grassland, coast to mountains (a).
Ledebouria apertiflora (Common squill)	Common in dry, woodland (a).

(a) Pooley (1998)

Appendix 3 Recorded locations of *G. scabridus* at Itala Nature Reserve (C.R. Scott-Shaw, pers. commun., 2001)

Record ID	Latitude (S)	Longitude (E)	Herbarium
6205	27°28'23"	31°13'0"	BRI
7693	27°30'0"	31°15'0"	CPF
9508	27°31'23"	31°18'28"	CPF
9509	27°31'23"	31°18'28"	CPF
10833	27°32'43"	31°12'47"	CPF
10834	27°32'43"	31°12'47"	CPF

Appendix 4 Rating system for determining *G. scabridus* postharvest floret quality

Quality rating	Floret requirements
1	Faded; dark purple colour Tight bud Deformed Crinkled and dry
2	Light purple colour Slightly open Deformed Slightly crinkled and dry
3	Pink with purple blotches Half open florets Slightly deformed
4	Light pink colour Almost fully open Very few deformations
5	Bright pink colour Perfect shape Wide, fully open florets No deformations

Appendix 5 Standard curves for ferricyanide sugar analysis

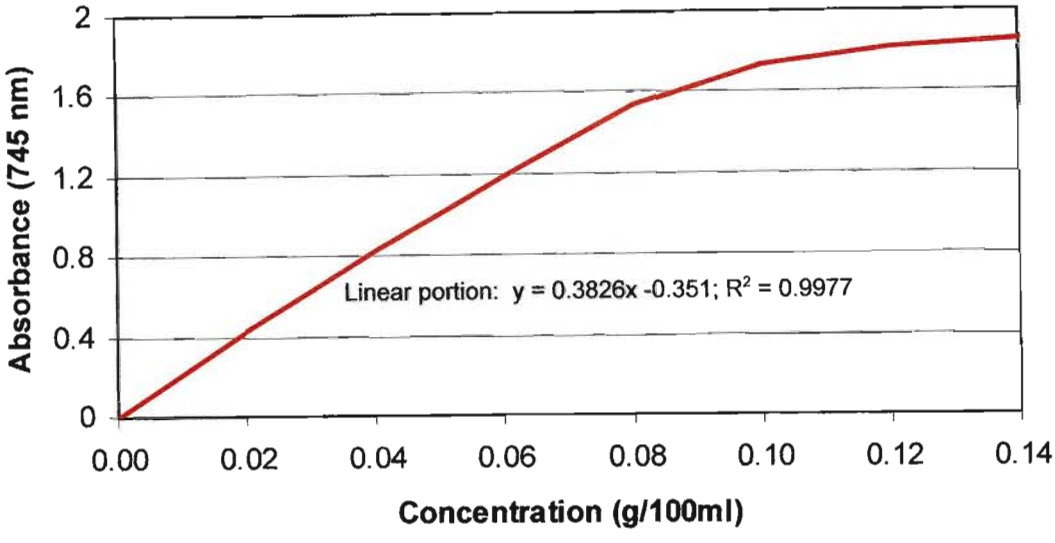


Figure A5: 1 Fructose standard curve for ferricyanide sugar analysis

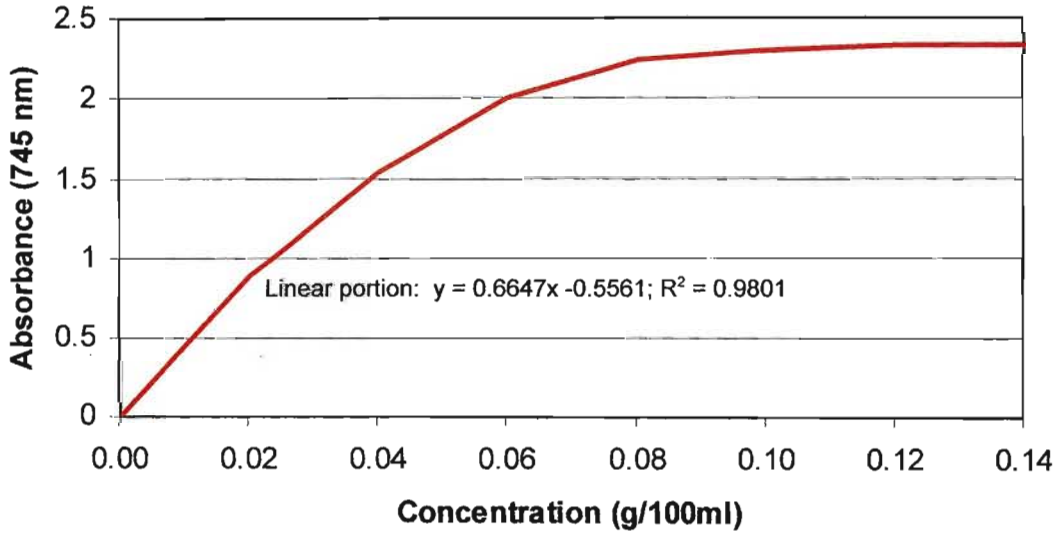


Figure A5: 2 Total reducing sugar standard curve for ferricyanide sugar analysis

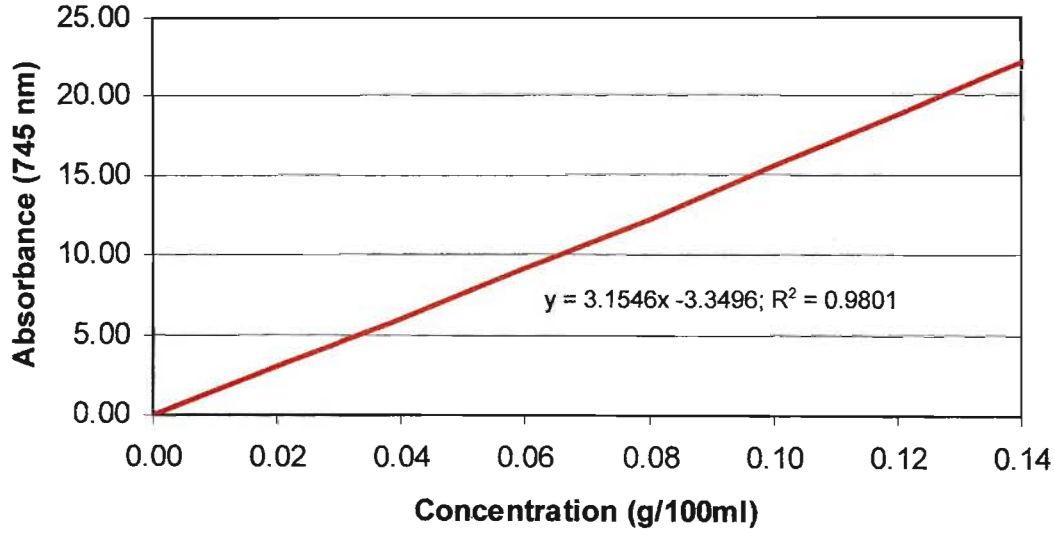


Figure A5: 3 Total sugar standard curve for ferricyanide sugar analysis

Appendix 6a *G. scabridus* retail marketability questionnaire

Gladiolus scabridus is an endangered species indigenous to northern Kwazulu-Natal. My PhD has involved studying the species to determine its growth requirements with the aim of commercialisation and as a result the improvement of its conservation status in South Africa. As a florist and / or retail nursery we would really value your opinion and impressions on this species as a saleable product.

Thank you for your time.

Tracey Campbell

Shop name: _____

Shop description: _____

1. Do you sell many indigenous wild flowers? Give details. (Please indicate on a scale of 1 = never to 5 = all the time as well as with your comments.)

_____

_____

_____

2. Do you ever sell any hybrid gladioli? Give details. (Please indicate on a scale of 1 = never to 5 = all the time when in season as well as with your comments.)

_____

_____

_____

3. What are your initial impressions of *G. scabridus*? (Please indicate on a scale of 1 = totally unimpressive to 5 = very impressive as well as with your comments.)

_____

_____

_____

4. What characteristics of the species would help promote the sale thereof?

5. Any negative comments?

6. On a scale of 1 = no potential to 5 = excellent potential, do you think there is a market for this species to be sold as:

   (i) a cut flower?

   (ii) a flowering staked plant in a bag?

   (iii) seed?

(i)

(ii)

(iii)

7. Any other comments?

Appendix 6b *G. scabridus* consumer marketability questionnaire

Gladiolus scabridus is an endangered species indigenous to northern Kwazulu-Natal. My PhD has involved studying the species to determine its growth requirements with the aim of commercialisation and as a result the improvement of its conservation status in South Africa. As a consumer we would really value your opinion and impressions on this species as a saleable product.

Thank you for your time.

Tracey Campbell

Name: _____

1. Do you buy any indigenous wild flowers? Give details. (Please indicate on a scale of 1 = never to 5 = all the time as well as with your comments.)

_____

_____

_____

2. Do you ever buy any hybrid gladioli? Give details. (Please indicate on a scale of 1 = never to 5 = all the time when in season as well as with your comments.)

_____

_____

_____

3. What are your initial impressions of *G. scabridus*? (Please indicate on a scale of 1 = totally unimpressive to 5 = very impressive as well as with your comments.)

_____

_____

_____

4. What characteristics do you look for in a horticultural product, and in particular, cut flowers when purchasing them?

5. In your opinion, what characteristics does *G. scabridus* have that would help promote the sale thereof or encourage you to purchase this product before any others?

6. Any negative comments?

7. On a scale of 1 = no potential to 5 = excellent potential, do you think there is a market for this species to be sold as:
   (i) a cut flower?
   (ii) a flowering staked plant in a bag?
   (iii) seed?

(i)
(ii)
(iii)

8. Any other comments?

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