Carbohydrate Partitioning and Developmental Physiology of *Nerine bowdenii* Will. Watson

by

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

University of Tasmania
Hobart

May 1999

Declaration

I declare that this thesis contains no material which has been accepted for the reward of any other degree or diploma in any other tertiary institution and, to the best of my knowledge and belief, contains no copy or paraphrase previously published or written by any other person except where due reference is made in the text of the thesis.

Martin R. Blake

21 May 1999

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Summary

Nerine spp. are a bulbous genus that is grown commercially in Australia on a limited scale. However, environmental conditions in the south-eastern region of the continent, including the island state of Tasmania, are ideal for production of cut-flowers. However, large scale cultivation of the genus has been precluded to some extent not only by unpredictable flowering under field conditions but also unsatisfactory inflorescence scape lengths for export markets. The objective of these studies was to begin to elucidate some of the factors involved in the control of flowering in *Nerine bowdenii* Will. Watson, and to investigate the process of scape elongation.

The *Nerine* bulb consists of a number of recently initiated and previously emerged annual units. The concentric leaf bases are retained and serve as a source of carbohydrates for the development of the units. Dry weight analysis of the bulb components over time showed that all outer units were utilised for current season leaf and inflorescence growth. Photoassimilates and carbon from senescing leaves appeared to predominantly accumulate in these organs but were not critical to flowering under glasshouse conditions.

The major carbohydrates detected in bulbs were starch, sucrose, fructose, glucose, and fructans. Sucrose was found to be the major form of carbon transport in the vascular tissue of mature leaves. Fructan was present in very high concentrations in all

organs, and the presence of this polymer has not been previously reported in this genus.

To investigate the role of the reserve carbohydrate supply in inflorescence abortion ^{14}C-sucrose was applied to the outer scales of bulbs. Bulbs containing an aborting inflorescence showed increased sink activity by leaf/floral primordia. ^{14}C data indicated that outer-scale derived sucrose rapidly became dispersed throughout the bulb, with accumulation of radio-actively soluble sugars in the roots and basal plate.

Expansion of leaves and scape was shown to be initially dependant on cell divisions within a plate meristem located at the base of the organs. This was followed by expansion of the cells. Control of flower height was able to be manipulated by the imposition of shading on plants, which resulted in longer scape lengths as a consequence of increased cell divisions. The data also suggested a potential role of shading in the regulation of leaf number.

Acknowledgments

The author wishes to thank Dr. Ron Crowden and his family (Channel Bulbs Pty Ltd). Ron was the motivation, initial academic supervisor, and industry partner for this project and without whom this project would not have been possible.

Special thanks must go to my very tolerant wife Anne, and to my even more tolerant supervisor Dr. Phil Brown. Words cannot express my gratitude for your perseverance, nor can I ever repay the debt I owe you both for your direct and indirect contribution to this project.

Financial assistance was provided by the Australian Postgraduate Research Council through an Australian Postgraduate Research Award (Industry) Scholarship, for which the author has been most grateful.

Table of Contents

Abbreviations

CPM	Counts per minute
DMSO	Dimethyl sulphoxide
DP	Degree of polymerisation
DW	Dry weight
EtOH	Ethanol
FEH	Fructan exohydrolase
Fru	Fructose
GC-MS	Linked gas chromatography/ mass spectrometry
Glc	Glucose
HCl	Hydrochloric acid
MOPS	Morpholinopropane sulfonic acid
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced *NADP*
NSC	Non-structural carbohydrate
RSA	Relative specific activity
SE	Standard error
SS	Sucrose synthase
SST	Sucrose:sucrose fructosyltransferase
Suc	Sucrose

I

Introduction

Nerine spp. are bulbous monocots of the family Amaryllidaceae. They are mostly Autumn flowering perennials that are indigenous to southern Africa (Huxley *et al.*, 1992b). The four species that dominate cut-flower production are: *N. sarniensis* (L.), *N. bowdenii* W. Wats., *N. undulata*, and *N. flexuosa* Jacq. (van Brenk & Benschop, 1993). Cultivars of *N. bowdenii* are grown worldwide (Landon-Lane, 1988; van Brenk & Benschop, 1993). Typically they are amenable to harsh European winters because of their winter "dormancy" period (Landon-Lane, 1988).

Nerine spp. first appeared in European gardens during the seventeenth century (Custers & Bergervoet, 1992). However, commercial utilisation of the genus did not occur until 1903 following the introduction of *N. bowdenii* (van Brenk & Benschop, 1993). Cut-flower production of *Nerine* has expanded in recent years, particularly in the Netherlands where the harvest has increased from 156 000 stems in 1960 to over 28 million by 1990 (van Brenk & Benschop, 1993).

The current success and potential for future expansion of *Nerine* as a cut-flower crop can be at least in part be attributed to the factors described below:

1. There is a wide range of colours in the genus, particularly in *N. sarniensis*, including peach, orange, purple and all shades of red (Zuidgeest, 1975; Sherriff, 1994). Excellent whites and pinks can also be produced and bicolours are becoming increasingly common (Huxley *et al.*, 1992b; van Scheepen, 1991).

2. There may be up to 22 florets in an umbel (Smithers, 1990), which produce a large and attractively shaped flower head.

3. Flower scapes may attain a height 90 cm and they are leafless and lightweight (Smithers, 1990). Since they are harvested while the flower is still unexpanded inside the spathe, they are therefore economical to transport (Smithers, 1990; Cooper, 1993).

4. Flowers have a long vase life, with florets typically lasting seven to twelve days (Genders, 1973; Cooper, 1993).

5. *Nerine* spp. flower during autumn/winter (Cooper, 1973). This is a period when other cut-flowers are often scarce and, thus, are able to fill a market gap.

6. Manipulation of environmental conditions permit year-round forcing of *N. bowdenii* in the Netherlands (van Brenk & Benschop, 1993).

Currently there are relatively few growers in Australia. However, the potential for the genus in south-eastern Australia could be large. *Nerine* spp. can be produced for expanding European and North American markets at a time of the year when these flowers are comparatively scarce (Landon-Lane, 1988). Even though year round forcing is possible in Europe, abortion of the inflorescences during the spring forcing period makes production economically infeasible (Groen & Kok, 1997). The export potential of *Nerine* spp. inflorescences has been long recognised (Landon-Lane, 1988).

Cool temperate climates with mild winters are ideal for *Nerine* cultivation (Systema, 1982; Landon-Lane, 1988). Also, the genus prefers low fertility soils (Smithers, 1990), which are common in many Australian localities. *N. sarniensis* is an ideal cut flower crop and is currently under utilised (Smithers, 1990). Hybrids of this species are arguably the most attractive cultivars, yet commercial cultivation is restricted in Europe due to frost sensitivity (leaves are emergent during winter). Therefore, they currently command only a minor market share (van Brenk & Benschop, 1993). In south-eastern Australia, this species can be cultivated outdoors where bulbs can be left to establish and increase (Landon-Lane, 1988).

However, flowering problems have limited the economic viability of the genus as a commercial cut-flower crop in Tasmania, the most southerly state of Australia. The primary one has been unpredictable flowering caused by abortion of the inflorescence when rapid elongation of the scape and emergence is about to occur (R. Crowden pers. com.). Due to the high unit value of these

types of crops, the tolerance for loss of cut flower production is small (Baker & Linderman, 1979). Prerequisites for quality scapes, particularly those intended for export markets, include long stem lengths, which, in the case of *Nerine*, is considered to be a minimum of 50 cm (R. Crowden, pers. com). The primary purposes of these studies were to elucidate the reasons why inflorescence abortion occurs in *N. bowdenii* and to examine factors controlling scape length with a view to controlling scape growth to maximise production of export length flowers.

II

Literature Review

Nerine and the Amaryllidaceae

The Amaryllidaceae (Jaurne St.-Hil.)

Introduction

Deriving its name from the South African genus *Amaryllis*, the Amaryllidaceae is one of the most horticulturally important monocotyledonous families (Huxley *et al.*, 1992a). It consists of approximately 60-85 genera and 850 - 1390 species (Everett, 1980; Huxley *et al.*, 1992a; Meerow & Snijman, 1998). The amaryllids are widely distributed, particularly in the tropics and sub-tropics (Everett, 1980; Huxley *et al.*, 1992a) and are usually xerophytic (Huxley *et al.*, 1992a). The genera consist of perennial or biennial herbs (Dahlgren *et al.*, 1985), which can be deciduous or evergreen in habit, and usually possess subterranean storage organs such as bulbs, corms, or rhizomes (Everett,1980; Huxley *et al.*, 1992a). Roots are contractile and in all known cases incorporate vessels possessing scalariform perforation plates (Dahlgren *et al.*, 1985; Kawa & De Hertogh, 1992). Leaves are usually few, sheathing at the base, and rarely occur on the

scape (Huxley *et al.*, 1992a; Dahlgren *et al.*, 1985). The lamina is characteristically flat, dorsiventral with parallel veins and linear to virtually orbicular in some cases. Stomata are anomocytic (lacking guard cells) and other leaf cells may contain mucilage and elongate sacs with raphides of calcium oxalate (Dahlgren *et al.*, 1985).

Taxonomy

The major taxonomic characteristics of the Amaryllidaceae are the formation of terminal, helicoid cymes contained in a spathe and borne by a scape (Huxley *et al.*, 1992a; Dahlgren *et al.*, 1985; Meerow & Snijman, 1998). Umbels are always subtended by bracts (Clarke & Lee, 1987) and branching is sympodial (Dahlgren *et al.*, 1985), with the possible exception of one Australian species of *Crinum* (Howell & Prakash, 1990). The perianth is petaloid (two whorls of three), stamens are also arranged in two whorls of three and the ovary is inferior (rarely half-inferior), consisting of 3 fused carpels (Huxley *et al.*, 1992a). The ovary is usually three loculed with axile placenta and few to many ovules, from which develop loculicidal capsules or baccate, indehisent fruits (Clarke & Lee, 1987; Dahlgren *et al.*, 1985). The family is closely related to the Liliaceae and some of the amaryllids are included in this family by some authors (Clarke & Lee, 1987), although Dahlgren *et al.* (1985) place them in the separate orders of Liliales and Asparagales respectively.

The Nerine *genus (Herb.)*

Introduction

Approximately thirty species of *Nerine* have been recognised (Traub, 1967; Everett, 1981). All are native to the southern regions of Africa, including tropical areas (Rees, 1985), and most are autumn-flowering perennials (Huxley *et al.*, 1992b). The genus is closely related to *Brunsvigia, Amaryllis, Crinum, Strumaria, Hessea,* and *Carpolyza* (Tribus Amaryllideae) (Dahlgren *et al.*, 1985). The name *Nerine* is generally considered to be derived from the sea nymph *Nereis* of Greek mythology (Genders, 1973; Everett, 1981; Huxley *et al.*, 1992b). It may, however, have originated from the phonetically similar local South African name of *Nuhree nah* (Coombs, 1948). The genus was recognised for its ornamental properties as early as the seventeenth century. At that time *N. sarniensis* (L.) was found in Paris (van Brenk & Benschop, 1993) and on the island of Guernsey where it became naturalised, apparently through the agency of a Cromwellian exile of the Restoration who introduced the plant to the island from the English mainland (Huxley *et al.*, 1992b; Smithers 1993). It was the occurrence of *Nerine* on Guernsey that caused its association with the sea (*Nereis*) and the specific naming *sarniensis* (*Sarnia* being the Isle of Guernsey) (Traub, 1967). It was initially proposed (perhaps conspiratorially by royalists at Kew Gardens) that it had arrived there via a wrecked trading ship (Traub, 1967; Huxley *et al.*, 1992b).

Taxonomy

Nerine have tunicated bulbs, globose or ovoid-pyriform in shape and sometimes continued into a neck (Traub, 1967; Huxley *et al.*, 1992b).

Leaves are deciduous or evergreen, strap-shaped to narrowly linear (Everett, 1981) and arranged in two opposing ranks (Genders, 1973). The timing of scape emergence relative to that of the leaves varies among species, however, all species flower during late summer to autumn. The umbel ranges from 2 to more than 20 flowers (Everett, 1981; Huxley *et al.*, 1992b) and is formed on a leafless scape which is 40-80 cm in height depending on the species (van Brenk & Benschop, 1993). The perianth is funnel shaped, usually zygomorphic, and colours include white, pink, red, magenta, mauve, orange and bicolours (Traub, 1967; Zuidgeest, 1975; Huxley *et al.*, 1992b; van Scheepen, 1992). Perianths are divided into six lobes (tepals) of various shapes, which may be crisped to varying degrees and the tips are usually recurved or rolled (Traub, 1967; Huxley *et al.*, 1992b). There are six stamens inserted at the bases of the perianth lobes and these are positioned in a suberect to declinate-ascending fashion, with three each being of two sizes (Traub, 1967; Huxley *et al.*, 1992b). The style is filiform, straight or declinate-ascending and bears a simple or three-lobed (trifid) stigma (Traub, 1967). Ovaries are globose and three-lobed, containing few ovules per cell (Traub, 1967; Huxley *et al.*, 1992b). The membranous capsule is loculicidally dehiscent, three-valved, variously shaped and often ruptures with the expansion of maturing seed (Traub, 1967).

Classification

Species of *Nerine* can be classified using standard biosystematic classifications, of which there are several (Traub, 1967; Norris, 1974), or by groupings based on ontogenetic characteristics, which is more common among commercial producers (van Brenk & Benschop,

1993). The biosystematic classification of Traub (1967) divides the genus into the subspecific ranks of Laticomae, Nerine, Bowdeniae and Appendiculatae. These groupings are based largely on positioning and/or size of filaments, style and scape (Traub, 1967). Classification according to life cycle is more practical from a growers perspective and may prove to be physiologically relevant. Differentiation of these groups is based on emergence of the inflorescence relative to the leaves.

Group 1: Species that flower before leaf emergence, i.e., summer rest period (van Brenk & Benschop, 1993). These species are native to the South-western Cape region of South Africa, where summers are hot and dry and rainfall is predominantly in the winter months, e.g., *N. sarniensis* (Coertze & Sorour, 1990; van Brenk & Benschop, 1993).

Group 2: Species in which lamina emergence precedes the inflorescence, i.e., winter rest period (van Brenk & Benschop, 1993). These forms originate from the northern areas of the distribution of the genus where rainfall tends to be in the summer, e.g., *N. bowdenii* (W. Wats.) (Coertze & Sorour, 1990; van Brenk & Benschop, 1993). *N. bowdenii* is considered the hardiest cultivated *Nerine* species (Everett, 1981) and when planted is able to withstand temperatures to -15°C (Huxley *et al.*, 1992b).

Group 3: Species in which flowering and leaf emergence are simultaneous, i.e., they are evergreen (van Brenk & Benschop, 1993). These species grow in intermediate areas and are able to tolerate a wide range of climates, e.g., *N. flexuosa* (Jacq.) can be found at

elevations of 800-1200 meters as well as in hot, dry regions (Fortanier *et al.*, 1979; van Brenk & Benschop, 1993).

Commercial aspects

Traditionally, the significance of the genus as an ornamental flower has been derived largely from the characteristic "sparkling" of the flowers (van Brenk & Benschop, 1993), caused by what have been best described as crystalline structures in the petal substance (Smithers, 1993), and for the attractive flower colours (van Brenk & Benschop, 1993). Recent interest by Dutch commercial cut-flower producers is due to flower shape, high keeping quality, and the potential for year-round forcing of *N. bowdenii* (Genders, 1973; Custers & Bergervoet, 1992; van Brenk & Benschop, 1993). The commercial potential of *Nerine* was initially realised after the introduction of *N. bowdenii* into Europe by Cornish Bowden in 1903 (van Brenk & Benschop, 1993). Studies on the horticultural viability of *Nerine* as a cut flower began in The Netherlands during the 1950s and were based on cultivars of *N. bowdenii*, *N. samiensis*, and *N. undulata* (L.) (van Brenk & Benschop, 1993). In 1990, total acreage of *Nerine* in The Netherlands was 66.5 ha., of which 95.5% was *N. bowdenii*, mostly comprising of the cultivars 'Favourite' and 'Van Roon' (van Brenk & Benschop, 1993). The predominance of *N. bowdenii* is attributable to relative frost tolerance (winter dormancy), disease resistance, amenability to forcing, and long scape lengths (Landon-Lane, 1988). Other species and cultivars currently of commercial importance in The Netherlands are: *N. samiensis* var. *corusca* 'Major', *N. undulata*, and *N. flexuosa* var. *alba* (van Brenk & Benschop, 1993).

It is generally recognised that the most attractive species of *Nerine* is the *N. samiensis* hybrids (van Brenk & Benschop, 1993). However, maximum scape lengths of 45 cm, irregular flowering, disease susceptibility, and unsuitability for forcing have precluded this species from large scale utilisation in Europe (Landon-Lane, 1988).

Nerine production in Australia

The current land area dedicated to *Nerine* spp. in Australia may be hundreds of hectares (Sherriff, 1994), of which most is *N. bowdenii* (Cooper, 1993). The potential for *Nerine* in Australia is related to the fact that crops can be grown without lifting for long periods, as cool, temperate climates are ideal for *Nerine* cultivation (Systema, 1982; Landon-Lane, 1988). Aside from being economically efficient, allowing the bulbs to remain *in situ* is advantageous since it is known that *Nerine* roots should not be disturbed (Everett, 1981). *Nerine* spp., particularly *N. samiensis*, prefer nutrient poor soils (Coertze & Sorour, 1990; Smithers, 1993) which is also appropriate for many Australian sites. Finally, export markets have already been identified for Australian-grown *Nerine* cut flowers and the scapes are economical to transport, making them amenable to small quantities in mixed consignments (Landon-Lane, 1988).

Obstacles to efficient outdoor production in Australia

The use of *Nerine* as a major cut flower crop in Australia has been limited to some extent by unpredictable flowering in all cultivars which is exacerbated by an apparent sensitivity to disturbance.

Floral abortion frequently occurs at a late stage of inflorescence development and just before rapid elongation of the scape. Although links have been established with growing temperatures (van Brenk & Benschop, 1993), elucidation of the phenomenon has been hampered by delayed expression of treatments and associated physiological complexities. Flower buds of *N. bowdenii* are initiated two growing seasons prior to flowering (van Brenk & Benschop, 1993; Theron & Jacobs, 1994a). Thus, after senescence of the current years' flower, inflorescences for the following two years have already been initiated. These buds and their associated scales/leaf primordia which comprise the growth unit are termed 'N' for the current bud, N+1 and N+2 for the next two years respectively (Theron & Jacobs, 1992; van Brenk & Benschop, 1993). Similarly growth units of outer leaf bases during the development of N are termed N-1 and N-2 scales. Evergreen species such as *N. flexuosa* initiate 2 to 4 flower buds per growing season, which elongate and flower the following year (van Brenk & Benschop, 1993).

Storage temperatures for lifted bulbs directly effect inflorescence development (van Brenk & Benschop, 1993). However, practices regarding *Nerine* storage appear to differ markedly in the Netherlands (glasshouse culture) and South Africa (shadehouse culture) (van Brenk & Benschop, 1993; Theron & Jacobs, 1994b). Light has been shown to have no direct effect on bud initiation and development (van Brenk & Benschop, 1993), but photosynthetic measurements have shown that *N. bowdenii* and *N. sarniensis* perform optimally under low light intensities (van Brenk & Benschop, 1993; Warrington *et al.*, 1989). Furthermore, *N. bowdenii* and *N. flexuosa* are day-neutral, making these species highly amenable to year-round

production in the glasshouse which has been achieved in the Netherlands (van Brenk & Benschop, 1993).

The commercial significance of *Nerine* has been limited by low flowering percentages caused by abortion of the inflorescence prior to emergence from the bulb (Fortanier *et al.*, 1979; Berghoef & van Brenk, 1983; Theron & Jacobs, 1992; Theron & Jacobs, 1994b). Factors responsible for inflorescence abortion include; unfavourable growing conditions (Berghoef & van Brenk, 1983; van Brenk & Benschop, 1993), improper storage conditions (Systema, 1971), bulb circumference (Rees, 1985; Theron & Jacobs, 1992), and underdeveloped inflorescences (van Brenk, 1988 cited in Theron & Jacobs, 1994b). However, Theron & Jacobs, 1996a have indicated a possible relationship between abortion and sink competition between organs. Inflorescences containing a high proportion of aborted florets are reduced in terms of their capacity to compete with other sink organs such as leaf primordia and daughter bulbs (Theron & Jacobs, 1996a). A hierarchy of competing organs at this stage of development has been suggested: formation of leaf primordia > N+2 > formation of floret primordia N+1 > inflorescence N > daughter bulbs > roots > storage tissue (Theron & Jacobs, 1996a).

Similar observations of increased assimilate partitioning to daughter bulbs at the expense of an inflorescence have been documented in Dutch iris (*Iris x hollandica* Tub) (Elphinstone *et al.*, 1987; de Munk & Schipper, 1993). Some antagonism between leaf and inflorescence development has also been suggested (De Munk & Schipper, 1993). If *Tulipa* daughter bulbs develop before root formation by the mother

bulb, abortion of the flower bud occurs (Le Nard & De Hertogh, 1993). However, this is considered to be temperature induced developmental anomaly rather than a sink competition effect. A developmental factor determining inflorescence abortion in *Hippeastrum* has been observed (Rees, 1972). The third inflorescence in a growth period is unable to grow unless it has reached a size of 2-3 cm inside the bulb before rapid elongation is due to take place. Hayashi & Suzuki (1970 cited in Okubo, 1993) also observed that *Hippeastrum* fails to initiate inflorescences at high temperatures, corresponding to a marked increase in leaf growth. Competition between vegetative growth and floral development has also been reported in a range of other species including *Bougainvillea* (Tse *et al.*, 1974), tomato (*Lycopersicon esculentum*) (Kinet, 1977), and grapevines (*Vitis vinifera*) (Mullins, 1968).

The *Nerine* genus has substantial potential as a cut flower crop in south-eastern Australia. However, factors such as high rates of inflorescence abortion and scape length inconsistency have limited the success of the crop particularly in regards to export markets. The problem of abortion in *Nerine* is not confined to Australian conditions, but applies to most *Nerine* crops (Theron & Jacobs, 1992; Groen & Kok, 1997; Shillo *et al.*, 1997). The current hypothesis to explain irregular flowering in *Nerine* is at least partly derived from observations from other flower bulb species. It is based on the concept that competition between bulb organs for a limited carbon supply is the probable cause. The following sections provide a brief and truncated overview of some aspects of flower bulb physiology to provide a framework by which this concept may be investigated.

Flowering in Ornamental Bulbs

Introduction

The body of literature pertaining to *Nerine* species is relatively small compared to that of other ornamental bulb species. It has, therefore, been necessary to examine the literature on a much wider scale in order to address the issue of flowering in *Nerine*. Information has been included not only on flowering in *Nerine*, but also, for the purpose of comparison, on the closely related *Hippeastrum* and the two comprehensively studied ornamental bulb genera, *Narcissus* (Amaryllidaceae) and *Tulipa* (Liliaceae). In regards to the regulation of flowering, the *Iris* and *Lilium* are also included due to enormous databases on the regulation of flowering in these species. All are "true" bulbs, i.e., those possessing a modified, shortened stem (basal plate) to which is attached several fleshy scales and which contains adventitious root initials (De Hertogh & Le Nard, 1993a). In this discussion of flowering, some attention will be paid to other bulb processes and organs, such as foliage production and daughter bulbs because these organs and their processes are often interrelated and cannot be considered as separate issues (De Hertogh *et al.*, 1983).

Periodicity

Bulbs are extremely heterogeneous and considerable differences exist in structure, morphology, development, and periodicity. Rees

(1972) considered bulbs to be divisible into three broad groups based on the origins of storage organs viz. the presence of true scales/leaf bases. The three archetypical genera are: *Hippeastrum* (storage tissue comprising entirely of leaf bases, the group to which *Nerine* belongs), *Tulipa* (scales not producing leaves), and *Narcissus* (combination of leafless scales and leaf bases) (Rees, 1972)[1]. However, any systematic analysis of bulbous geophytes could also examine evergreen (e.g., *Hippeastrum*, *Clivia*) as compared to seasonal foliage patterns (e.g., *Nerine*, *Lilium*, *Tulipa*, *Narcissus*), or differentiate truly perennial bulbs (e.g., *Nerine*, *Muscari*, *Narcissus*, *Hippeastrum*) and bulbs that are replaced by daughter bulbs in a relatively short time (e.g., *Tulipa*, *Crocus*, *Iris*). Therefore, the following genera share various aspects of growth and development with *Nerine* and provide interesting contrasts.

Hippeastrum

The three archetypes described by Rees (1972) also represent three different structural forms of bulb development. *Hippeastrum* (Amaryllidaceae) bulbs develop via a sympodial branching system (Rees, 1969). The apical meristem in the centre of the bulb typically produces foliar initials in units of four leaves followed by an inflorescence (Rees, 1972; Okubo, 1993). Each leaf has a fully sheathing base encircling the apex except the innermost, which is

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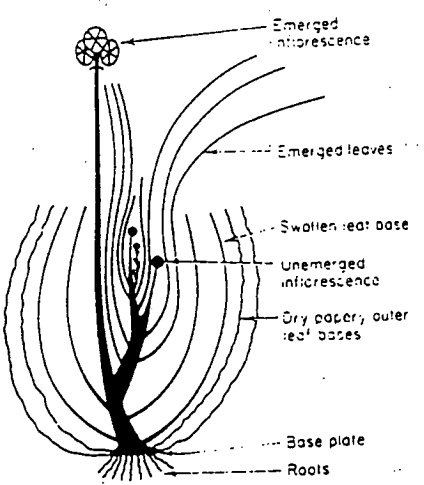
[1] Attempts have been made to separate the usage of "scale" and "leaf base" (Rees, 1972). Although De Hertogh and Le Nard (1993a) in their comprehensive treatise on flower bulbs state that "...scales can be *either* [authors italics] enlarged leaf bases or enlarged scale leaves" (p. 7), usage in this review and thesis will follow the definitions by Rees (1972) as the author believes the distinction between leaf bases and lamina-less scales to be a useful one.

semi-sheathing and precedes the inflorescence (Okubo, 1993). At the time of flower initiation, a lateral growing point is formed on the side of the apex which becomes the new apical meristem (Okubo, 1993). The leaves emerge considerably before their associated inflorescence, with the emerged foliage usually coinciding with the flower from an older growth unit (Rees, 1972: Okubo, 1993). Emergence of foliage is not unit-dependant, however, at any time expanded leaves may originate from a number of growth units (Rees, 1972). Typically, a large mature bulb is composed of six units, the outermost two having no aerial parts (leaf bases), two have emerged leaves, and two are yet to emerge (Rees, 1972; Okubo, 1993). Usually it is the fourth inflorescence from the centre that is emergent, although variations to this ontogenetic pattern (including bud abortion) may be brought about by seasonal factors (Rees, 1972). Vegetative reproduction occurs via daughter bulbs formed in the axils of older scales which are released by the degeneration of the surrounding mother bulb (Rees, 1972).

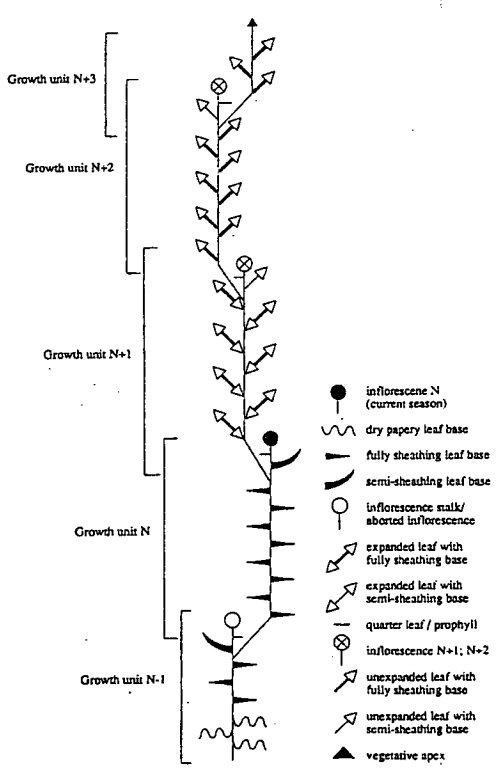
Figure II.1: Diagrammatic representation of *Hippeastrum* (from Rees, 1972).



Nerine

Nerine has a sympodial branching structure of growth units (van Brenk & Benschop, 1993), although these are annual units (as discussed previously) and some species are herbaceous (also discussed previously). The pattern of development and emergence of aerial parts is very similar to *Hippeastrum*, although seasonal periodicity is usually more pronounced. The bulb of *N. bowdenii* is comprised of up to five units, the two outermost have no aerial parts, one with emerged leaves (quiescent during "rest") and at least one developing unit (Theron & Jacobs, 1996b). The leaf number preceding the inflorescence is not as regular as *Hippeastrum*. The number of leaves per unit varying from 4-15 in *N. flexuosa* (Fortanier *et al.*, 1979) and 8-14 in *N. bowdenii* (Systema, 1982; Theron & Jacobs, 1994a).

Figure II.2: Diagrammatic representation of *Nerine bowdenii* at anthesis (from Theron & Jacobs, 1994a).



The number of leaves that elongate is considered predetermined and not influenced by conditions during the current growing season (van Brenk & Benschop, 1993). Although fall flowering and synanthous in its native country (Berghoef & van Brenk, 1983; Theron & Jacobs, 1994a), *N. bowdenii* has been found to flower at various times of the year without forcing and may exhibit an evergreen or hysteranthous (flowering before leaf emergence) growth habit under some environmental conditions (Shillo *et al.*, 1997). Thus, growth phenology and periodicity are determined by environmental factors to a large degree in this species.

Tulipa

The ontogenetic cycle of *Tulipa* (Liliaceae) differs considerably from the sympodial growth form. The bulb is composed of 2-6 scales, which under normal growing conditions do not form leaves (Rees, 1972; Le Nard & De Hertogh, 1993b). Leaves are borne on a scape which terminates in an inflorescence if more than one leaf is present (Rees, 1972). The mother bulb is replaced by up to eight daughter bulbs within 2.5 years due to the limited lifespan of the apex after initiating an inflorescence, although the time in which this takes place and the number of daughter bulbs produced is cultivar dependant (Rees, 1968; Rees, 1972; De Hertogh *et al.*, 1983). A bulblet is typically produced in the axil of each scale (although there may be more than one associated with older and larger scales), however, only inner bulblets survive to become daughter bulbs (Rees, 1968; De Hertogh *et al.*, 1983). The fastest growing bulblet is the last initiated closest to the apex and is initiated simultaneously with the inflorescence (Rees, 1968). It has

been hypothesised that the breaking of apical dominance allows accelerated growth of the innermost bulb first because of a centrifugal response (Rees, 1968). When bulbs are harvested, this innermost bulb is larger than the other bulblets (Rees, 1968). Because bulblets are able to produce their own bulblets in a relatively short time, there may be up to two generations of bulblets inside a mother bulb (Rees, 1972). The apex of a mature bulb is vegetative during summer and a large sized bulb must initiate 3-5 leaves before an inflorescence - two inflorescences are formed in some species and cultivars (Le Nard & De Hertogh, 1993b) - is formed (Shoub & De Hertogh, 1975; De Hertogh *et al.*, 1983). The shoot develops during the winter rest, or "dormant",[2] period (Rees, 1972) and the initiation of internodes is concurrent with flower organogenesis (Shoub & De Hertogh). A cold period is required for shoot elongation to occur, although flowering and rate of shoot growth are dependant upon prevailing environmental conditions (Rees, 1972). Elongation occurs first at the lowermost node and continues up the shoot (De Hertogh *et al.*, 1983). All post-cooling elongation of the scape is due to cell elongation from 30-45 μm to an eventual 200 μm in *T. gesneriana* cv. Paul Richter (Shoub & De Hertogh, 1975). The number of vascular bundles in the scape were not affected by light intensity, however, scape diameter was increased by high light intensity (Wassink, 1965 cited in De Hertogh *et al.*, 1983).

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[2] As is the case with a number of other bulb species, *Tulipa* bulbs are never truly "dormant" (De Hertogh *et al.*, 1983) (hence the term "rest" - which also is not strictly correct), there always being organs either growing, senescing or differentiating (De Hertogh, 1974; Aung & De Hertogh, 1979; Le Nard & De Hertogh, 1993b).

Narcissus

The third type of bulb that should be considered is the *Narcissus* (Amaryllidaceae) type. *Narcissus* bulbs are perennial, the internal branching system producing very abbreviated lateral as well as replacement terminal axes each year resulting in up to four generations being in the bulb (Rees, 1969; Rees, 1972). There is some question as to whether branching is monopodial or sympodial, the inflorescence is produced in the axil while the apex continues growth if the former is correct, while sympodial growth suggests that the inflorescence is terminal, as in *Hippeastrum* and *Nerine* (Rees, 1972; Hanks, 1993).

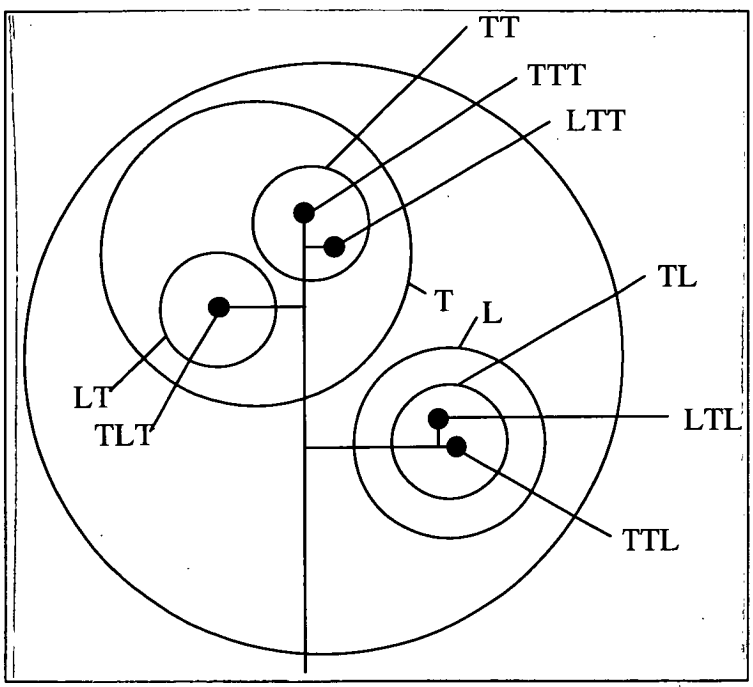

Figure II.3: Diagrammatic representation of a "double-nosed" Narcissus bulb. Shaded circles represent current seasons growth units, concentric circles represent older units. T = terminal unit, L = lateral unit (from Rees, 1969). The number of letters designates the age of the unit. The three labelled generations are enclosed by an incomplete fourth generation. See following text for a full description of relationship and properties of units.

As in *Nerine*, each bulb is comprised of annual "units" originating in the centre of the bulb (Rees, 1969). The bulb unit consists of 2-3 leaf bases and 2-4 scales which enclose the apex and these are followed by an inflorescence (Rees, 1972; Hanks, 1993). Within some cultivars a high degree of flexibility exists in the combinations of leaves and scales, and combinations are sometimes species specific (Rees, 1969; Rees, 1972). Terminal bulb units are initiated beside the inflorescence in the axil of the second leaf from the centre of the bulb at approximately the same time as floral initiation at the end of autumn, whilst lateral units usually originate in the axil of the third leaf from the centre during winter (Rees, 1969; Hanks, 1993). At this time, or a year later, other terminal units may originate next to this lateral, in the axil of an outer leaf, or in the axil of an inner scale (Rees, 1969). Terminal units are replaced by another terminal unit plus a lateral set of units; whereas, lateral units rarely differentiate other lateral units (Rees, 1969). A bulb unit survives for about four generations before carbohydrate reserves in the scales and leaf bases are exhausted and form a dry papery tunic (Rees, 1972). Therefore, a range of bulb types (based on their terminal or lateral origins) may be present (T = terminal, L = lateral) (from Rees, 1972):

Generation	**First**	**Second**	**Third**	**Fourth**
	T	TT	TTT	TTTT
	L	TL	TTL	TTTL
		LT	TLT	TTLT
			LTT	LTTT
			LTL	LTTL
				LTLT
				TLTT

TLTL

This idealised model of *Narcissus* bulb unit production means that bulb units increase in a Fibonacci series (1,1,2,3,5,8,13...) (Rees, 1969), a real increase in bulb terms of 1.6-fold per generation, 38.2% of which will be laterally originating bulbs (Rees, 1969; Hanks, 1993). However, daughter bulb unit production may be suppressed under certain unfavourable environmental conditions (Rees, 1972). Actual average unit production has been reported at 2.2 units per annum, of which 19% originated in the axils of bulb scales (not accounted for above) (Okada & Miwa, 1958 cited in Hanks, 1993). Composition of lateral bulbs may differ from terminal units, the former possessing fewer parts in 'Fortune' and it has a lower flowering rate (Rees, 1969). It is possible that the differences between terminal and lateral units is due to the later initiation date of lateral units (up to 40 weeks depending on cultivar), the consequences which are evident even in the next generation (Rees, 1969).

Scales and leaves of terminal units are fully initiated during summer and develop rapidly until they slow during winter followed by another period of rapid growth in the spring (Rees, 1969). Following initiation of the leaves the apex is inactive until the spring when the inflorescence is initiated (Rees, 1969). The inflorescence reaches anthesis in the next spring (Rees, 1969). If floral initiation does not occur, leaf initiation by the apex is increased (Rees, 1969). Development of laterals continues at a steady rate from the time the unit is initiated ten months after the terminal unit, until floral initiation occurs, a few months after the terminal unit (Rees, 1969). It has been suggested that the later

initiation of lateral inflorescences is due to suppression of the lateral apex by the terminal unit (Rees, 1972).

Following differentiation, cell division occurs in the base of scales and in the sheaths and an intercalary region of the blades (Rees, 1972). Most cell division in the blade occurs in the basal 40 mm and is more rapid in the palisade cells than in the parenchyma or epidermis (Rees, 1972). Elongation of cells ceases approximately 50 mm up the blade, with intercalary division slowing when the leaf reaches about 50% of its final length (Rees, 1972). Single or multiple inflorescences (cymes) are borne on a leafless scape (Rees, 1972). Initially this internode is mostly meristematic, but subsequent division is restricted to a basal meristem (Chan 1952 cited in Hanks & Rees, 1975). Elongation of the scape is partially controlled by auxins originating from the gynoecium (Hanks and Rees, 1975; 1977).

Periodicity of aerial organs is probably related to seasonal changes which cause stress to the plant. This assertion is highlighted by the evergreen habit of bulbs such as *Hippeastrum* and *Clivia* (Amaryllidaceae) when found in the relative climatic uniformity of mountainous equatorial and subtropical zones (Rees, 1972; Le Nard & De Hertogh, 1993a). If, however, *Hippeastrum* bulbs are grown in conditions of low temperature or low moisture, seasonal periodicity of inflorescence and foliage development may be observed (Okubo, 1993). Recently, *Nerine* has been demonstrated to be highly plastic in regards to its periodicity (Shillo *et al.*, 1997), however, patterns of leaf emergence and the impact of leaf number and/or turnover have not been described for environments in

which the bulb phenology is hysteranthous or evergreen. This is probably due to an assumption that leaf numbers in the Amaryllidaceae are largely species dependant (Theron & Jacobs, 1994a). As has been demonstrated above, vegetative and reproductive phases of apical activity have been observed to be related in other species. Therefore, the phenology of foliage emergence may be an important factor in flowering periodicity in *Nerine*. A large proportion of the information available on flowering in bulbs relates to the artificial manipulation of the flowering process in controlled conditions. These techniques and the associated research provide some valuable insights into the processes controlling flowering in bulbs. In order to examine these in some detail, a general understanding of the process of inflorescence initiation in bulbs is required because treatments are often based on this process.

Inflorescence Initiation and Development

Development of the inflorescence in bulbs follows a similar pattern in most species once initiation has occurred. However, patterns of initiation may vary. Variability in flower initiation and subsequent development of the inflorescence is illustrated in the following examples, based on the classification of the behaviour of commercial bulbs by Hartsema (1961).

1. Initiation in spring or early summer shortly after anthesis. New buds reach anthesis in the following year, e.g. *Narcissus*.
2. Initiation occurs after the aerial organs are senescent ("rest"), e.g. *Tulipa*.

3. Initiation occurs at low temperatures in winter or early spring, e.g. *Iris*.
4. Initiation more than a year before flowering, e.g. *Nerine*.
5. Initiation of flower primordia alternates with leaf initials during growth period, e.g. *Hippeastrum*.

This generalised classification is not, however, always applicable even at a generic level. *Lilium*, for example, has been divided into four categories based on the time of flower initiation (Baranova, 1972). The first group contains those types which begin floral initiation in summer and development continues until autumn. In the second group are those *Lilium* spp. which initiate flower development in summer but do not finish till the following spring. The third group is those species which initiate buds in spring prior to flowering in late spring or early summer. The fourth group comprises of those that initiate flower buds after the scape has begun elongating in early summer, flowering in late summer to early autumn.

It is clear that the periodicity of initiation and flowering fluctuates widely in bulbous geophytes and even within a genus. It is also evident that in many instances flower formation occurs over a long period of time and may be simultaneous with other processes such as leaf formation, root growth, or vegetative reproduction. It is unlikely, therefore, that there are uniform physiological conditions or states that result in inflorescence initiation in all species. In view of the diversity of temperatures and physiological states required for this process, it is likely that there are a wide variety of mechanisms in bulbs which could entail variations ranging from

enzyme systems and carbohydrate metabolism to genetic sensory mechanisms.

When floral initiation does occur, subsequent development of bulb inflorescences may be described in terms of five successive stages (De Hertogh *et al.*, 1983; Le Nard & De Hertogh, 1993a):

1. Induction
2. Initiation
3. Organogenesis
4. Maturation and growth of floral organs
5. Anthesis

Flower maturation includes differentiation of sporogenous tissues, meiosis, and pollen and embryo sac development (Beattie & White, 1993). Anthesis refers to flowering, or the opening of the flower bud to the setting of seed (Blackmore & Toothill, 1984).

Abbreviation Symbol	Stage of Development
I	Vegetative meristem
II	Transition of meristem - evident as doming
Pr	First flower primordia visible
Sp	Development of spathe (if present)
Br	Development of bracts (if present)
Bo	Development of secondary bracts (if present)
P_1	First whorl of perianth
P_2	Second whorl of perianth

A_1	First set of androecium
A_2	Second set of androecium
G	Development of gynoecium
Pc	Paracorolla (if present)

Table II.1: Standardised abbreviations for stages of floral initiation and development (Beijer, 1942; Le Nard & De Hertogh, 1993a).

Where induction is the stimulation of the apical meristem that leads to initiation. Initiation describes the conversion of the apical meristem from vegetative to reproductive (Beattie & White, 1993). Organogenesis (differentiation) is the development of amorphous cells into specialised cell types resulting in the formation of floral parts or organs (Blackmore & Toothill, 1984). The stages of inflorescence initiation and development are represented by standardised abbreviations formulated by Beijer (1942). Although originally used to describe organogenesis in *Tulipa*, they have been applied to a range of other bulbous species, particularly to the Liliaceae and Amaryllidaceae families (e.g. De Hertogh *et al.*, 1976). These are listed in the table above.

Where required, the terminology and the flowering process may be species specific where required such as for *Hippeastrum* spp. (Blauw, 1931). The following table of standardised abbreviations for flower initiation may also be applied to each individual *Hippeastrum* flower (Okubo, 1993):

Stage	Description
I	Vegetative meristem
II	Last leaf and new growing point formed. Old growing point oval-shaped and becomes inflorescence
III	First of two spathe leaves formed at old growing point
IV	Development of second spathe leaf
V	Inflorescence primordium divided to form flower one and two with associated bracts. Also possible lateral flowers originate from this point
VI	Bracts have split from flowers.
VII	Development of three outer tepals
VIII	Development of inner tepals
IX	Development of outer whorl of stamens
X	Emergence of all stamens
XI	Carpels present as three distinct primordia

Table II.2: Stages of flower initiation in *Hippeastrum* spp.

The understanding of the flowering process in bulbs has provided a valuable framework on which to base the control of the flowering process by artificial systems. This is largely due to the ability to accurately assess and describe stages of inflorescence initiation and development. This has become increasingly important, as it will be shown in the following section, because the climatic requirements for maintaining the optimum phenological periodicity in flower bulbs may vary between these stages of development. Although the information that may be obtained from these studies is not necessarily biochemical or even physiological in nature,

some valuable insights into the nature and mechanisms of flowering may be gained.

Regulation of flowering

The control of the flowering process may not only produce early flowering, but also it may be used to delay flowering, preventing flowering, or inducing abortion of the inflorescence (Le Nard & De Hertogh, 1993). A prerequisite for control of flowering is the knowledge of the timing of initiation in relation to the life cycle of the bulb (Le Nard & De Hertogh, 1993). There are only a very limited number of bulb species that have been investigated sufficiently to provide a comprehensive overview of the regulation of the flowering process. Control of the flowering process in these species invariably has commercial significance. In this review, control of flowering will be discussed in terms of the stages of flowering listed previously, i.e., induction, initiation, organogenesis, maturation, anthesis, as well as artificial control of this process. Because the process is continuous there is little distinction between initiation and organogenesis stages (Beattie & White, 1993). The following review is intended to be a background for later discussion of aspects of the flowering process in *Nerine*

Tulipa

The flowering process in *Tulipa* has been extensively described (e.g., Hartsema, 1961; Rees, 1972; Shoub & De Hertogh, 1975; De Hertogh *et al.*, 1983; Le Nard & De Hertogh, 1993). Provided the bulb is large enough, temperature is the primary factor influencing

development of the inflorescence (De Hertogh *et al.*, 1983), although light intensity and nutrient levels impact upon flower quality (De Hertogh *et al.*, 1983). Moisture stress and ethylene have been found to produce inflorescence abortion (bud blasting) (De Munk & Hoogeterp, 1975).

Tulipa is harvested with the apical meristem in a vegetative state (Hartsema, 1961; Shoub & De Hertogh, 1975). Induction of the apex to initiate reproductive organs is controlled by the number of leaves and bulb size (Hartsema, 1961). As discussed previously, a minimum of 2 leaves must be produced by the meristem if a flower is to be initiated (Shoub & De Hertogh, 1975), usually non-flowering size bulbs produce only a single leaf (Hartsema, 1961). The minimum size bulb required for floral initiation to occur is 6-7 cm in circumference (8 g), with a standard commercial requirement of at least 10 cm (De Hertogh *et al.*, 1983). This general size requirement does not, however, apply to so-called "H" bulbs located inside the tunic (De Hertogh *et al.*, 1983). Vascular connections with the mother bulb are required for floral induction in this small bulb and its ability to initiate and develop a flower (De Hertogh *et al.*, 1983). Whether this is due to translocation of growth regulators (promoting initiation), availability of carbohydrate from the mother bulb, or a weakening of apical dominance with distance from the apical meristem is unknown (De Hertogh *et al.*, 1983; Le Nard & De Hertogh, 1993b). A similar effect of accelerated bulblet flowering (less than a year from initiation) has been observed in *N. bowdenii* under some environmental conditions (Shillo *et al.*, 1997).

Commercially bulbs are stored when inflorescence initiation and organogenesis occurs and, therefore, the annual warm-cool-warm cycle must be simulated artificially. The rates at which organogenesis and maturation take place is determined by the storage temperature used (Hartsema, 1961). Normally flower initiation and organogenesis takes place at 15-20°C (Hartsema, 1961). The initial stage of inflorescence maturation also requires this temperature, although a period of 14-22 weeks of chilling is required for complete maturation to occur (De Hertogh *et al.*, 1983). If the bulb is exposed to low temperatures (2°C) at an early stage of differentiation the bud will not elongate and eventually abort, even though organogenesis may later be completed (Le Nard & De Hertogh, 1993). If this temperature is applied later during bud initiation, the stem that develops is slender and shows aborted flowers (Le Nard & De Hertogh, 1993). Since a detailed discussion of storage temperatures follows, it is sufficient to say that at this stage that storage temperatures can have a variety of effects on flowering, e.g., such as determining the number of flower parts, scape length, and date of anthesis (Le Nard & De Hertogh, 1993). The rate of inflorescence differentiation is increased if the bulb is initially exposed to high temperatures (30-35°C) before being returned to the optimal 17-20°C (Le Nard & De Hertogh, 1993). Prolonged exposure to high temperatures has the effect of delaying bud differentiation, suggesting that this pre-treatment has a residual effect on the bud that is only realised once the bulb is placed at low temperatures (Le Nard & De Hertogh, 1993). No such effect is found if high temperatures are applied to bulbs possessing a developed flower bud, which is possibly related to observations

that the higher temperatures appear to promote leaf initiation (Le Nard & De Hertogh, 1993).

The final stages of inflorescence development are completed after planting in the field or glasshouse, ideally at temperatures of 10-17°C (De Hertogh *et al.*, 1983). Higher temperatures for extended periods may result in problems, such as floral abortion, stem topple or generally reduced yields (Dosser & Larson, 1981; De Hertogh *et al.*, 1983). The ability of a scape to extend is apparently directly affected by the gynoecium, as removal of this organ from the flower bud results in much reduced internodes - similar to those of blind stems (op den Kelder *et al.*, 1971; Hanks & Rees, 1977).

The period from harvest to placing bulbs in a greenhouse is called the "programming phase" (De Hertogh, 1974). The bulb is then "forced" to flower in a temperature controlled environment (De Hertogh *et al.*, 1983). The primary objectives of a forcing program are the prevention of abortion, achieving an optimum stem height and flower size, uniform flowering, and to minimise the time bulbs occupy greenhouse space (De Hertogh *et al.*, 1983). Physiological activities which are directly affected during this period are flower development, root growth, low temperature mobilisation of storage carbohydrate, and leaf and scape growth (Hartsema, 1961). Forcing and the degree of forcing that can be achieved is species and cultivar dependant (De Hertogh *et al.*, 1983). In *Tulipa,* bulbs that are larger than the minimum flowering size are used (usually larger than 12/up cm) to promote larger flower sizes (De Hertogh *et al.*, 1983). It should be noted, however, that very large bulbs of

some cultivars are susceptible to blindness or "antholyse" (Schenk, 1971 cited in De Hertogh *et al.*, 1983). This condition causes a full leaf complement to be formed without an inflorescence and has been found to effect 'Apricot Beauty,' 'Demeter', and 'Yokohama' bulbs in the 16/18 cm size range (De Hertogh *et al.*, 1983).

Forcing techniques simulate annual seasonal thermoperiodic cycle of the bulbs native habitat in either a compressed or extended sequence to shorten or lengthen time to anthesis (De Hertogh *et al.*, 1983). Normal forcing for spring flowering involves regularly decreasing temperatures after an initial storage period at warm temperatures (>20°C) (De Hertogh *et al.*, 1983). These applications of artificial temperatures may be referred to as "programming" (De Hertogh, 1974). Bulbs are planted and subjected to a rapid temperature drop from 18°C to 9°C for rooting to occur (De Hertogh *et al.*, 1983). The rooting temperature of 9°C represents a compromise between chilling the bulb and the optimal rooting temperature (De Hertogh *et al.*, 1983). When roots have reached at least 5 cm the temperature is reduced to 5°C. If shoots reach 5 cm, the temperature is further reduced to 0-1°C to retard further development of the shoot (De Hertogh *et al.*, 1983). Bulbs are given a total of 13-22 cold weeks followed by 13-17°C greenhouse conditions where the bulb will reach anthesis after approximately 14-25 days (De Hertogh *et al.*, 1983). A modification of this standard forcing technique is to store bulbs initially for a week at 34°C followed 20 and 17°C until the inflorescence has reached or passes "Stage G" (De Hertogh *et al.*, 1983). The high initial temperature (34°C) followed by a significantly lower one (17-20°C) results in accelerated floral and root organogenesis (Le Nard & De

Hertogh, 1993). A precooling treatment is then administered, followed by a 12 week rooting period and three weeks at 5°C (De Hertogh *et al.*, 1983). Bulbs are then planted in the greenhouse for early summer flowering (De Hertogh *et al.*, 1983). However, as this process uses all accelerating temperature optima combined many *Tulipa* cultivars cannot be forced in this manner (De Hertogh *et al.*, 1983).

Bulbs also may be chilled at 5°C immediately or shortly after reaching "Stage G" in a process known as special precooling (De Hertogh *et al.*, 1983). This technique does not include the rooting of bulbs during the chilling phase; instead the stored bulbs are cooled dry and rooting occurs in the greenhouse (De Hertogh *et al.*, 1983). With this system glasshouse temperatures are lower (13-15°C) and the period to anthesis after planting is longer (45-50 days) (De Hertogh *et al.*, 1983).

Alternatively, the freezing technique may be used which follows the procedure used for early summer flowering (standard precooling), except after bulbs are well rooted they are placed in temperatures as low as -4°C and kept moist (De Hertogh *et al.*, 1983). After defrosting, they will be forced quicker than bulbs subjected to the previous two systems and the number of cultivars suitable for this freezing method of forcing are low (De Hertogh *et al.*, 1983).

Many aspects of the forcing process involve compromises between optimal temperatures for different processes. For example colder temperatures and extended chilling produces an inflorescence with a longer scape, however, scape diameter and flower size become

reduced (De Hertogh *et al.*, 1983; Le Nard & De Hertogh, 1993). Similarly, extended initial periods of high temperature will increase the rate of later root development, while an adverse effect can be detected with regard to the rate of bud development (Le Nard & De Hertogh, 1993). Greenhouse conditions can also be optimised, warm day temperatures up to 18°C will accelerate flowering and can be combined with a low night temperature to increase scape height (Dosser & Larson, 1981).

Despite observations that application of gibberellins (GAs) could substitute some of the cold requirement of *Tulipa* (Hanks, 1982), there appears to be no correlation between cold-treatment and endogenous GA levels indicating that they are not the cold-induced trigger for scape elongation (Rebers *et al.*, 1995; Rebers *et al.*, 1996). However, there is evidence to suggest that GAs do have a role in the elongation response (Rebers *et al.*, 1995).

Iris hollandica (Dutch iris)

Iris hollandica are hybridised *I. xiphium* and *I. tingitana* varieties and are collectively known as Dutch irises (van Scheepen, 1991; De Munk & Schipper, 1993). Bulbs are used either for cut-flowers or for multiplication, in which case flowering is suppressed (De Munk & Schipper, 1993). Flowering plants (minimum bulb size of 8/9 cm in circumference) produce a scape with at least five leaves and a number of buds which reach anthesis in the spring (De Munk & Schipper, 1993). Primordial scales are initiated after flowering in the summer and have a lifespan of 21-22 months, after which the mother bulb is replaced by daughter bulbs (De

Munk & Schipper, 1993). Discussion of flowering in the group will be based on the growth cycle under commercial conditions..

Bulbs require an annual sequence of warm-cool-warm to develop normally (Hartsema, 1961). Flower bud induction is completed after the formation of 3-4 leaf primordia at high temperature, followed by a period of low temperature (Hartsema, 1961; Elphinstone & Rees, 1990). If a maximum of ten leaves is reached the apex will automatically become reproductive (Elphinstone *et al.*, 1988). If the bulb is not exposed to an adequate low temperature treatment, flowering will not occur and leaf production will continue (Hartsema, 1961), although this temperature must be as high as 26°C (Rees, 1972). Flower initiation and differentiation is optimal at low temperatures (9-13°C) and may occur after planting or in storage (Hartsema, 1961). Unlike *Tulipa*, the first stage of initiation is A_1, (stamen formation - see "Inflorescence Initiation and Development"), possibly indicating the importance of these organs in the regulation of initiation and development of other floral organs (De Munk & Schipper, 1993). Several floral primordia may be initiated, however, most of these inflorescences will abort, although the reason for this is unknown (De Munk & Schipper, 1993).

Differentiation and growth of floral organs are significantly effected by temperature in storage (De Munk & Schipper, 1993). As has been previously mentioned, a warm post-harvest treatment (> 20°C but usually > 30°C) is necessary for floral induction, but must be followed by a cool period (< 17°C) for initiation to occur (Hartsema, 1961; De Hertogh, 1974; Atherton *et al.*, 1988). The warm

temperature period also promotes leaf formation and suppresses the formation of a daughter bulb at the apex (Atherton *et al.*, 1988). Bulbs will not initiate inflorescences at temperatures of 25-30°C and may be maintained at this temperature for prolonged periods, making year-round glasshouse forcing possible (Hartsema, 1961; De Munk & Schipper, 1993). Three types of programming regimes exist for producing early, normal, and year-round flowering times (De Hertogh, 1974). A short period of heat treatment (< 15 days) is used to accelerate development of the bulb for early flowering and retard development of the terminal bud (De Hertogh, 1974). If no warm treatment is applied an apical daughter bulb is formed instead of aerial organs (De Munk & Schipper, 1993). Following the warm treatment a two week period at 17 °C may be applied which can increase plant quality and reduce flower blasting (De Hertogh, 1974). At this stage, meristems are vegetative and must be placed at a cultivar dependant optimum temperature for the apex to become reproductive (De Hertogh, 1974). Early transfer to low temperatures results in only scale and leaf production (De Munk & Schipper, 1993). Length of this treatment (9-13°C) can vary between six and thirteen weeks (De Hertogh, 1974; De Munk & Schipper, 1993). Low temperatures also have the effect of promoting rapid shoot growth and increasing bulb size (De Munk & Schipper, 1993). Slightly longer periods at low temperature will increase the rate of shoot development and flowering time at the expense of shoot height and satisfactory flower development (De Munk & Schipper, 1993). A substantially longer duration will produce bulbs in which no root or shoot growth occurs (De Munk & Schipper, 1993).

Heat treatments can be partly replaced by treating bulbs with ethylene gas, or by dipping bulbs in a solution containing ethephon, which have a significant effect on floral induction and flowering (De Munk & Schipper, 1993). Preliminary studies have also found favourable effects of gamma rays and infra-red irradiation on bulbs at this stage of programming (De Munk & Schipper, 1993).

Temperature, moisture, and light intensity are critical factors for controlling growth and development in the greenhouse (De Hertogh, 1974). Optimal day temperature is 15-16°C, night temperatures are not as critical and may be as high as 23°C (Fortranier and Zevenbergen, 1973 cited in De Hertogh, 1974). Bud abortion can occur in *Iris* under low greenhouse light intensities; although genotype, morphology and temperature are contributing factors (De Munk & Schipper, 1993). The use of large bulbs and ethylene curing during programming also reduces the risk of abortion (De Munk & Schipper, 1993) In addition, plants possessing long leaves are more susceptible to poor light conditions (De Munk & Schipper, 1993).

Low irradiances and high temperature and water stress can cause inflorescences to abort, which can be partly avoided by the injection of cytokinin into the bud (Atherton *et al.*, 1988).

Lilium longiflorum

Lilium longiflorum (Liliaceae) is a perennial herbaceous bulb that generally produces a single multi-leafed stem bearing several flowers at its apex. Two stems are developed concurrently, one

which develops in the current year and the other will flower the following season - these are referred to as the primary and secondary axes respectively (Miller, 1993). The secondary axis is initiated at the base of the primary axis in early summer, floral induction occurring during winter and inflorescences are initiated in early spring. Bulbs have a cumulative vernalisation requirement (De Hertogh, 1974), after which the degree of temperature increase determines the rate of shoot growth and flower development (De Hertogh, 1974; De Hertogh *et al.*, 1976). In the field buds reach anthesis in the summer after elongation of the stem and expansion of leaves (Miller, 1993). Flowers are classified according to their point of initiation (De Hertogh *et al.*, 1976). Initial primary buds arise from the apical meristem, secondary flowers are produced in the axil of the pedicel bracts and raised primary inflorescences are a product of a short stem produced by the apical meristem above the original primary buds (De Hertogh *et al.*, 1976; Miller, 1993). The number of leaves produced is dependant on environmental conditions, and does not effect bud development (Miller, 1993). Organogenesis is described using the inflorescence differentiation description used for *Tulipa* (see previous section "Inflorescence Initiation and Development") (De Hertogh *et al.*, 1976). Floral organogenesis and development in *L. longiflorum* follows a logarithmic pattern correlated with micro- and megasporogenesis (Erickson 1948 cited in Miller, 1993). Floral organs of *L. longiflorum* have been used as a model for the study of floral cell division, elongation, and differentiation (Gould & Lord, 1988; Gould & Lord, 1989). Growth of anthers was found to follow a basipetal wave of mitotic activity and cell elongation (Gould & Lord, 1988). Hypotheses for the waveform pattern of division and

elongation include: (1) Pulses of a stimulator (or inhibitor) of growth is transported down the organ, stimulating firstly mitotic activity then promoting cell elongation; (2) The wave may represent a trigger that is propagated autonomously and caused by the loss of turgor accompanying cell division which in turn stimulates mitotic activity in the cells below; and (3) Waveforms are generated in electrical impulses similar to those measured in roots (Gould & Lord, 1988). Investigation of tepals revealed that the waveform may be replaced by strong basal growth during the most rapid phase of elongation followed by apical cell elongation and reduction of growth rate (Gould & Lord, 1989).

As is the case with most bulbs, processes in *L. longiflorum* are significantly influenced by temperature (Miller, 1993). This permits control of the growth cycle. An annual warm-cool-warm temperature regime is required which is achieved by lifting bulbs in late summer (with a vegetative meristem), placing them at 2-10°C for a six weeks (1000 hour) followed by planting in the glasshouse (16-18°C) (De Hertogh, 1974; Miller, 1993). Precise programming temperatures are dependant on bulb size and cultivar (Miller, 1993). Unlike *Tulipa* and *Iris*, floral initiation, organogenesis, maturation and anthesis occur during forcing in the glasshouse (De Hertogh, 1974). Programming remains important, however, as the uniformity and timing of the crop can be regulated by determining the maximal rates of bulb development (De Hertogh, 1974).

Cold temperature programming can be accomplished by natural cooling, in which the bulbs are shipped directly to a grower who

plants them outdoors to accumulate the required six weeks of chilling (De Hertogh, 1974). Alternatively, they may be "precooled" by placing the bulbs at 2-5°C followed by 5-7°C for a total of six weeks (De Hertogh, 1974). Thirdly, in a process known as controlled temperature forcing, bulbs are planted and placed at 17°C for 2-4 weeks for roots and shoots to develop followed by six weeks at 2-5°C (De Hertogh, 1974). The presence of an established root system is probably the cause of the increased floral bud numbers and increased leaf growth observed in bulbs treated with cold temperature forcing (Miller, 1993). Low temperature programming promotes shoot bolting, reduces leaf number and plant height, reduces time to flowering, and promotes uniformity of flowering times (De Hertogh, 1974). However, flower numbers are also reduced by the period of cold temperature treatment, which is a compromise to achieve optimal height (causes a reduction leaf number and internode length) (De Hertogh, 1974). The maintenance of strict moisture levels must be observed in all of the above treatments or time of flowering will be effected (Miller, 1993).

The first stage of development after transfer to the greenhouse is the transition of the apical meristem(s) to a reproductive state (De Hertogh, 1974). The date of this event is dependant upon the programming temperature and duration and greenhouse conditions (De Hertogh, 1974; De Hertogh *et al.*, 1976). The number of floral primordia produced is a function of greenhouse temperatures and can vary greatly (De Hertogh, 1974). Inflorescence number in 'Croft' and 'Ace' is highest between 16°C and 18°C (De Hertogh, 1974). Because leaf production ends with inflorescence initiation, it is possible to force stems to flower with a

known number of leaves by dissecting representative plants to determine the time in which the flower forming stimulus is applied (Blaney *et al.*, 1967 cited in De Hertogh, 1974). Optimal flower development is achieved with a 12 hour photoperiod and a temperature of 21°C (De Hertogh, 1974). Cool temperatures applied early in the day or warm night temperatures may also be applied to further regulate stem height (Miller, 1993).

Because *L. longiflorum* is daylength sensitive, photoperiod and light intensity are also critical for initiation and development of the flower (De Hertogh, 1974). Interrupting the night period or lengthening the day period allows the length of bulb cooling to be curtailed during the first stage of greenhouse forcing (De Hertogh, 1974). The bulb itself is also sensitive to photoperiod indicating the presence of active phytochrome in the scales (Roh & Wilkins, 1974 cited in Miller, 1993). Bud weight and size can be increased by increasing levels of irradiance (Miller & Langhans, 1989).

Height control can also be achieved by the use of exogenous plant growth regulators (Miller, 1993). Gibberellin synthesis inhibitors such as ancymidol [α-cyclpropyl-α-(ρ-methoxyphenyl)- 5-pyrimidine- methanol] and uniconazole [(E)-1-(ρ-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] effectively reduce internode growth (Miller, 1993). In contrast, paclobutrazol, another well known gibberellin synthesis inhibitor that is closely related to uniconazole, has no effect on stem height (Miller, 1993).

Therefore, it is evident that the impact of environmental factors such as light and temperature on the development of bulb

inflorescences is marked. It is usual for a combination of temperatures to be used in bulb storage and forcing in order to reproduce the normal annual seasonal variation of the bulb. Although some progress has been made into the replacement of temperature requirements with chemical treatments, it is evident that the degree to which these processes are understood at a physiological and biochemical level is very limited. A forcing program is also used for *N. bowdenii* which utilises the bulbs 12 month cycle - bulbs are grown in the glasshouse for 8-9 months and stored at 2-5°C for 3-4 months (Groen & Kok, 1997). However, bulbs cannot reliably be forced from late winter to early spring possibly due to warm temperatures encountered before storage. This effect could be counteracted to some extent by chilling bulbs at a lower temperature (to 0°C) for a longer period and using a precooling treatment of 13°C (Groen & Kok, 1997). The effect of high temperature on the bulb (if this is indeed the problem) may be mitigated by the lower storage temperature or the longer storage period. Unfortunately, growth of bulbs for flower production outdoors does not allow this type of temperature regulation to be used. It is evident that the bulbs have a cold requirement during the "rest" period and also that bulbs may require an intermediate storage period when being transferred between warm outdoor environments to cold storage. It is possible, therefore, that precise temperature treatment regimes may be able to be applied to further enhance growth and development of the inflorescence as has been demonstrated for other bulb species. One way of addressing this issue and the application of beneficial treatments to field grown crops is to obtain a complete knowledge of bulb physiology; i.e., a study of bulb physiology beginning with the basics and progressing

towards specific areas in order to determine what treatments or conditions may be required to achieve superior and reliable flowering.

Partitioning of Carbohydrates to Inflorescences

Introduction

A review of the literature in development and flowering in ornamental geophytes has demonstrated the influence of bulb size, temperature, light, and developmental state on flower bud development and shoot growth. These examples are, however, simply observations of cause and effect. They lack an understanding of the underlying processes which these factors directly effect and how these changes impact inflorescence development. Chilling, for instance has been implicated in the increase of enzyme activities and levels of carbohydrate in the bulb scales and producing faster growth and longer scapes (Moe & Wickstrom, 1973; Nowak *et al.*, 1974; Thompson & Rutherford, 1977; Moe & Wickstrom, 1979). However, neither the observed carbohydrate changes nor programming conditions definitively controlled the process of stem elongation (Thompson & Rutherford, 1979). There are aspects of organ status and interrelationships that must be investigated even in highly studied bulbous crops. In fact, Le Nard and De Hertogh (1993a) have gone as far to say that the "...practical approach has been an obstacle to detailed physiological and fundamental approaches on growth and development." As the role of carbon partitioning has been implicated in flower bud abortion in *Nerine* (Theron & Jacobs,

1996a) it appears to be a logical initial step. In the following discussion it has been necessary to refer to many studies outside the specific area of bulbous geophytes in order to achieve a basic understanding of the processes controlling plant partitioning and flower bud development. As a background to some of the concepts encountered in my study, the factors regulating assimilate supply as pertaining to geophytes are briefly examined beginning with the carbon source, carbon transport, phloem unloading at the sink, and competition between sinks.

Storage Carbohydrates

Often carbon assimilated at a sink has been derived from long-term or short-term storage carbohydrates. This is particularly true, of course, for geophytic species in which rapid seasonal growth is supported, possibly even initiated in some instances, by the mobilisation of stored carbon. It is necessary, therefore, to examine the method by which this carbon is stored and the events characterising their hydrolysis.

Starch

It is used as a short term storage polysaccharide in chloroplasts during photosynthesis and as a long term storage carbohydrate in seeds and storage organs (Goodwin & Mercer, 1990). Starch is always stored and formed in plant plastids. In photosynthesising organs this is the chloroplast, while it is the amyloplast in storage tissue (Goodwin & Mercer, 1990). Starch polysaccharides are highly organised structures built up by the accumulation of layers of α-D-

glucopyranose residues (Goodwin & Mercer, 1990). Shapes include spherical, ovoid, lens shaped or irregular, specific shapes and sizes of the molecules is often a characteristic of the plant species (Goodwin & Mercer, 1990). Starch granules have two components which form complexes; the linear polymer amylose and the branched chain polymer amylopectin (Duffus & Duffus, 1984). Amylose usually accounts for approximately 25% of starch content (Duffus & Duffus, 1984). It is slightly branched, but a mainly linear polymer of 1000-2000 D-glucopyranose units joined by α-(1→4)-glycosidic linkages which are packed and folded in a helical manner within the starch granule, making it readily accessible to hydrolytic enzymes. Amylopectin are branched chains of 20-25 D-glucopyranose units bonded by α-(1→6)-glycosidic linkages and contain a large number of non-reducing sugars per molecule.

Sucrose is the most common source of starch carbon, which is either converted to glucose and fructose or directly to the sugar nucleotides ADPglucose or UDPglucose and fructose (Preiss, 1982). There are only two known cytosolic enzymes capable of metabolising imported sucrose: alkaline invertase and sucrose synthase (ap Rees, 1996). Alkaline invertase is distinct from vacuolar invertases, which are discussed in the following section, and acid wall invertases, and are inhibited by very high levels of fructose (ap Rees, 1996). The resultant hexoses as then phosphorylated to 6-phosphate esters by kinases which are either virtually exclusive to fructose or have a preference for glucose (ap Rees, 1996).

As mentioned previously, sucrose synthase (SS) catalyses conversion of sucrose and UDP to UDPglucose and fructose and vice versa (ap Rees, 1996). SS will also catalyse the conversion of ADP and sucrose to ADPglucose at a lower rate, however, production of ADPglucose by a cytosolic SS *in vivo* has not been proven (ap Rees, 1996). The questions of how the glycosidic unit becomes available from UDPglucose and why two sucrose hydrolysis pathways exist have not been established (ap Rees, 1996). Data indicates that ADPglucose is the direct precursor of storage starch and it is synthesised in the amyloplast following the conversion of hexose monophosphates to glucose-1-phosphate (ap Rees, 1996). There is also evidence of an alternative pathway in sycamore (*Acer pseudoplatanus*) involving cytosolic production of ADPglucose and transport of these triose-phosphates into amyloplasts (Pozueta-Romero & Akazawa, 1993). A number of starch synthase and branching enzymes have been identified. However, the mechanism by which two complex polymers are formed in characteristic shapes and in a precise ratio from the single precursor is poorly understood, as is regulation of starch synthesis in storage organs in general (ap Rees, 1996).

Hydrolysis of starch involves several enzymes which produce the monosaccharide component, glucose (Goodwin & Mercer, 1990). Degradation of amylose is catalysed by α-amylase, producing maltodextrins (α-1,4-dextrins), which are in turn hydrolysed to maltose, maltotriose and glucose (Duffus & Duffus, 1984). α-Amylase also hydrolyses amylopectin chains excepting α-(1→6)-branching junctions producing glucose maltose, maltotriose and α-1,4-:α-1,6-dextrins (Duffus & Duffus, 1984). Maltose inhibits the binding of α-amylase to amyloplasts and the breakdown of maltose by maltase

may be an important prerequisite of starch hydrolysis (Duffus & Duffus, 1984). Some species also contain a β-amylase to hydrolyse the very last α-1,4 glycosidic linkage from the non-reducing end of an α-1,4 glucan (Goodwin & Mercer, 1990). Thus, there is the ability to cleave off successive maltose units until chains are reduced to within two or three residues of the α-1,6 linkage. Starch phosphorylase catalyses the formation of D-glucose-1-phosphate from maltodextrins, completely converting all starch residues to this form, except those associated with α-1,6 branching points (Goodwin & Mercer, 1990). Hydrolysis of this linkage is catalysed by amylopectin 6-glucanohydrolase and oligo-1,6-glucosidase, leaving only glucose and short chain α-1,4-dextrins (Goodwin & Mercer, 1990). The remaining α-1,4-linked glucans and maltose are reduced to D-glucose through the action of α-glucosidase (Goodwin & Mercer, 1990). Some D-glucose 1-phosphate is also produced from glucose by starch phosphorylase (Goodwin & Mercer, 1990).

Fructan

Although starch is the most widely known storage polysaccharide it has been estimated that about 15% of angiosperm flora contain fructans (formerly fructosan) (Hendry, 1993). Utilisation of both storage forms is not unknown (Albrecht *et al.*, 1997). Where both occur the fructan and sucrose synthesis pathways usually dominate over those that result in starch formation (Lewis, 1984; van der Meer *et al.*, 1994), although this not the case in *Tulipa* (Ohyama *et al.*, 1988; Lambrechts *et al.*, 1994). Fructans (β-D-fructofuranose polymers releasing fructose when hydrolysed) are found in a wide range of temperate, mainly perennial,

monocotyledonous and dicotyledonous plants as well as bryophytes, fungi, bacteria and algae (Pollock, 1986). Plants containing genes for fructan synthesis may be considerably more widespread, though not expressed, particularly in tropical grass species (Hendry, 1993). The degree of polymerisation (DP) of these molecules in plants ranges from trisaccharides (e.g., Livingston *et al.*, 1993) to a DP of c.170 (Isejima & Figueiredo-Ribeiro, 1993). However, a strict terminological analogy with glucan would indicate a polymer of more than ten units (French, 1989).

The ecological significance of fructan is often unclear as species accumulating this form of carbohydrate often coincide with others that do not (Pollock, 1986). Plants with the ability to form fructans are significant components of ecosystems that experience environmental instability (Albrecht *et al.*, 1997). Thus roles in carbon storage, osmotic regulation, drought and cold tolerance have been suggested. The well documented function of imparting cold tolerance has come into question in regards to a direct response or principal role (Hendry, 1987; Hendry 1993).

Fructans are categorised into two principal types according to the linkages between fructofuranose residues. Firstly, there is the inulin-type, which usually are longer polymers bonded by β2→1 glycosidic linkages and can be considered extensions of the trisaccharide isokestose (1-kestose or 1-F fructosyl sucrose). Second, there is the levan-type comprising of β2→6 glycosidically linked moieties, based on the trisaccharide kestose (6-kestose, 6-F fructosyl sucrose). In both cases the terminal fructofuranose molecule is linked to an α-D- glucopyranose residue via an α1→β2

glycosidic linkage - effectively a terminal sucrose unit (Pollock, 1986). However, at least two naturally occurring fructans lack terminal glucose residues and this structure is not definitive (French, 1989; Ernst *et al.*, 1995).

Inulin is a group of molecular species of roughly 30-35 units (MW 4900-5700) and this type of polymer is present in the underground storage organs of members of the Asteraceae, Compositae, and Campanulaceae (Nelson & Smith, 1986). This form of polymer functions as short- to medium-term storage carbohydrates in these species, and structurally may be shorter polymers than inulin (Goodwin & Mercer, 1983) or much larger (French, 1989).

A third form of oligosaccharides exists based on another isomer of fructosyl sucrose - neokestose (6-G fructosyl sucrose) - in which the glucose residue is linked directly to both fructose moieties. The chain may elongate from either fructose group resulting in inclusion of the glucose unit within the oligomer (Pollock, 1986).

Branched fructans with inulin and levan backbones as well as fructans containing mixed linkages have also been described, as have mixtures of both types (Pollock, 1986).

The synthesis of fructans from sucrose occurs in the plant vacuole and is catalysed by the actions of at least two enzymes (Wagner *et al.*, 1983; Pollock & Cairns, 1991). However, the precise compartmentalisation of fructan synthesis at the subcellular level has not been thoroughly investigated (Koroleva *et al.*, 1997; Turk *et*

al., 1997), and the interactions with fructan polymerases, also found in the vacuole, are not understood (Cairns *et al.*, 1997). The activity of acid invertases, if located in the vacuole would normally hydrolyse sucrose and inhibit fructan synthesis, may be inhibited by free fructose (Oberland *et al.*, 1993; Walker *et al.*, 1996). Alternatively, there is evidence to suggest invertase activity may be located outside the protoplast (Koroleva *et al.*, 1997).

The simplest model of fructan synthesis is found in *Helianthus tuberosus* (Jerusalem artichoke) (Edelman & Jefford, 1968), in which both enzymes, sucrose:sucrose fructosyltransferase (SST) and fructan:fructan fructosyltransferase (FFT) are active. FFT may also be responsible for redistributing fructosyl units among polymers during *H. tuberosus* tuber dormancy and sprouting (Koops & Jonker, 1994). Some invertases will also synthesise all three fructan trisaccharides (Pollock & Cairns, 1991), but they appear to be insignificant with regards to fructan accumulation in leaves of *Lolium* (Guirrand *et al.*, 1997).

However, in plants containing mixtures of fructosyl polymers instead of the simple linear β(2-1) inulin of *Helianthus tuberosus*, synthesis is more complex. Construction of fructans with a DP>5 *in vitro* requires the action of at least two different FFT activities, SST and possibly invertase from *Lolium rigidum* (St. John *et al.*, 1997). Furthermore, SST activity was shown to increase three-fold when the second FFT preparation was added to the first. This could have been caused by unidentified activity in the preparation, the removal of an SST inhibitor, or addition of a SST activator or possibly the synthesis of a fructan necessary for DP>5. However,

this preparation only synthesised two fructan series of the three found *in vivo*, suggesting an unidentified fructan synthesising activity. In contrast SST and FFT preparations from *Viguiera discolor* readily synthesise longer chain polymers from a number of substrates, although the high stability of these preparations enabled incubation for extended periods at high temperatures (Itaya *et al.*, 1997).

A number of possible ecological functions for fructan have been established or hypothesised. These include both long and short term storage of carbon, osmoregulation, and the directing of assimilate supply, cold acclamation and flooding tolerance. Most vascular plant species store photoassimilate as chloroplastic starch in the photosynthetic organs. However, a number of species have higher levels of extrachloroplastic reserves such as fructan, which is usually associated with low starch/sucrose ratios (Pollock, 1986). Formation of fructan is a secondary metabolic event since sucrose is the sole substrate from which fructans can be formed. In temperate Gramineae, fructans are only formed when supply of sucrose in the leaves exceeds demand (Pollock, 1984). They also play a role by buffering temporary imbalances between photosynthetic carbon supply and developmental demands (Roth *et al.*, 1997).

Fructans can be found in extremely high concentration in the storage organs of some plants, e.g., *Viguiera discolor* (Asteraceae) may contain up to 80% fructan (dry weight) (Itaya *et al.*, 1997).

Chromatic characteristics and chain lengths of stored fructan polymers occur during the ontogeny of the plant. For instance, the fructan stored by *Viguiera discolor* is primarily oligomers of a DP<5 during sprouting and flowering (Isejima & Figueiredo-Ribeiro, 1993). However, during dormancy a series between sucrose and high molecular mass (28 kDa) fructans was found. The storage of fructan for long-term utilisation is sink-limited in some cases (Schubert & Feuerle, 1997).

Unlike starch, which is osmotically inert, fructan is soluble to various degrees, depending largely on the type of linkage between moieties. Therefore, it has an effect on water movement within the plant. The most obvious role of fructan in the directing of water and assimilate movement is in actively photosynthesising organs. Through synthesis and compartmentation of fructan in photosynthetically inactive parts of the organ, the mesophyll cells may be able to avoid negative feedback of photosynthesis by high sugar concentrations and prevent excessively negative, and potentially damaging, osmotic potential (Koroleva *et al.*, 1997). Fructans also play a role in the expansion of leaves and petals through the maintenance of osmoregulation and assimilate supply (Schnyder & Nelson, 1989; Bieleski, 1993; Guirrand *et al.*, 1996). In addition it is temporarily deposited in wheat kernels during grain filling (Schnyder *et al.*, 1988).

Another of the ecologically significant aspects of fructans is the relative insensitivity of fructan synthesis to cold temperatures (Pollock & Jones, 1979; Simmen *et al.*, 1993), allowing plants to accumulate carbohydrates even though growth is retarded (Roth *et*

al., 1997). These reserves can be either rapidly mobilised in the spring allowing rapid development of assimilatory organs (Hendry, 1993) or buffered against potentially detrimental temporary reductions in assimilate supply (Roth *et al.*, 1997).

Although glycolysis is the most commonly observed adaptive response to hypoxia (Crawford & Brandle, 1996), accumulation of fructans has also been found in response to flooding in *Triticum*, *Iris*, *Senecia*, and *Myosotis* (Albrecht *et al.*, 1993; Kubin, 1992 cited in Albrecht *et al.*, 1997; Albrecht *et al.*, 1997). Compared to starch the advantages of fructan accumulation may be a lack of negative feedback on photosynthetic rates and a more energy efficient storage of carbohydrate (Albrecht *et al.*, 1997). Stored fructan can mobilised immediately after aeration leading to rapid regrowth of previously flooded roots (Albrecht *et al.*, 1993).

Temperature effects and phloem unloading

The effects of temperature on promoting or retarding floral bud development in bulbs has already been reviewed in the previous section dealing with flowering in ornamental bulbs. However, the specific effect of temperature on the bud that causes the response in bulbs has received little attention. It has been reported that the unloading of assimilate in sink tissue may act as a mechanism by which source-to-sink transport and intraplant competition may be controlled (Gifford *et al.*, 1984). Despite its importance, this area has not been extensively studied, possibly due to the fact that the unloading process varies considerably between species and even between tissues (Sonnewald *et al.*, 1994). A number of theoretical

pathways for carbohydrate unloading at sinks have been proposed (Hawker *et al.*, 1991). These are based on; (1) invertases (cell wall, cytosolic and vacuolar), (2) sucrose synthase, and (3) an SST/FFT model in which fructan is stored and glucose released (Nelson & Spollen, 1987; Hawker *et al.*, 1991). Sucrose synthase activity in tomato fruit and two invertases in maize kernels are effected by heat stress, resulting in altered sugar metabolism and consequently lower sink activity (Wang *et al.*, 1993; Cheikh & Jones, 1995). The existence of a variety of unloading mechanisms within a plant with various and distinct temperature tolerances and a demonstrated effect on sink strength, may explain the mechanisms behind temperature based periodicity and development in bulbs.

Pressure-flow hypothesis

The pressure flow hypothesis which is regarded as applying at both a cellular and whole plant level, predicts that bulk flow of assimilate through the phloem is driven by an osmotically generated hydrostatic pressure gradient (Minchin & Thorpe, 1993). This gradient is maintained by the hydrolysis of sucrose at the sink sites (Ho, 1988; Wardlaw, 1993). If sucrose arriving in the developing sink is not immediately utilised (eg respiration, starch synthesis) movement of sucrose into the sink is decreased (Doehlert, 1993). The expression of sucrose synthase is induced by carbohydrates and is probably is the factor controlling rates of import (Stitt, 1993).

The importance of water in the assimilate transport process cannot be overlooked. This is demonstrated by the growth of onion shoots in dry versus moist storage (Pak *et al.*, 1995). Despite increasing sucrose synthase activity and sufficient stored carbohydrate, growth rates of onion shoots decline if water supplies were inadequate. Similarly, abortion of cucumber fruit occurred when water flow was not be maintained (Chamont, 1993). Water flow control through turgor has been shown to be responsible for assimilate allocation in some species (Pomper & Breen, 1996) and in seeds (Patrick, 1993).

Competition between plant organs

The ability of an inflorescence to compete effectively as a sink with other organs is considered to be one of the primary factors that regulate flower bud development. While patterns of dry matter accumulation effectively define "apparent" sink strength, it does not account for substantial demands that may be made by sink organs for respiration (Ho, 1988). "Effective" sink strength may be defined as the ability of a sink organ to attract assimilate necessary for its growth, development and maintenance (Chamont, 1993), whilst "potential" sink strength is genetically conferred and is only realised when supply of assimilate is adequate and environmental conditions for maximum metabolic activity are met (Ho, 1988).

Flower bud development is highly dependant on the supply of carbohydrate and metabolites (Halevy, 1988). Therefore, its ability to attract assimilate is critical. The flower bud may not require large amounts of assimilate (although sink strength relative to the

bud size may be high), however, continuity of supply can be the key issue (Robinson *et al.*, 1980; Halevy, 1988). A number of factors may effect sink status. These are not necessarily momentary environmental conditions but may relate to the long term states of organs (Sachs *et al.*, 1993). An extremely pertinent point in case is the establishment and reorientation of vascular connections. If certain environmental conditions occur for a long period that benefit only some potential sinks, the vascular connectivity between these organs and their source will become well established (Sachs *et al.*, 1993). It is simpler for an organ to maintain these connections than for a developing organ to reorientate vascular connections and effectively compete with the established sink. Although the complex and inter-connected vascular system of monocotyledons somewhat reduces this effect, increased physiological competition between organs may occur in these plants. In *Nerine,* the possibility exists for competition between a number of organs for the same supply of assimilate at any time. The development of sufficient, or complete, vascular connections during development could also become important, if they cannot be reorientated at a later date. This has significance in the programming of flower bulbs since the establishment of vascular connections to the bud may be one of the limiting factors controlling development. Another aspect determining the establishment of sink hierarchies is the effect of relatively minor advantages in determining the dominant sink between two sinks with similar potential sink strength (Novoplansky, 1996). He found that a *Pisum* bud released from apical dominance just two hours before the other on a two-shoot pea plant resulted in the differences increasing with time and the first bud dominating the

plant. Furthermore, it was the *developing* bud, not the larger of the two, which was able to compete successfully for assimilates. This indicates that in a multiple bud system, such as *Nerine*, that interruption of development in the N unit bud may confer a decisive advantage to the younger bud N+1 in terms of their respective ability to attract assimilates. However, the current season bud is benefited by two morphological factors which may also have a role in determining relative sink strength. Distance from a source often can determine relative success of wheat ears and where developmental rates are similar size of the sink is important (Cook & Evans, 1976). Sink strength can be altered in wheat by removal of kernels from ears (Cook & Evans, 1976). Aborting inflorescences of *Nerine* have been observed to possess a higher number of aborted florets than those in which scape elongation is successful (Theron & Jacobs, 1996a). The effect may be related to a depletion of gynoecium-derived auxin and/or androecial acid invertase (Hanks & Rees, 1975; 1977; Russell & Morris, 1982; Morris & Newell, 1988). However, it is not known what causes individual floret abortion which may ultimately be the determining factor in the maintenance of relative sink activity. The period of time in which abortion occurs is short, coinciding with rapid stem elongation (Theron & Jacobs, 1996). A similar phenomenon has been observed in wheat (Gifford *et al.*, 1984). Floret competency to set grain was dependant on a period of only ten days, beginning twenty days before pollination, in which 60-70% loss of competency occurs if assimilate supply was limited.

Competition between vegetative parts and reproductive organs is a well documented phenomena (e.g., Tse *et al.*, 1974; Kinet, 1977;

Mullins, 1968). Correlative interactions between shoots and buds in the tomato (*Lycopersicon esculentum*) are mediated, to some extent, by endogenous plant growth regulators such as gibberellins, cytokinins, and auxins (Morris & Newell, 1988). Direct application of N^6-benzylaminopurine (BAP) and GA_3 resulted in a substantial increase in acid invertase activity in tomato flower buds under low light conditions that caused abortion to occur (Morris & Newell, 1988). Further examples of species in which correlations have been found between acid invertase activity and plant growth regulators are cited by Morris & Newell (1988). Acid invertase activity in the bud has been related to androecium development, which possessed high levels of the enzyme and levels of acid invertase fluctuate with development and anther dehiscence (Russell & Morris, 1982; Morris & Newell, 1988). Shading in tomato also causes a corresponding increase in apical (vegetative) invertase activities (Morris & Newell, 1988). Enzyme activities are significantly affected by the sugars themselves, often as a result of carbohydrate mediated gene expression (Koch, 1996). Such genetic regulation provides a means not only of integrating cellular responses to transport sugars but also coordinating resource utilisation and allocation among sinks, as well as developmental changes at a whole plant level (Koch, 1996). Carbohydrate fluxes enhance gene expression, resulting in suppressed or enhanced photosynthesis, reserve mobilisation and export processes (Koch, 1996). These changes are slow processes consistent with observed time frames for sink and source adjustments (Koch, 1996). Levels of sugars can compliment or replace growth regulators often associated with the control of sink strength, cell differentiation, and cell division such as auxin (Koch, 1996). However, the

complex interactions of carbohydrate modulated genes with growth regulators such as auxin, gibberellin, and cytokinin are poorly understood (Koch, 1996). Growth regulators are often implicated in assimilate partitioning between plant organs, particularly to apical dominance (Cline, 1994). However, it is not known what controls levels of endogenous plant growth regulators or how they are able to influence assimilate allocation (Halevy, 1988).

The ontogenetic periodicity of bulbous geophytes produces an interdependence of timing for organ development. For example, leaf emergence may be linked to inflorescence development in some way, whether it is a requirement for a minimum number of leaves for flowering to occur (Rees, 1972; Shoub & De Hertogh, 1975), or increased foliage resulting from an inability to flower (Rees, 1969). Foliage emergence from bulbs composed of leaf/leaf base units does not necessarily follow patterns of unit initiation (Rees, 1972). It is known that patterns of foliage emergence from *Nerine* vary significantly under different environmental conditions (Shillo *et al.*, 1997). However, the impact of leaf emergence and turnover on the development of the inflorescence is unknown. A cold period may be required for elongation of the flowering shoot (Rees, 1972). Stem or scape elongation may be due solely to cell expansion (Shoub & De Hertogh, 1975), or to a reduction of mitotic activity from the entire organ to the basal region, which continues throughout elongation (Hanks, 1993). The mode of scape elongation in *Nerine* is unknown.

The early stages of initiation and development of the inflorescence are able to be categorised based on a system devised by Beijer (1942). Nomenclature describing inflorescence initiation and development in *Nerine* are based on this system (Theron & Jacobs, 1994a). Using such descriptions, control of flowering based on stages of inflorescence and root development are possible by applying artificial temperature and/or chemical treatments. The diversity of species specific treatments necessary for control of flowering in bulbous crops indicates that the mechanisms inducing the flowering response vary considerably. Generally, periods of warm and cool temperatures are required for the stimulation of various processes, which may also result in compromise temperatures being used when divergent processes coincide (Le Nard & De Hertogh, 1993). One of the major effects of temperature change is on carbon partitioning, whether due to enzyme activities at the sink or source or to its effect on transport of assimilate in the phloem (Wang *et al.*, 1993; Cheikh & Jones, 1995). Temperature can also be used to control periodicity and the flowering response in *Nerine* (Groen & Kok, 1997). Failure of the inflorescence to develop in *Nerine* has been linked to assimilate partitioning, specifically to a low ranking of the current season flower bud in an assimilate sink hierarchy (Theron & Jacobs, 1996a). It has been shown that the process of assimilate partitioning can be influenced by a wide range of factors, e.g., hydrolysis at the source can be complex, phloem loading and unloading may be involved, there may be vascular constraints, development of other organs, or ability of the sink itself to metabolise incoming carbohydrate. Therefore, most of the applied studies in this area of bulb physiology have concentrated on the

application of treatments to achieve a desired outcome rather than understanding the process responsible for the response.

The basic goal of this project was to address some of the fundamentals of *Nerine* physiology in order that additional research may be carried out on target areas which have been identified as possible causes of unsuccessful inflorescence development. The first objective was to understand patterns of assimilate movement in the bulb. Once patterns of carbon distribution were ascertained, the factors responsible for these distributions and the timing of assimilate movement were studied. The second objective was to elucidate some of the factors involved in the elongation of the flower scape.

III

Materials and Methods

INTRODUCTION

The general objectives of this study were to obtain an understanding of assimilate partitioning within the *Nerine* bulb and the factors controlling inflorescence scape elongation. Initial indications of assimilate movement were made by assessing dry weight changes of the various bulb parts. Subsequently, the carbohydrate composition of the bulb, the implications of the carbon form, and patterns of carbohydrate mobilisation were studied. Once completed, labelled carbon was applied in the appropriate form to the known source of assimilate for the developing bud to study partitioning of carbon to this organ.

Scape elongation in *Nerine* was studied by examining the effect of various treatments on cell elongation and number. Studies were conducted in the field and under controlled glasshouse environments. Zones of cell division in the scape were identified. The effect of foliage and leaf turnover on development of the

inflorescence was investigated by the application of treatments in the glasshouse.

PLANT MATERIAL

Flowering size bulbs (12/up cm in circumference) of *Nerine bowdenii* 'Line 63' were used in all experiments. The bulbs were originally purchased from Holland in 1990 by Channel Bulbs Pty Ltd, Kettering, Tasmania, Australia and grown under field conditions at a location near Woodbridge, southern Tasmania (coordinates 43°15'S, 147°24'E, elevation 100m). The environmental data for Kettering (approximately 10 km from Woodbridge) is given in the Appendix.

Bulbs were sampled from the field planting in a random manner for periodic assessment of dry weight distribution and carbohydrate status. Only bulbs free of mechanical damage or obvious signs of disease infection were used. Bulbs used for glasshouse trials were lifted during the winter rest period (July-September) and planted in pots at the Horticultural Research Centre, University of Tasmania, Australia. Plants were grown in a glasshouse maintained at 21°C and watered via an automated overhead watering system.

POTTING MEDIUM

Plants removed from field conditions were placed in 20 cm diameter pots containing a potting medium consisting of:

70% composted pine bark
20% coarse river sand
10% peat moss

The potting medium also contained the following nutrient additives:

6 g l^{-1} (w/v) dolomite
6 g l^{-1} (w/v) slow release Osmocote™ macronutrients (Scotts, Australia)
0.5 g l^{-1} (w/v) $FeSO_4$
0.4 g l^{-1} (w/v) Micromax™ micronutrients

Bulbs were planted with the neck of the bulb protruding above the potting medium.

DRY WEIGHT ANALYSIS

Changes in dry weight distribution over a one year growth cycle were investigated in bulbs grown under commercial field conditions at Woodbridge, Tasmania. Ten bulbs were collected from the field

planting near Woodbridge, Tasmania at approximately 3 week intervals from July 1995 to August 1996. Harvested bulbs had a circumference of between 12/up cm. Plants were selected randomly and carefully removed from the ground to avoid root damage. They were placed on ice after harvest and immediately dissected upon return to the laboratory. Bulbs were divided into annual growth units (demarcated in the outer bulb by remnants of previous inflorescences and associated semi-sheathing leaf bases), daughter bulbs, and the current season inflorescence. Bulbs typically contained leaf bases from two preceding growth units (N-1 and N-2). Where leaf bases apparently originated from a third outer unit (N-3) (occurring outside of a second scape residue), they were incorporated with the N-2 unit. Bulb components were dissected and placed in liquid nitrogen and lyophilised using a Dynavac FD16 freeze drying unit (High Vacuum Pty Ltd, Melbourne, Australia) and then weighed with a Mettler AE200 balance (Mettler Instruments, Switzerland). Bulb components were stored at -20°C prior to quantification of carbohydrate content.

CARBOHYDRATE DETERMINATIONS

Bulbs were collected, dissected and stored as described above prior to carbohydrate analyses. In addition, Locally grown *N. sarniensis* 'Rosea', 'Salmonea' and 'Fothergillii Major' as well as *N. alata*, *N. fothergillii*, *N. bowdenii* 'Pink Jewel', and *N. undulata*, were also collected from Channel Bulbs Pty Ltd, Kettering, Tasmania to test for the presence of ethanol-soluble and -insoluble fructans. These

bulbs had been cultivated under plastic tunnel in a well-drained medium.

Determination of mono- and disaccharides using GC-MS

Initial analysis of bulb tissue was conducted to identify the mono- and disaccharide carbohydrate forms using a combined gas chromatography/mass spectrometry technique (GC-MS) (Kristic, 1997). Lyophilised ground tissue (0.10 g) was extracted overnight in 18 ml of 60% ethanol at 60°C in an agitated water bath. One ml of trehalose (disaccharide) and arabinose (monosaccharide) standards were subsequently added (1 mg/ml), and ethanol removed in a vacuum concentrator (Savant, USA). The remaining aqueous phase was partitioned twice against diethyl ether (5 ml) before filtering with Whatman No. 1 filter paper into a volumetric flask. Solids were washed several times with deionised water. The extract was diluted in 10 ml of deionised water and homogenised. A 5 µl aliquot was transferred to a 150 µl glass insert within a GC vial and lyophilised.

Freeze-dried soluble carbohydrate extracts were silylated with 20 µl of Tri-Sil Z (Pierce Chemical Co., Rockford, USA) and incubated at 60°C for 10 minutes prior to injection of 1 µl into a Hewlett-Packard 5890A series gas chromatograph linked to a Hewlett-Packard 5970 series mass selective detector. The GC column used was a HP-1 (cross-linked methyl silicon gum) with a length of 25 m, 0.32 mm internal diameter and a 0.52 µm film thickness. The head pressure was 15 psi with a splitless column flow rate of 5 ml/min at 60°C and helium carrier gas. The oven temperature was set at 260°C and the detector temperature at 290°C. Oven

temperature was increased from an initial 50°C to 150°C at a rate of 30°C/min and from 150°C to 300°C at a rate of 15°C/min. The oven temperature remained at 300°C for four minutes. Compounds were detected in selected ion monitoring (SIM) mode with a dwell time of 40 ms/ion. Solvent delay was set at 6.5 min. All peaks eluted between 6.5 and 16.5 min were integrated with Hewlett-Packard ChemStation software. Sugar quantities could be calculated by comparison with the area of peaks representing internal standards.

Extraction and fructan determination

Extraction of freeze dried bulb tissue and partitioning of supernatants was based on the methodology described by Lambrechts *et al.* (1994). Fifty mg of finely ground tissue was extracted at 60°C three times for one hour. After each extraction samples were centrifuged at 2000 g using a Beckman model J2-21 centrifuge (Beckman Instruments, Fullerton, USA). Supernatants were combined and the ethanol evaporated, followed by partitioning against chloroform (chloroform:water 5:8 v/v). The pellet was retained and lyphilized for analysis of ethanol-insoluble fructans and starch. These insoluble fructans were extracted in 2.5 ml of 1% (v/w) oxalic acid at 80°C for 2 h in 3.5 ml vials. Fructose content of the supernatant and total fructose content of the ethanol-soluble fraction were quantified colorimetrically using an alcoholic resorcinol/HCl procedure (McRary and Slattery, 1945). The resorcinol reagent was prepared by dissolving 1.0 g resorcinol in 1000 ml 95 % ethanol. HCl used to hydrolyse fructan polymers was 32% analytical grade. Five ml resorcinol was dispensed into 3.5 ml scintillation vials and 2.5 ml HCl added. A

20 µl aliquot of the extract (containing soluble or insoluble carbohydrate, but no starch) and 480 µl of de-ionised water were pipetted into the reagent and thoroughly mixed. Vials were loosely capped and placed in a water bath at 80°C for 45 min. After cooling, the absorbance of samples was measured at 540 nm at room temperature using a Perkin-Elmer Lambda 20 UV/Vis spectrophotometer (Bodenseewerk Perkin-Elmer GmbH, Ueberlingen, Germany) with water as a reference. Absorbance of a standard curve containing values of 5 to 40 µg of fructose (fru) (5 to 40 µl of a 1 mg/ml standard) was measured with every group of samples for conversion of absorbance to weight values. Soluble sample absorbance included endogenous fructose and fructose derived from hydrolysed sucrose which was subtracted from the total fructose found after hydrolysis of fructans.

Starch determination

Starch was solubilised in 1 ml of DMSO at 100°C for 5 min. Then, 1.5 ml (150 U) of α-amylase in MOPS buffer (50 mM, pH 7.0) was added and the samples vigorously shaken at 2 min. intervals during a 6 min. incubation at 100°C. Subsequently, the solution was washed into 20 ml vials with 2 ml of sodium acetate buffer (200 mM, pH 4.5) followed by 0.05 ml (20 U) of thermostable amyloglucosidase. The solution was mixed and incubated at 50°C for 30 min.

The final volume was adjusted to 15 ml with deionised water. An aliquot of this solution was centrifuged at 3000 rpm for 10 min. A further 0.05 ml aliquot of the supernatant was combined with 1.5 ml of chromogen reagent containing glucose oxidase (12 Uml^{-1}),

peroxidase (0.65 Uml^{-1}) and 4-aminoantipyrine (0.4 mM) (McCleary *et al.*, 1997). The absorbance of the solution was read at 510 nm against the reagent blank. The colorimetric reaction was quantified using glucose (glc) standard curves. All enzymes were supplied by Megazyme, Australia.

Free reducing sugars and sucrose determinations

Free reducing sugars and suc were assayed using the stoichiometric reduction of NADP to NADPH initiated by the oxidation of Glc-6-P dehydrogenase (Bergmeyer *et al.*, 1974). Fifty µl extracts containing free reducing sugars were phosphorylated using 0.5 ml ATP (5.8 $mgml^{-1}$) in triethanolamine buffer (pH 7.6) (also containing 2.4 $mgml^{-1}$ NADP). This reaction was catalysed by 0.01 ml hexokinase (290Uml^{-1}) added concurrently with Glc-6-P dehydrogenase (146 Uml^{-1}). Fru-6-P was converted to Glu-6-P by the addition of 0.01 ml phosphoglucose isomerase (700 Uml^{-1}) (Bernt & Bergmeyer, 1974), reacting with NADP in the presence of Glc-6-P dehydrogenase to form gluconate-6-phosphate, H^+ and NADPH. The NADPH formed was quantified spectrophotomically at 420 nm (Bergmeyer *et al.*, 1974). Suc concentrations in 50 µl aliquots were determined by enzymatic inversion of suc with 0.1 ml β-fructosidase (72 Uml^{-1}), following which glc content was calculated. Differences between glc content before and after inversion were doubled to give total suc weight (Bergmeyer & Bernt, 1974). Determination of weight was achieved by comparing sample absorbance with a glc standard curve containing values of 1 µg to 40 µg glc (from a 1 mg/ml glc stock solution). Standard curves were performed with each group of samples.

^{14}C-SUCROSE APPLICATION

Methods for feeding ^{14}C-sucrose and assessment of partitioning were based on those of Neo *et al.* (1991). With all bulbs remaining *in situ*, soil was carefully removed from around one side of the bulb prior to emergence of the inflorescence. The outer tunic (desiccated leaf bases) were removed from the exposed bulb portion and the surface of the outermost intact scale lightly abraded with wet paper towelling. A well was constructed from adhesive Blu-Tack (Bostick) and attached to the scale. A 100 µl dose of ^{14}C-sucrose (Amersham) with a total radioactivity of 0.37 MBq was then applied and sealed in the well. After a period of 36 h, bulbs were harvested and dissected into components of two scale groups, flower bud, leaf primordia, stem, roots and bulblets and weighed. The freeze-dried powder was extracted three times in 80% ethanol as described for soluble carbohydrate.

Three ml aliquots were centrifuged at 7000 rpm in a Beckman J2-21 centrifuge. Sub-samples of 250 µl were taken in triplicate and combined with 2.5 ml of Beckman Ready Safe scintillation fluid and counts per minute (cpm) of samples assayed using a Beckman LS5801 scintillation counter (Beckman Instruments, Fullerton, USA).

^{14}C label incorporated into insoluble or structural matter were assayed by separating solids from the supernatant with Whatman no. 54 filter paper. Solids were rinsed twice with 80% ethanol and divided into triplicates of 10 to 50 mg, depending on the sample

size. The triplicate samples were suspended in Insta-Gel scintillation fluid (Packard, St. Kilda, Australia) and water (1:1 v/v) (Beveridge *et al.*, 1992) and assayed as described for the ethanol soluble fraction.

CELL MEASUREMENTS

Scape cell lengths were measured in longitudinal sections taken from areas at the top, middle, and base of the structure. Sections were stained in toluidine blue for approximately 40 sec to highlight cell walls and rinsed with de-ionised water. Sections were then mounted and viewed with a Zeiss Stemi 2000-C stereomicroscope (Zeiss, Oberkochen, Germany). Parenchymatous cells immediately posterior to outer collenchyma and fibre cells were used as a standardised comparative indicator of cell size within the scape.

IV

Dry Weight Analyses

INTRODUCTION

Since a major proportion of the dry weight of plants consists of carbon compounds (Gifford *et al.*, 1984), dry weight analysis of a plant over an entire growing cycle can be used to give an indication of patterns of carbon distribution and assimilate movement. Dry weight increases provide an indication of apparent sink strength, but actual sink strength can only be determined by accounting for processes expending carbon such as respiratory processes (Ho, 1988). Such an approach is particularly relevant when a large proportion of the plant is comprised of reserve carbohydrate as is the case in bulbous geophytes. Bulbs of *N. bowdenii* consist of a number of annual growth units which are comprised of retained leaf bases at the outside of the bulb, and leaf and floral primordia at the centre. Dry weight distribution changes over time have been previously investigated in *N. bowdenii* (Theron & Jacobs, 1994b). The major findings of this study were that bulb organs could act as sinks or sources at various stages of development, foliage development depended upon previously stored reserves in older leaf bases and photosynthate was directed to both new and old leaf

bases. Their experiments used *N. bowdenii* commercially cultivated in the Elgin region of the Western Cape, South Africa. The climate was described as Mediterranean with mild, wet winters (winter minimum soil temperature of 9°C) and dry, hot summers. Bulbs grown for cut-flower production in South Africa are cultivated in shadehouses. In contrast, southern Tasmania has a temperate climate with cool winters, mild summers, and relatively even rainfall throughout the year. The habit of 'Line 63' used in this study differs significantly to the unnamed cultivar used in the studies of Theron & Jacobs (1994b). Their cultivar was synanthous (leaf and inflorescence emergence coinciding) under local growing conditions; whereas, senescence of leaves in 'Line 63' observed growing in open fields in southern Tasmania have occurred prior to emergence of the inflorescence from the bulb. The timing of leaf senescence relative to the rapid elongation and emergence of the inflorescence may be significant in terms of the fate of photoassimilate in the two contrasting forms. Defoliation at an early stage of foliage emergence of *N. bowdenii* exhibiting a synanthous habit has been demonstrated to cause increased inflorescence abortion (Theron & Jacobs, 1996a). This poses some interesting questions in relation to the role of photosynthate in bulbs with a hysteranthous growth form. It is has been proposed that carbon for rapid inflorescence development in synanthous *N. bowdenii* partly originates from N+1 foliage photosynthate (Theron & Jacobs, 1994b). The purpose of this study was primarily to identify the sources of assimilate for rapid elongation of the inflorescence. Other areas of interest were the distribution patterns of photoassimilate in the leaf bases and to clearly

establish the timing of events within the bulb in relation to the synchronisation of growth processes.

RESULTS

Leaf emergence occurred during late October to early November (spring, *c.* day 81) in all bulbs. Foliage of bulbs became fully senescent during the following March (*c.* day 204). Rapid elongation of the flower bud and scape inside the bulb commenced in March and plants reached anthesis during April (*c.* day 267). This pattern of development was typical of the seasonal growth pattern of *N. bowdenii* 'Line 63' grown under field condition in Tasmania.

Total leaf base dry weight initially declined possibly due to increased respiratory metabolic activity and/or growth of roots. The total dry weight of bulbs reached a maximum during December/January (days 150-160), which was after the foliage had become established, and was lowest 3-4 months after anthesis, possibly as a result of continued respiratory loss and subterranean root development.

Dry weight declined during senescence and continued to decline over the period preceding and during rapid elongation of the inflorescence and scape. While bulb weight more than doubled over two months prior to foliage senescence, there was no significant difference in bulb weight at the beginning and end of

the trial indicating that carbon utilisation and fixation were equal over the annual cycle.

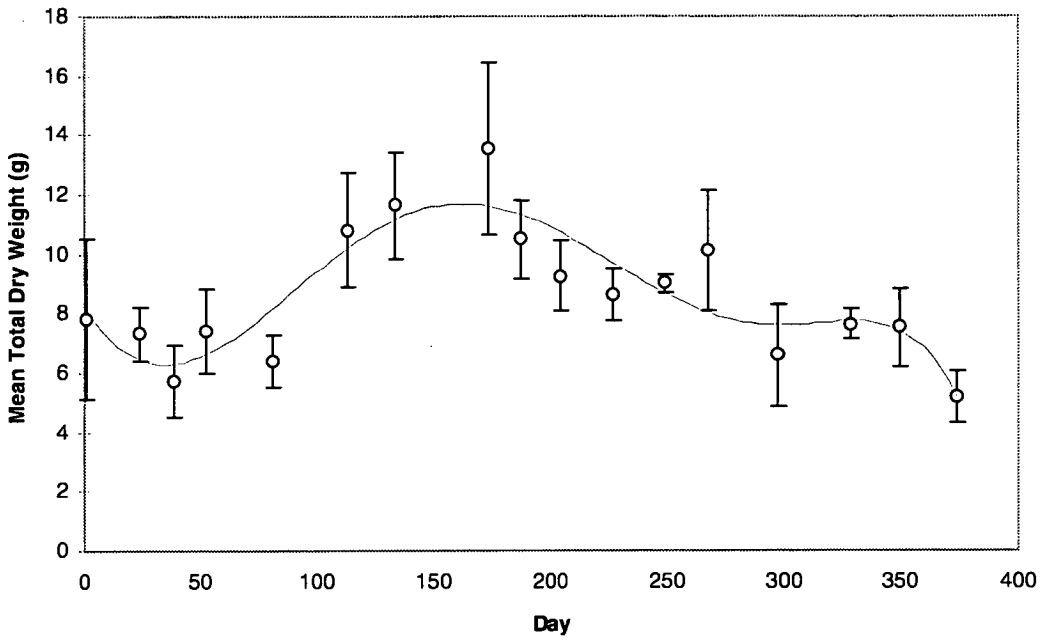

Figure IV.1: Mean total weight of bulb over an annual growth cycle. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

Weight increases due to photosynthesis were balanced by losses through respiration and abscission of senescent leaf and floral tissue. A large proportion of total leaf base weight is composed of the mass of N unit leaf bases in the middle of the bulb throughout the year, particularly just prior to senescence. A decrease in mean dry weight of outer (N-2/N-1) leaf bases occurred during the beginning of foliage emergence and an increase in the dry weight of these same units was recorded in the period immediately following foliage expansion. It was largely responsible for the peak in total

bulb dry weight. The dry weight of leaves was a major contributing factor in total bulb weight at this time.

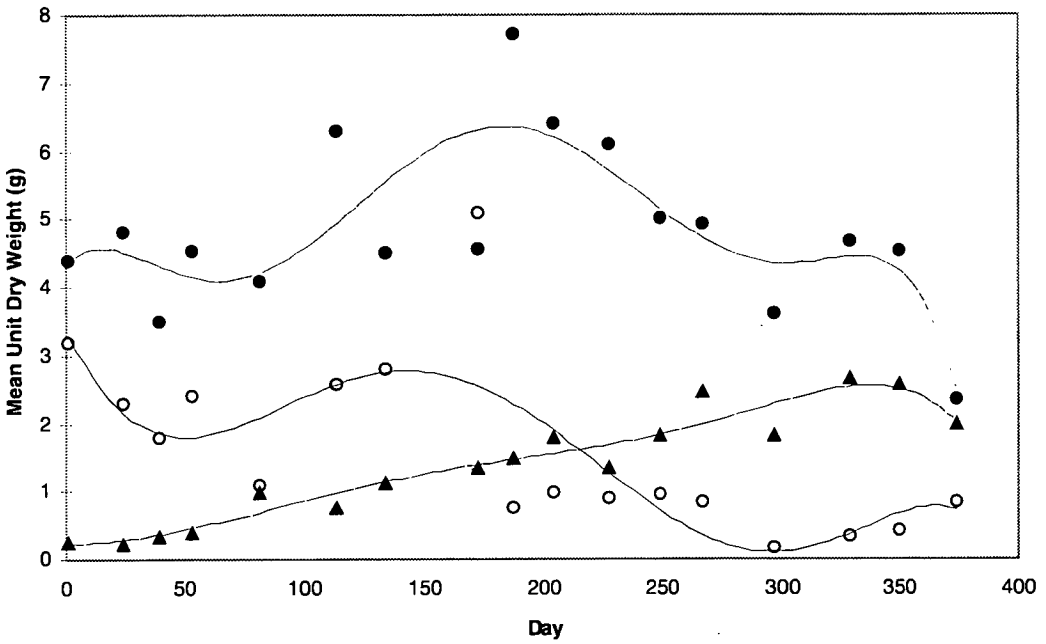

Figure IV.2: Mean dry weight of various leaf base units over an annual growth cycle. Mean dry weight of N-1/-2 leaf bases (o), N leaf bases (•) and N+1 leaf bases (▲) are shown in grams. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329.

Senescence of foliage coincided with a dry weight gain in N leaf bases and a significant reduction of N-2/N-1 dry weight. Coinciding with the decrease in outer scale dry weight, rapid elongation of the scape and inflorescence occurred. This was accompanied by a gradual decrease in the weight of the N growth unit. The dramatic decline of dry weight from the N-1/-2 leaf bases immediately followed an increase in total carbon within these organs. This pattern of development suggests that the metabolic processes with the leaf bases are reversed from the storage of

carbohydrate to utilisation in a relatively short period of time. Total dry matter declined at a much slower rate in the N leaf bases, and appeared to occur following the depletion of leaf bases anterior to them. The weight of N+1 leaf bases increased in a generally linear fashion during the growth cycle, although some decreases were evident at the time of rapid inflorescence growth and senescence.

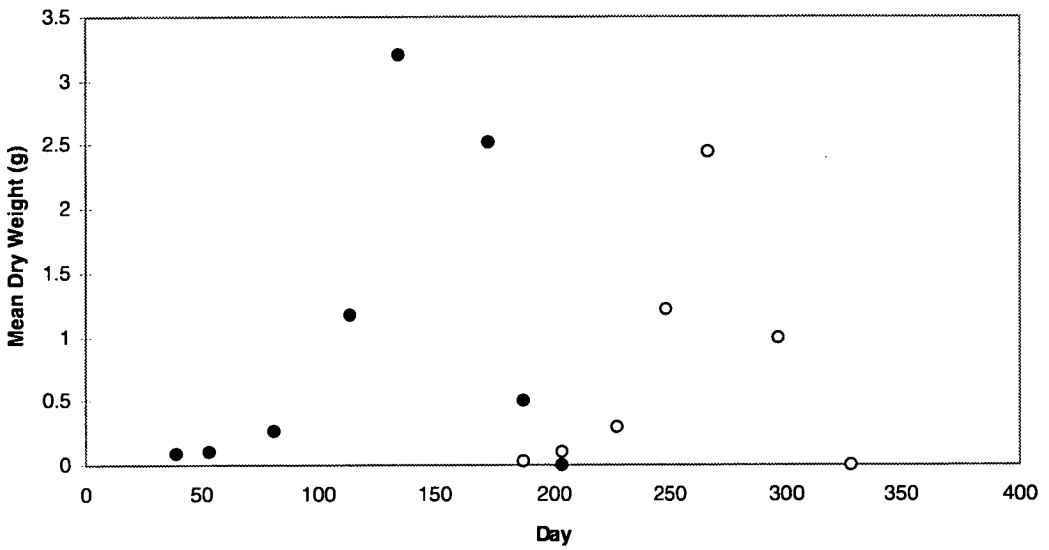

Figure IV.3: Mean dry weight of aerial foliage and inflorescence/scape. Foliage (•) and inflorescence/scape (o) are shown as grams. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329..

The dry weight distributions of the foliage and inflorescence/scape clearly demonstrates the synanthous habit of Tasmanian-grown *N. bowdenii* 'Line 63.' The dry matter content of the foliage was asymmetric in nature, taking a longer period to accumulate total dry weight than to lose it during senescence. During the period of

foliage emergence, some turnover of leaves occurred, however, there was a trend toward increasing amount of biomass until general senescence occurred. Growth of the inflorescence/scape was also rapid, and almost exponential in nature. Dry matter accumulation and loss was more symmetrical. It slowed to some extent, perhaps by the availability of carbon sources, either through transportation from the bulb or retained in the parent tissue itself, and for development of the seeds following fertilisation.

DISCUSSION

The results (Figure IV.2) indicate that the outer leaf bases contain the major sources of carbon used for development of the aerial foliage. Once the foliage was established, weight increases in all bulb units were recorded, indicating that all leaf bases are photoassimilate sinks. The outer leaf bases were also a major source of carbohydrate during rapid elongation of the inflorescence and scape following leaf senescence. Once the mean dry weight of the N-1/-2 growth unit fell to a weight of approximately one gram, the weight of the next N bulb unit also began to decline rapidly. Surprisingly, very little dry weight was regained by the bulb following senescence of the inflorescence. This may be due, in part, to utilisation of mobilised carbon by the developing seeds and respiration in the aerial organ.

A reduction in outer scale dry weight prior to expansion of foliage indicates that these outer scales may provide the source of

carbohydrate for this developmental process. However, the results suggest after foliage became net exporters of assimilate, these leaf bases become a major sink for photosynthetically-derived carbon. Therefore, the leaf bases have the capacity to both synthesise and hydrolyse storage carbohydrate according to sink/source changes within the plant. This must require precise regulation of carbohydrate metabolism within the storage tissue. The fact that no major loss in dry weight of leaf bases can be observed during later leaf development suggests that carbon may be provided by the expanded leaves. During this period dry weight gains in N+1 leaf bases were negligible.

Carbon transported out of foliage during senescence appears to accumulate in N leaf bases. Leaf bases in this unit may also be recipients of carbohydrate lost from the outer scales at this time. It is likely assimilate from these outer scales is also being transported to the inflorescence. Although rapid weight gains were not discernible, it would be reasonable to assume that metabolic activity in this organ would be accelerating prior to rapid growth. Rapid growth of the inflorescence was accompanied by a gradual decline in the mean dry weight of N leaf bases, apparently largely as a result of exhaustion of reserves in outer units. A similar centripetal pattern of mobilisation of reserve carbon has also been observed in *Allium cepa*, a geophyte of similar structure (Pak *et al.*, 1995), and can be correlated with the distribution of polysaccharide hydrolysing enzymes (Henry & Darbyshire, 1979). Dry weight increases observed in N and N+1 units following senescence of the inflorescence indicate that some carbon partitioned to the flowers and scape is recycled to the storage

organs. The relative contribution of scape derived photosynthate to floral and/or vegetative organ development, or indeed the capacity of the scape to photosynthesise, cannot be determined from these results. Likewise, the extent to which photosynthate produced by the scape, if any, is immediately utilised by the scape cannot be determined from these data.

Comparison of total bulb dry weight patterns with South African shadehouse-grown *N. bowdenii* (Theron & Jacobs, 1994b) showed similar development, notwithstanding a more prolonged period of maximum weight as a consequence of the synanthous habit. Loss of dry weight from outer scales was less marked in these bulbs, although the effect of chilling accelerated carbon movement from these leaf bases to some extent (Theron & Jacobs, 1994b). Photoassimilate was unequivocally directed towards N leaf bases in the South African bulbs, which was not observed in this study. However, the presence of an outer N-2 unit associated with the bulbs used in this study could alternatively result in photoassimilate being partitioned to N-1 leaf bases (i.e., the unit inside the outermost leaf bases). It may also be of significance that the photoassimilate sinks in *N. bowdenii* (N-2/N-1 units) are also apparently the initial source of carbohydrate for rapid inflorescence development.

The evidence from dry weight analysis of the leaf base units throughout the growth cycle suggest that reserves utilised for rapid growth of the inflorescence/scape and the foliage are hydrolysed in a centripetal fashion. Furthermore, it was demonstrated that once the foliage has emerged and expanded a dry weight gain can be

observed in all leaf base tissue. This indicated that the foliage photoassimilate became a net exporter and is not directed to any specific sink. Although these results give a general indication of patterns of carbon partitioning, a more detailed perspective is possible by a closer examination of the carbohydrates themselves. Dry weight data alone cannot give an indication of changes in carbon forms within tissue. A more detailed analysis of the roles and processes operating within the storage tissue are required.

V

Carbohydrate Analyses

INTRODUCTION

The importance of carbohydrate changes in reserve tissue of bulbous geophytes has been well documented (e.g., Nowak *et al.*, 1974; Ohyama *et al.*, 1987, Miller, 1992; Pak *et al.*, 1995). When carbohydrates are stored in discreet cellular locations or in relatively insoluble forms, such as starch, mobilisation is characterised by an increase in the activity of hydrolysing enzymes (Moe & Wickstrom, 1973; Lambrechts *et al.*, 1994). Such activities are often sensitive to temperature changes, resulting in the periodic nature and cold requirement of many bulbous species (Rees, 1972). Temperature changes may cause changes in enzyme activities. Thus, altering the carbohydrate composition of the organ without significantly effecting total carbon weight (Pak *et al.*, 1995). Such fluctuations, however, may be critical for subsequent developmental changes (Nowak *et al.*, 1974).

Analysis of dry weight distribution in *N. bowdenii* 'Line 63' (Figure IV.2) indicated that carbon fixed by the foliage has partitioned to

fleshy leaf bases. They subsequently acted as a carbohydrate source for the development of the inflorescence after foliage senescence. Previous studies of an unidentified *N. bowdenii* cultivar showed that the dominant reserve polysaccharide in the leaf bases was starch (Theron & Jacobs, 1996b). However, sucrose, glucose, fructose and very low levels of fructan were also present. Despite the differences in growth habit between the unnamed commercial variety used in the previous study and 'Line 63,' it was assumed that the carbohydrate forms in both cultivars would be similar. However, preliminary investigations (unpublished data) revealed that this was not the case. All organs of Tasmanian grown 'Line 63' were found to possess fructan in significant quantities. In view of this finding, and the fact that Tasmanian-grown *N. bowdenii* 'Line 63' is not synanthous, a study was undertaken to determine changes in carbohydrate content and concentration in the various organs of the bulb in order to better understand carbon partitioning processes occurring through the growth cycle of *N. bowdenii*.

RESULTS

The most prevalent carbohydrate found in the leaf bases of *N. bowdenii* was ethanol soluble fructan. In plants these are often short chain (DP<5) β(2-1)-linked fructosylsucrose polymers (Ohyama *et al.*, 1984; Koroleva *at al.*, 1997). Ethanol-insoluble fructan (inulin) was also present in significant amounts, although generally less than the total amounts of starch. Fructose and sucrose were present in high concentrations, particularly in leaf bases belonging to the N-1/-2 (outer leaf bases) and N (current season leaf bases and leaves) growth units. Quantities of ethanol-soluble non-structural carbohydrate were found to be greater than that of insoluble carbohydrate in all bulb units.

Concentrations of soluble and insoluble non-structural carbohydrate (NSC) were inversely proportional in N-1/-2 leaf bases over the course of the growing season (Figure V.1a). The concentration of soluble NSC increased prior to rapid foliage and inflorescence development, corresponding with reductions in insoluble NSC concentration. This indicated that mobilisation was occurring. During the initial period that foliage was photosynthetically active, the concentration of insoluble NSC increased and was associated with a decline in soluble forms. Total NSC depletion ceased after foliage emergence, and increased slightly before senescence. Soluble NSC content decreased in the outer leaf bases at the onset of inflorescence emergence and continued until anthesis.

Decreases in soluble NSC concentrations were accompanied by slight increases in insoluble NSC. After anthesis, the N-1/-2 units were completely exhausted in some bulbs and soluble NSC increased in those remaining. This accounted for the high standard error values for the last three sample dates. Similar trends were observed in leaf bases of the N and N+1 bulb units (Figure V.1b and c), although total depletion of N-1/-2 leaf bases at the end of the study coincided with soluble NSC increases in the other bulb units. The degree of variation was generally reduced in the inner leaf bases, with the exception of soluble NSC in the N leaf bases at a single sample date immediately prior to total foliage senescence. The general trend in insoluble NSC concentration in N+1 leaf bases was a gradual increase until senescence of the foliage, after which levels gradually fell until anthesis. Soluble NSC in N+1 leaf bases followed a similar trend to those constituting the N unit. However, the concentration of soluble sugars was initially higher and showed less variation during the course of the trial.

Composition of NSC in N-1/-2 leaf bases is shown in Figures V.2-V.3. The major components of the soluble NSC fraction are ethanol-soluble fructan, fructose and sucrose. Glucose levels were low, usually in concentrations near 20 mg g^{-1}. Levels of soluble fructan appear related to the insoluble longer-chain storage form and starch. Mean total weight (therefore also reflecting mean unit weight) showed a dramatic fall in the levels of these compounds prior to anthesis and foliage emergence All of them increased for a short period after development of the leaves. Sucrose levels follow these trends, although levels of fructose and glucose initially

increased in contrast to levels of all other NSC, which decreased from the beginning of the study.

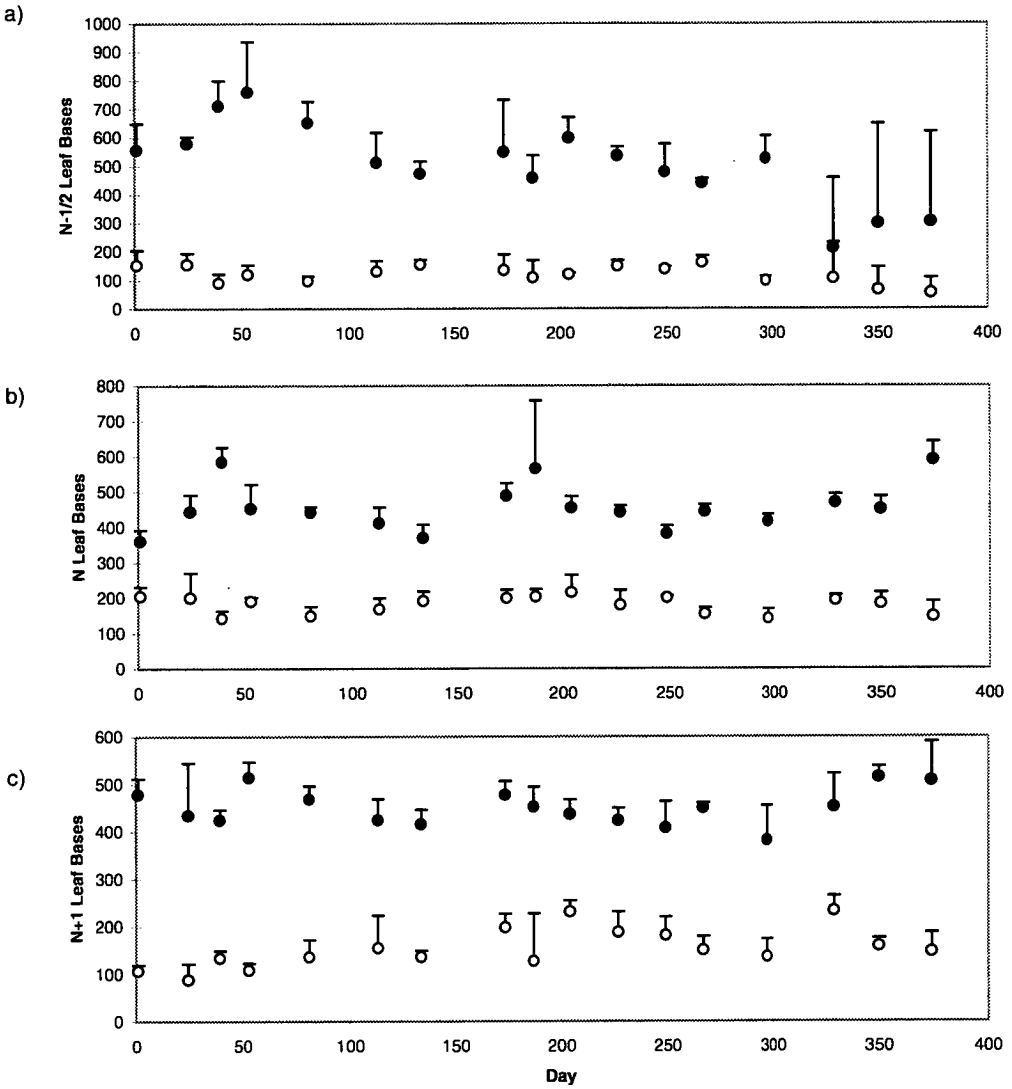

Figure V.1a-c: Mean total non-structural carbohydrate (NSC) composition of leaf bases in all *N. bowdenii* 'Line 63' growth units. Total soluble NSC concentration (•) and total insoluble NSC (o) are shown as mg g^{-1}. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

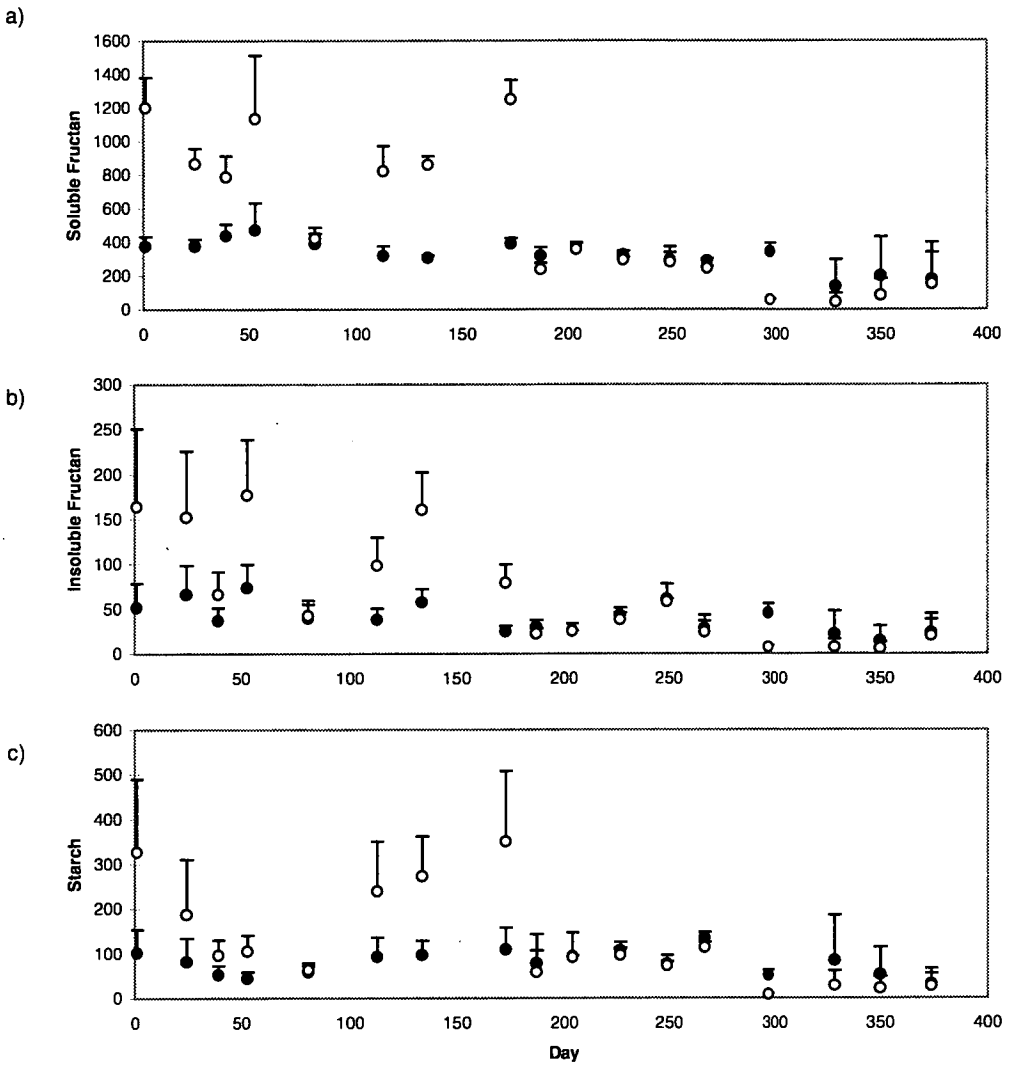

Figure V.2a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N-1/-2 growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

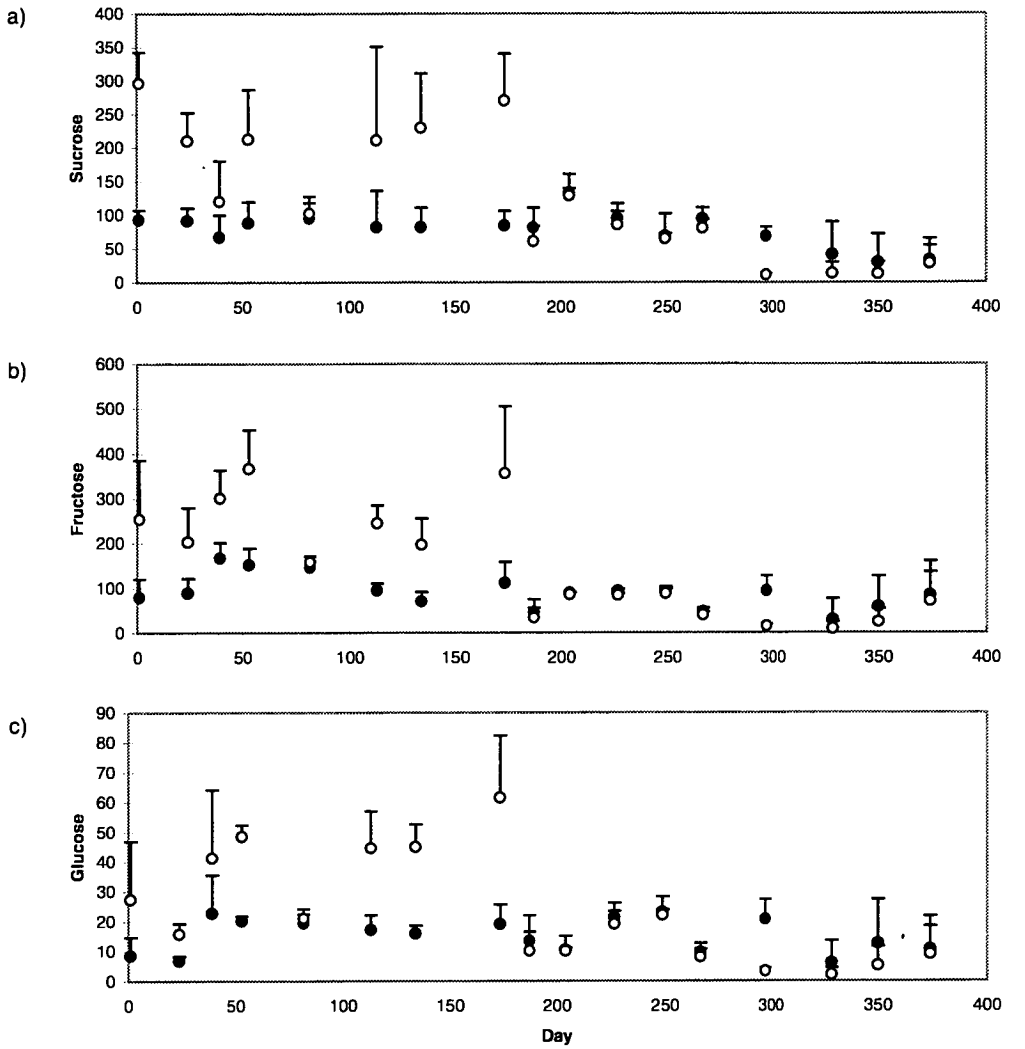

Figure V.3a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N-1/-2 growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

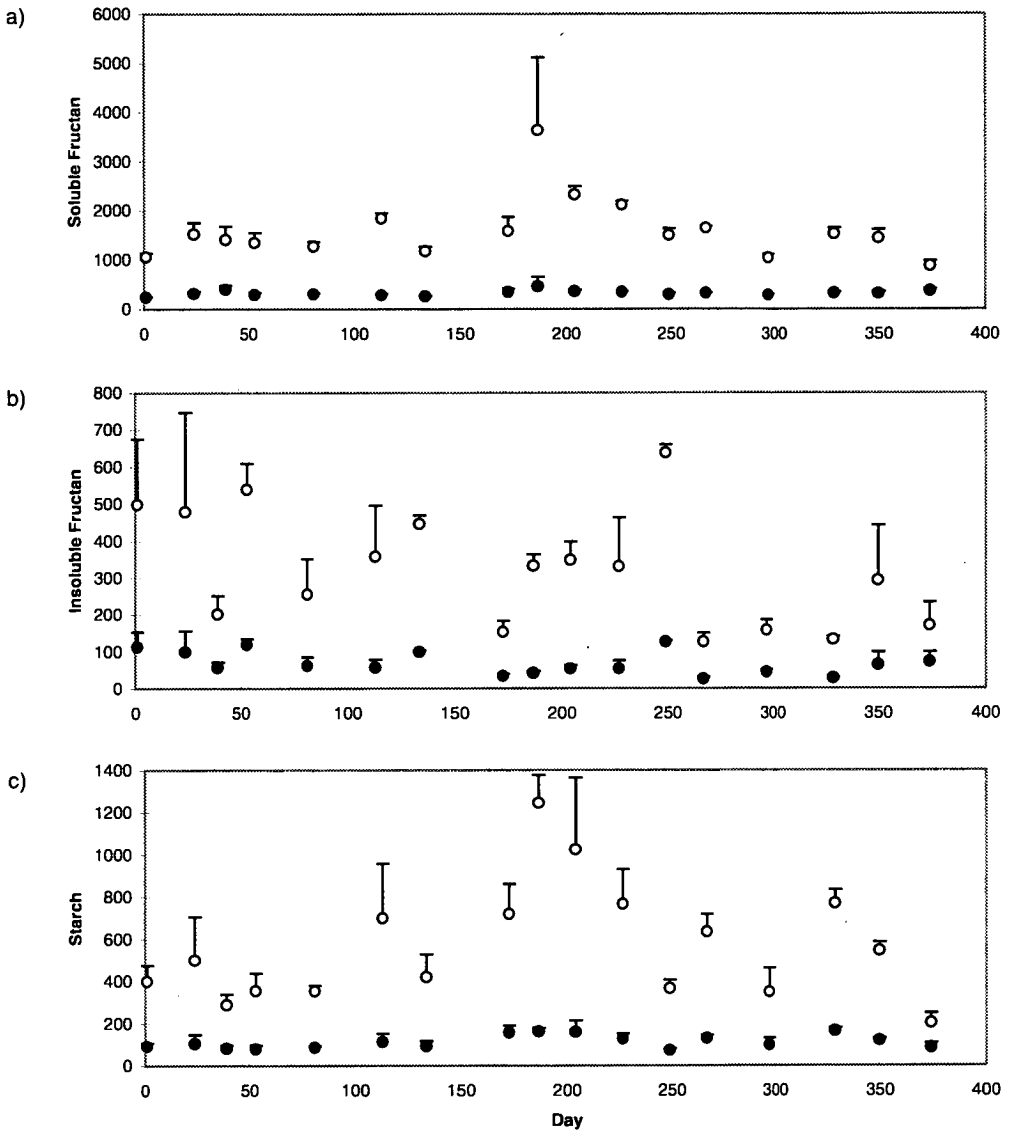

Figure V.4a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

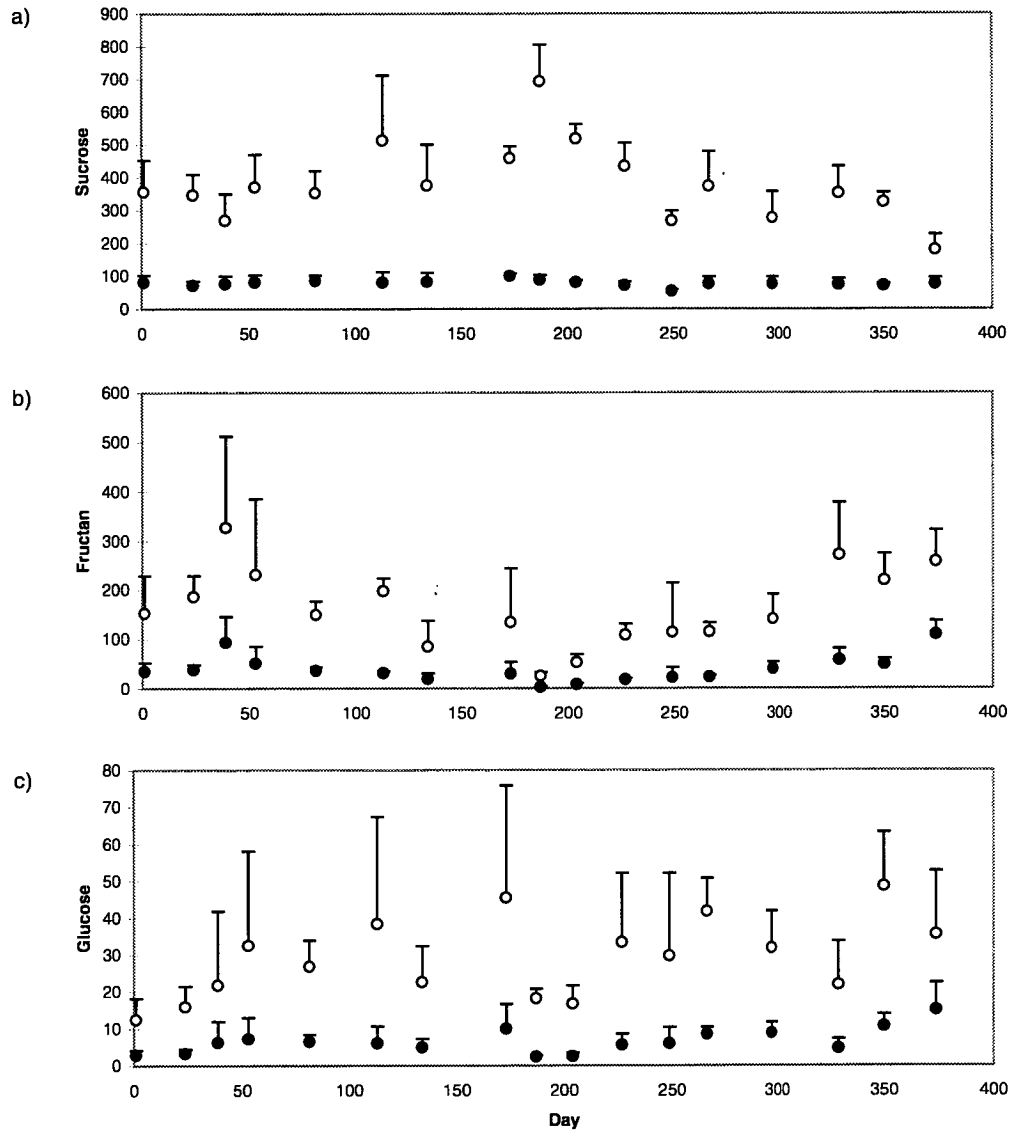

Figure V.5a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

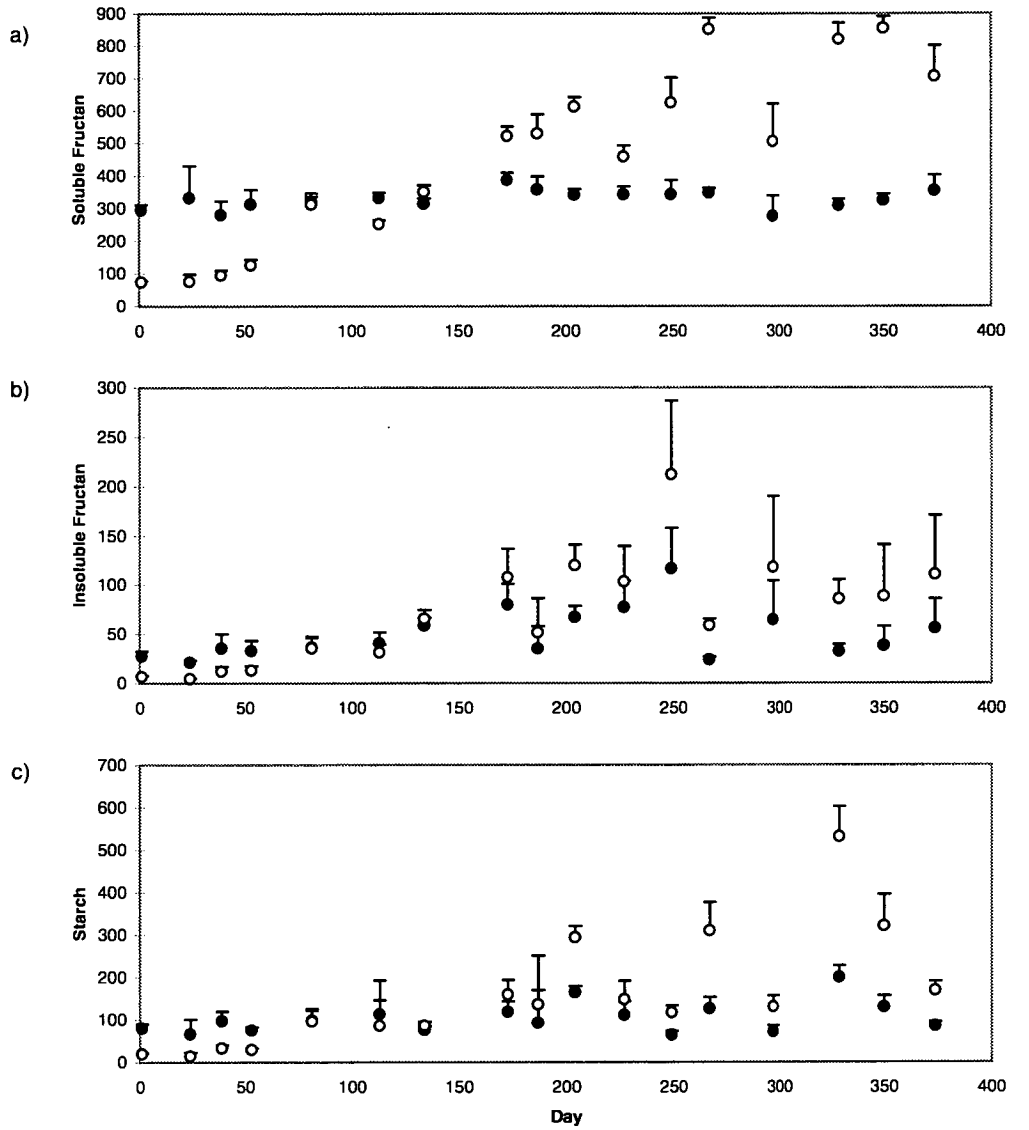

Figure V.6a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N+1 growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

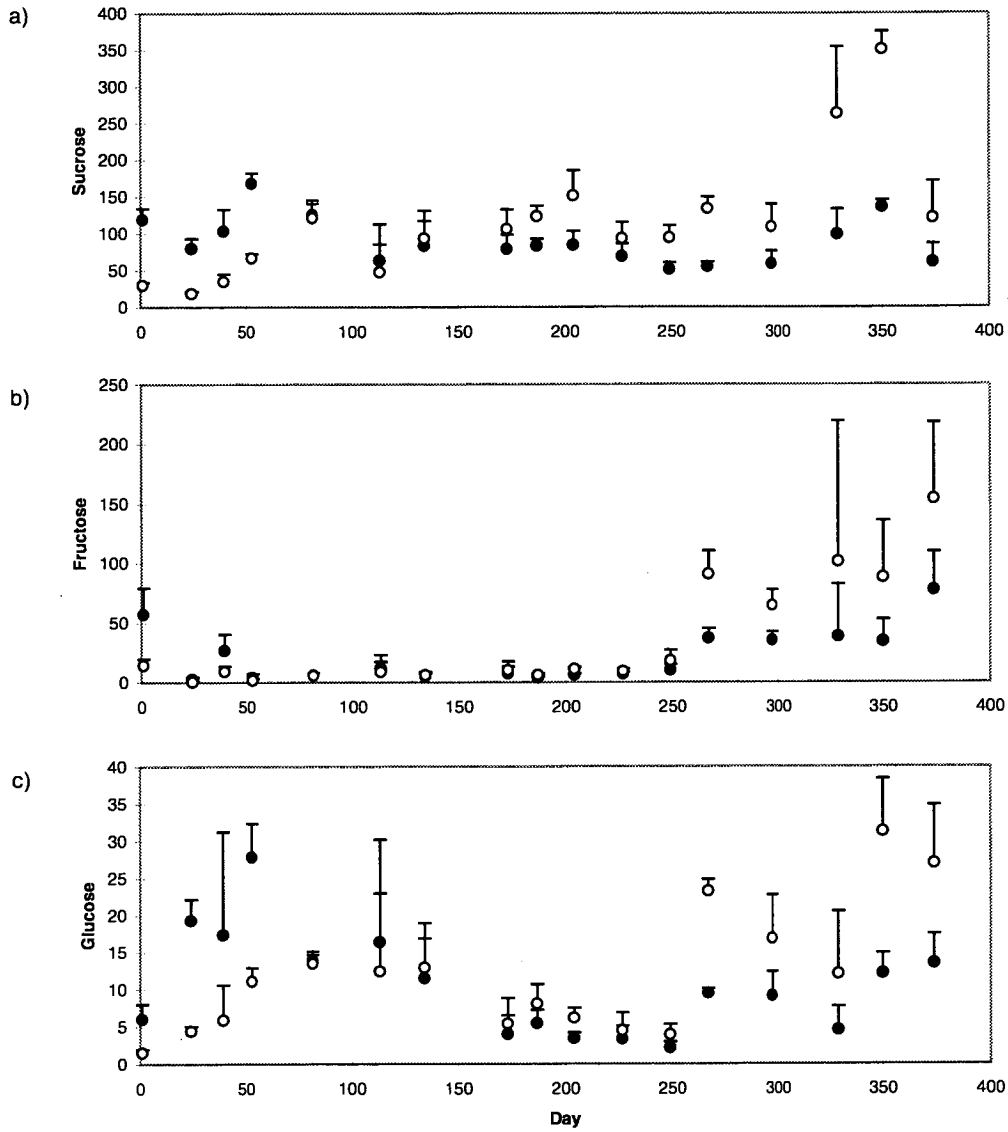

Figure V.7a-c: Mean non-structural carbohydrate (NSC) composition of leaf bases in *N. bowdenii* 'Line 63' N+1 growth units. NSC concentration (•) is shown as mg g^{-1} and total (o) as mg. Day 0 = 19 July 1995. Leaf emergence = day 81; full senescence of all foliage = 204; beginning of rapid scape elongation = 227; anthesis = 267; full senescence of all inflorescences and scape = 329. Error bars represent standard error.

All NSC were severely depleted at the time of foliage emergence from the ground and immediately prior to rapid scape elongation.

Due to the greater weight of the N unit, the mean total weight of NSC in these leaf bases was greater than that observed in the outer unit, although levels of glucose and fructose were generally lower. Depletion of carbohydrate was not as evident in these leaf bases during elongation of the foliage. However, there was indication of a substantial demand on stored carbon prior to and during inflorescence emergence. Starch levels showed a marked increase after foliage emergence, while fructose levels were exceptionally low. Insoluble fructan appeared to increase during the initial period of starch and ethanol-soluble fructan mobilisation, however, subsequently, there was a rapid decline.

The inner leaf bases from which the current season foliage originated, generally exhibited relatively low levels of NSC (Figures V.6-V.7). Although levels of free non-reducing sugars and sucrose appeared to decrease with elongation of the foliage, there was no evidence that storage carbohydrate from these leaf bases was being utilised for this process. After anthesis, levels of most types of NSC increased with the exception of starch and later sucrose, which decreased.

Preceding and following leaf emergence in the outer leaf bases, sucrose and soluble fructan concentrations followed a similar pattern. Leaf bases of the current season foliage exhibited increasing sucrose and glucose concentrations, but fructan concentrations became extremely low relative to the other storage

organs. Insoluble fructan concentrations during this period were highly variable in the outer bulb. Following development of the foliage, total amounts of storage carbohydrates (soluble and insoluble fructan and starch) increased dramatically in the outside leaf bases. However, after maximum dry weight of the foliage had been reached, levels of insoluble fructan in these leaf bases decreased. This may be associated with a simultaneous increase in soluble fructan levels. Soluble fructan, starch, sucrose, and glucose attained a concentration peak on day 134 in this growth unit. Afterward, total carbohydrate levels declined dramatically and again at day 300 subsequent to anthesis.

A slightly different pattern of carbohydrate distribution was observed in the N bulb unit. Total starch, soluble fructan, and sucrose levels reached a maximum during foliage senescence. However, insoluble fructan increased later during rapid scape elongation. During the period of rapid scape elongation, leaf bases in the outer N-1/-2 growth units became entirely expended in some bulbs. Increases in the standard error for mean values after day 297 (Figures V.1 & V.2) indicate the disappearance of these units in some bulbs. Total amounts of soluble and insoluble fructan, starch, and sucrose also declined in N leaf bases prior to, and during rapid scape elongation and anthesis. Fructose and glucose levels increased simultaneously. Some increase in storage carbohydrates was observed in these leaf bases following senescence of the inflorescence at day 329 (Figures V.3 & V.4). Levels of reserve carbohydrates in N+1 leaf bases (associated with current season foliage) increased after foliage emergence and again following senescence of the inflorescence (Figure V.5). Glucose

levels were high immediately prior to, and during, development of the foliage and following the termination of rapid scape elongation (Figure V.6c). Sucrose concentrations were highest before expansion of the foliage and in the period after inflorescence senescence (Figure V.6a). The substantial increase in soluble sugars observed in the outer N-1/-2 leaf bases prior to emergence of the foliage apparently occurred at the expense of starch (Figures V.1 & V.2).

Substantial fructan accumulation occurred in N leaf bases during active photosynthesis and declined in concentration in N-1/-2 units prior to rapid development of foliage and the inflorescence. This suggests that fructosyl sucrose polymers are being stored and utilised as a major reserve carbohydrate in *N. bowdenii* leaf bases. Decreasing starch and sucrose levels during winter were associated with increasing soluble fructan and fructose concentrations. Sucrose and fructan levels were highly correlated in tulip bulbs (Ohyama *et al.*, 1984) but this was not the case in *N. bowdenii* outer leaf bases. This suggests some differences in metabolic utilisation of carbohydrate between the bulbs. Levels of these carbohydrates were analogous in leaf bases of the N growth unit.

N. sarniensis 'Rosea', 'Salmonea', 'Fothergillii Major', *N. alata*, *N. flexuosa* 'Alba', *N. bowdenii* 'Pink Jewel' and *N. undulata* analysed for the presence of fructans indicated that fructosylsucrose polymers were present in similar quantities to those found in *N. bowdenii* 'Line 63' (Table V.1).

The concentration of storage carbohydrates varied relatively little. There were variations occurring between cultivars of the same species as between species.

Nerine species and cultivars	Soluble fructan (mg g^{-1})	Insoluble fructan (mg g^{-1})	Starch (mgg^{-1})
N. sarniensis 'Rosea'	325.7	44.8	147.9
N. sarniensis 'Salmonea'	333.6	22.8	128.1
N. sarniensis 'Fothergillii Major'	326.4	56.0	163.4
N. alata	196.6	28.4	253.8
N. flexuosa 'Alba'	297.1	35.4	203.7
N. bowdenii 'Pink Jewel'	310.0	30.8	196.9
N. undulata	218.6	38.8	232.9

Table V.1: Storage carbohydrates in the outer bulb leaf bases of several Tasmanian-grown *Nerine* species and cultivars.

DISCUSSION

The presence of fructans in the quantities recorded was surprising considering that they were reported to be insignificant in South African-grown *N. bowdenii* (Theron & Jacobs, 1996b). High levels of ethanol soluble fructosylsucrose polymers relative to longer chain insoluble forms have also been found in other bulb species that also contain starch, e.g., *Tulipa gesneriana*. However, in this species starch is the dominant reserve carbohydrate in the bulb scales (Ohyama *et al.*, 1984).

Rapid development of leaves and the inflorescence scape in *Nerine bowdenii* involves the utilisation of carbohydrate stored in the leaf bases. During the period preceding leaf emergence in the spring, the bulb is morphologically in a state of "rest" and no aerial developmental activity is visible. At a physiological level, however, it was evident that changes in carbohydrate composition of the bulb leaf bases occurred. Perhaps, as a precursor to rapid foliage development. Outer leaf base units showed a decline in starch and total soluble fructans at this time, with a corresponding increase in non-reducing sugars. Rapid foliage development appears to be supplied by hydrolysis of reserve carbohydrate from the outer leaf bases. The inner leaf bases appeared to be less effected.

Leaf bases in the N-1/-2 and N growth units exhibited a dramatic rise in concentration of the reducing sugars fructose and glucose. This trend was accompanied by a reduction in starch and the total soluble fructan, particularly in the outermost leaf bases. In contrast soluble fructan concentration increased. At day 81, as rapid foliage development is well under way, total content of all carbohydrates in the outer leaf bases was reduced to a low level. However, the content of the leaf bases immediately interior of these was less effected.

Carbohydrate metabolism and distribution patterns are known to be influenced by external environmental conditions. Exposure to chilling is known to increase the activity of starch hydrolysing enzymes such as α-amylase (Moe & Wickstrom, 1973; Lambrechts

et al., 1994) and to increase the activity of the fructan synthesising enzyme sucrose:sucrose fructosyltransferase (SST) (Pollock 1984; Koroleva *et al.*, 1997). This results in decreased starch concentrations and increased fructan concentrations (Figure V.2). Chilling also has the effect of causing a movement of sucrose out of the cytoplasm and into the vacuole (Tognetti *et al.*, 1989), the site of fructan synthesis (Pollock, 1986; Darwen & John, 1989). However, the activity of the enzymes responsible for the hydrolysis of fructan, fructan exohydrolases (FEH), are decreased in cold conditions in temperate grasses (Pollock & Cairns, 1991) and are often stimulated in the spring (Puebla *et al.*, 1997). Chilling does, however, induce net depolymerisation of fructan in Jerusalem artichoke (*Helianthus tuberosus*) tubers (Jefford & Edelman, 1968). High concentrations of fructose, such as those found in this study (Figures V.3b and V.5b), are an indication that rapid fructan depolymerisation exceeds the rate of sucrose synthesis (Machado de Carvalho & Dietrich, 1993; Pak *et al.*, 1995; Puebla *et al.*, 1997). In outer leaf bases, this appears to occur simultaneously with fructan synthesis (Figure V.2a/b), which uses sucrose as a substrate (Housely & Pollock, 1985; Pollock and Cairns, 1991). This apparent contradiction has been reported in *Veronia* (Machado de Carvalho & Dietrich, 1993), in which the depolymerisation of fructan occurred despite no change in temperature or SST activity. On one hand, the presence of an acid invertase in the vacuole complicates the process; while on the other hand it may be involved somehow in the resolution of the problem. Acid invertase catalyses the hydrolysis of sucrose and inhibits fructan synthesis, however, this enzyme is inhibited by fructose. (Oberland *et al.*, 1993; Walker *et al.*, 1996). The presence of such an enzyme would

promote hexose accumulation until negative feed back occurred. Any sucrose entering the vacuole as a result of cold induced starch hydrolysis would be hydrolysed by invertase until fructose levels accumulated, after which SST activity would presumably rise. The hydrolysis of fructan by FEH releases more fructose into the vacuole allowing sucrose synthesis to occur during fructan mobilisation. However, this integrated hypothesis for changes in carbohydrate levels found in *N. bowdenii* 'Line 63' is yet to be investigated. Such events involving accelerated activity of hydrolytic enzymes in source organs have been linked to the breaking of dormancy in other bulbous species (Halevy *et al.*, 1963; Nowak *et al.*, 1974; Ohyama *et al.*, 1984). However, in *N. bowdenii*, continuous NSC change (Figure V.1) suggests that there is no true dormancy period. The increase in the ratio between soluble and insoluble carbohydrate (Figure V.2) suggests a lowered osmotic potential in the vacuoles. Presumably this results in increased water flow to this tissue, despite a dry weight reduction. Water accumulation in the outer bulb may facilitate transport of assimilate out of these leaf bases and/or provide a source of water for rapidly expanding sink tissue. As soluble fructan is the largest component of the insoluble carbohydrate content, it is possible that this polymer is playing a direct role in the regulation of osmotic potential, as it does in the expanding tissue of some other species (Bieleski, 1993; Roth *et al.*, 1997). It has been argued that regulation of water within the plant is the greatest selective advantage of fructan synthesis (Hendry, 1993).

Although some depolymerisation of fructan appears to occur in N leaf bases, loss of dry weight and reductions in NSC concentration

in the N-1/-2 units indicate that these leaf bases provide the majority of carbohydrate required for foliage growth. Photosynthetically-derived assimilate from the foliage is accumulated by all leaf bases, principally those belonging to the N-1/-2 and N growth units, as soluble and insoluble fructan and starch. Insoluble fructan is the principle compound formed as a result of carbon translocation to the reserve leaf bases. Preferential metabolism of sucrose into fructan rather than starch has been demonstrated by the genetic transformation of potato with a microbial fructosyltransferase gene (van der Meer *et al.*, 1994). The presence of fructan synthesising enzymes in the vacuole of potato cells leads to substantially reduced starch synthesis. This suggests that the reduced starch levels observed in this study in relation to others (Theron & Jacobs, 1996b) may be the result of increased activity of fructan synthesising enzymes. Dramatic depletion in total carbohydrate from the outer leaf bases, after a sharp rise in all NSC, occurred prior to full leaf senescence in all bulbs. Mobilisation and depletion of NSC in the N leaf bases follows thereafter, although levels of insoluble fructan continue to rise until N-1/-2 units are almost entirely exhausted. It appeared that there is a centripetal distribution of hydrolytic enzyme activity in the bulb, similar to that described in onion (*Allium cepa*) (Henry & Darbyshire, 1979; Pak *et al.*, 1995), an outwardly morphologically similar bulb. These events (including dry weight loss) began before corresponding dry weight gains were observable in foliage or scape/inflorescence. It is possible, therefore, that a temporary sink, such as the basal plate (compressed stem), is holding assimilate for rapid utilisation, as occurs in onion (Pak *et al.*, 1995).

Mobilisation of NSC in N-1/-2 and N units prior to rapid scape elongation indicates that mobilisation of reserve carbohydrate probably is not controlled by assimilate demand from the sink. Perhaps, it is catalysed by an intermediate sink. The results demonstrate that the dry weight of reserve leaf bases may be composed of up to 90% NSC. Considering that photosynthetically derived assimilate is directed towards these leaf bases, ensuring that the NSC remains high following foliage expansion, there is apparently a large amount of carbohydrate available to the developing scape and other potential sinks. This suggests that any processes occurring in the bulb at this time would not be source-limited unless the demands were extreme.

Glucose levels in N-1/-2 leaf bases are generally analogous to those of fructose, albeit on a much reduced scale. This pattern was not repeated in N leaf bases where glucose distributions appeared to be erratic. Glucose concentrations were often variable and not considered to be indicative of physiological state in storage organs using starch exclusively a reserve carbohydrate (Merlo *et al.*, 1993). However, disparities between glucose concentrations in the units may be indicative of slightly different NSC metabolic processes. If this were the case, it could be postulated that the inner leaf bases are thermally insulated to some extent by those exterior to them, resulting in altered enzyme activities.

Although carbohydrate concentrations within the storage organs give an indication of NSC content and mobilisation they do not give an indication as to where the material is partitioned. Nevertheless,

it appears that the outer leaf bases are the primary source of carbon for elongating leaves, and that after this source becomes depleted the next unit is utilised indicating a centripetal mobilisation of stored carbohydrate. Therefore, it should be possible to determine the destination of sugars mobilised from these outer leaf bases. This issue will be examined in the following chapter.

VI

Assimilate Partitioning During Inflorescence Development

INTRODUCTION

Analyses of dry weight distributions and patterns of carbohydrate metabolism in the storage organs of *N. bowdenii* 'Line 63' revealed a centripetal pattern of carbohydrate hydrolysis and partitioning. Leaves were fully senescent at the time of rapid scape elongation in Tasmanian-grown *N. bowdenii* and, therefore, leaf bases were the only supply of assimilate for the developing inflorescence. The physiological state of possible sinks, however, has not been studied. In particular, the relative sink strength of the inflorescence itself and each of the florets within the spathe may be important in regulating the flower abortion process. Abortion of inflorescences may be the result of two possible mechanisms; (1)

an inability of source organs to supply the metabolites required for development, or (2) an inability of the sink organ to successfully attract, or compete for, assimilate (Ho, 1988).

Florets in the inflorescence of *N. bowdenii* are fully developed approximately three months prior to anthesis, and the number of florets at stages "Late G" (gynoecium elongated, carpels fused) and "Mid G" (carpels elongated) in mid-January is the same as at anthesis (Theron & Jacobs, 1996a). However, whole inflorescences appear only to abort at the onset of rapid scape elongation immediately prior to anthesis. The current hypothesis for inflorescence abortion is that the number of florets is instrumental in determining the ability of an inflorescence to compete with other bulb organs for assimilate supply (Theron & Jacobs 1996a; 1996b). It has been hypothesised that sink activity of the inflorescence increases as florets reach stage "Late G" (Theron & Jacobs, 1996b) and that a hierarchy of assimilate sinks exists within *N. bowdenii* bulbs (Theron & Jacobs, 1996a). The following hierarchy has been proposed: leaf primordia N+2 > floret primordia N+1 > inflorescence N > daughter bulbs > roots > storage tissue (Theron & Jacobs, 1996a). These conclusions were partially based on defoliation trails of synanthous *N. bowdenii* which were used to limit assimilate supply to bulbs for varying periods. In the following study, this hypothesis was tested by identifying the transportable carbohydrate forms in *N. bowdenii* and following the partitioning of this carbon transport form from the outer leaf bases to the various plant organs. Outer leaf bases rather than leaves were to be used since the Tasmanian-grown 'Line 63' is not synanthous and,

therefore, is not dependant upon photoassimilate in the period immediately prior to anthesis when inflorescence abortion occurs.

MATERIALS AND METHODS

Bulbs that had been transplanted two years previously into 20 cm pots were used in this study. Plants were maintained at Kettering, Tasmania, approximately 10 km north of the Woodbridge field site. Bulbs were treated *in situ*.

Prior to ^{14}Carbon feeds to examine carbohydrate partitioning the transport carbohydrate used in by *N. bowdenii* was ascertained by removing a mature leaf (considered to be a net exporter of assimilate) and placing the excised portion in a small amount of de-ionised water. Monosaccharide (arabinose) and disaccharide (trehalose) internal standards were added to aid identification and quantification of carbohydrate present in the sample. After 4 h a 5 µl aliquot was transferred to a 150 µl glass insert within a GC vial and lyophilised. Standards containing common sugars were also prepared to confirm the identity of sample peaks.

The aliquot was silylated with 20 µl of Tri-Sil Z (Pierce Chemical Co., Rockford, USA) and incubated at 60°C for 10 minutes prior to injection of 1 µl into a Hewlett-Packard 5890A series gas chromatograph linked to a Hewlett-Packard 5970 series mass selective detector. The GC column used was a HP-1 (cross-linked methyl silicon gum) with a length of 25 m, 0.32 mm internal diameter and a 0.52 µm film thickness. The head pressure used was 15 psi with a splitless column flow rate of 5 ml/min at 60°C

and helium carrier gas. The oven temperature was set at 260°C and the detector temperature at 290°C. Oven temperature was increased from an initial 50°C to 150°C at a rate of 30°C/min and from 150°C to 300°C at a rate of 15°C/min. The oven temperature remained at 300°C for four minutes. Compounds were detected in selected ion monitoring (SIM) mode with a dwell time of 40 ms/ion. Solvent delay was set at 6.5 min. All peaks eluted between 6.5 and 16.5 min were integrated with Hewlett-Packard ChemStation software.

Identification of the critical stage of inflorescence development was determined by frequent dissections during the rapid scape elongation phase. Radioactive labelled carbohydrate was fed into 10 bulbs as described previously in "Materials and Methods" chapter during April during the onset of rapid scape elongation when abortion was known to be occurring. ^{14}Carbon labelled sucrose was applied to three more bulbs 10 days later, to assess partitioning after this critical period had elapsed.

Partitioning of ^{14}carbon relative to the size of the organ was calculated by dividing radioactivity (cpm) by dry weight (g) of the organ. This in turn was divided by the total radioactivity per weight of the entire plant (cpm g^{-1}). This calculation gives the relative specific activity (RSA) of each organ (Mor & Halevy, 1979; Elphinstone *et al.*, 1988).

$$RSA = \frac{\text{cpm } g^{-1} \text{ of specified organ}}{\text{cpm } g^{-1} \text{ of entire plant}}$$

Counts per minute (cpm) were used for this study, instead of disintegrations per minute (dpm), due to a need to standardise

measurements of soluble and insoluble radioactivity. Cpm measurements account for differences in quenching levels that occur as a result of the presence of solids in the Insta-Gel™ scintillant preparation.

RESULTS

GC-MS analysis of the leaf exudate demonstrated the presence of sucrose, glucose, and fructose. Glucose and fructose were present only in trace quantities, with sucrose being the major carbohydrate. Therefore, ^{14}C-labelled sucrose was used to feed into outer leaf bases in order to follow partitioning of carbohydrate from these organs.

After dissection, bulbs from the first labelled sucrose application were divided into three categories: bulbs with inflorescence dry weight (DW) between 0.027 - 0.038 g (E, early stage of inflorescence elongation), aborting inflorescences with DW between 0.020 - 0.038 g (A, aborting inflorescence) and the remainder, which contained either no inflorescence or an inflorescence which was at an unsuitable stage of elongation, which were discarded. Aborting inflorescences were recognised from the desiccated appearance of the spathe and/or the florets within. Evidence of a physical separation at the base of the scape could also be observed (as a faintly discoloured line), not dissimilar to that which may be observed at the base of senescing scapes following seed set. DWs of inflorescences from bulbs to which labelled sucrose was applied ten days later ranged between 0.26 - 0.29 g (L, later stage of elongation).

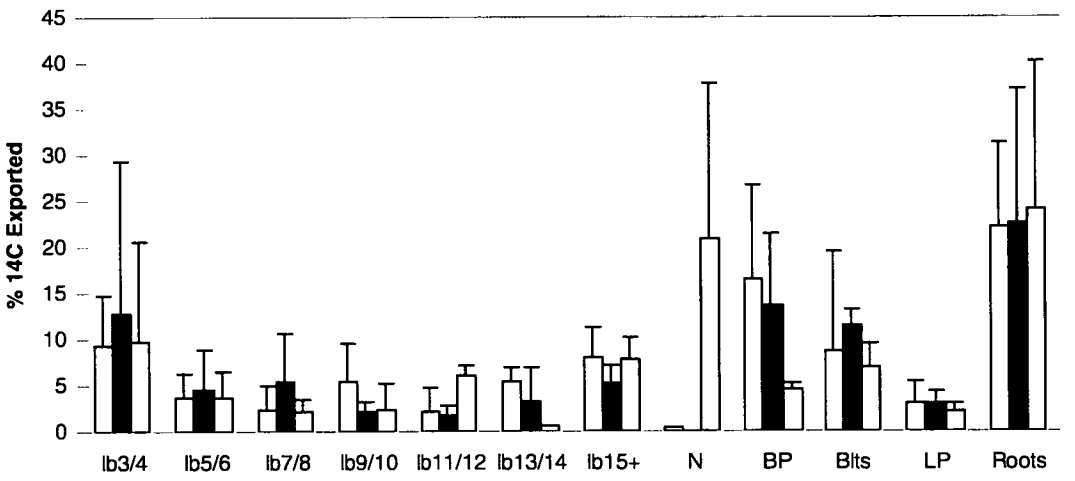

Figure VI.1: Soluble fraction containing partitioned ^{14}C originating from the outer leaf bases during inflorescence development in *N. bowdenii* 'Line 63'. Categories are leaf bases (LB) 3-15+, inflorescence (N), base plate (BP), bulblets (Blts), leaf primordia N+2 (LP) and roots. □ = 'E' bulbs with inflorescence between 0.027 - 0.038 g DW (n = 4), ■ = 'A' bulbs with aborting inflorescence between 0.02 - 0.38 g DW (n = 4), ⊠ = 'L' bulbs with inflorescence between 0.26 - 0.29 g DW (n = 3). Data are shown as means with SE bars.

Significant variation between categories was found between the basal plate in L bulbs and both other categories and between L inflorescence and both other categories (Table VI.1). The only significant variation found between A and E bulbs was in the N inflorescence.

With the exception of the roots in all categories, very little label was partitioned into ethanol insoluble matter after 36 h (Figure VI.2). Again, variation between bulbs of the same category was relatively high. Levels found in the basal plate of L bulbs were again lower, and exhibited less variation, than those found in E and A bulbs.

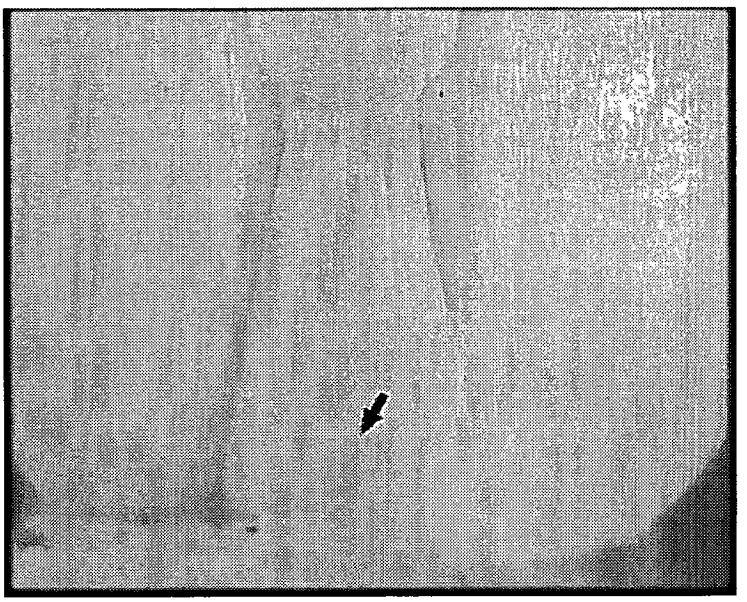

Plate VI.1: Aborting flower bud. Arrow indicates the point where a physical disjuncture forms at the scape base following abortion and senescence.

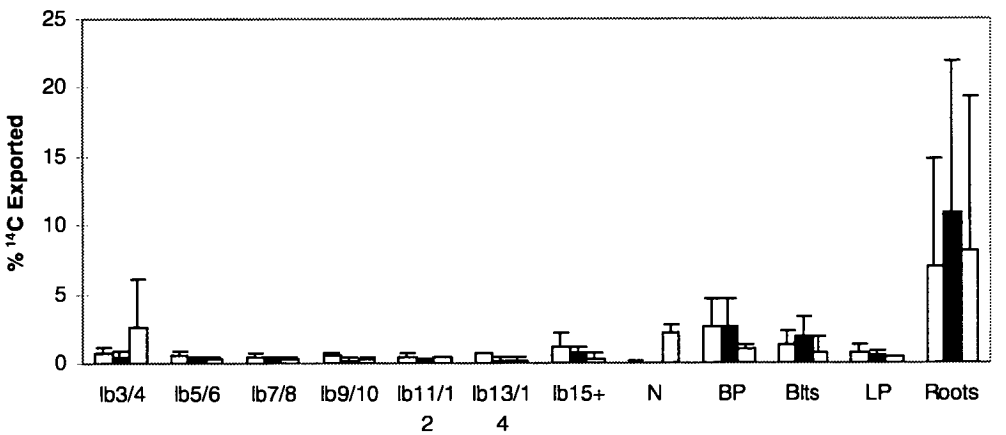

Figure VI.2: Insoluble fraction containing partitioned ^{14}C originating from the outer leaf bases during inflorescence development in *N. bowdenii* 'Line 63'. Categories are leaf bases (LB) 3-15+, inflorescence (N), base plate (BP), bulblets (Blts), leaf primordia N+2 (LP) and roots. □ = 'E' bulbs with inflorescence between 0.027 - 0.038 g DW (n = 4), ■ = 'A' bulbs with aborting inflorescence between 0.02 - 0.38 g DW (n = 4), ▩ = 'L' bulbs with inflorescence between 0.26 - 0.29 g DW (n = 3). Data are shown as means with SE bars.

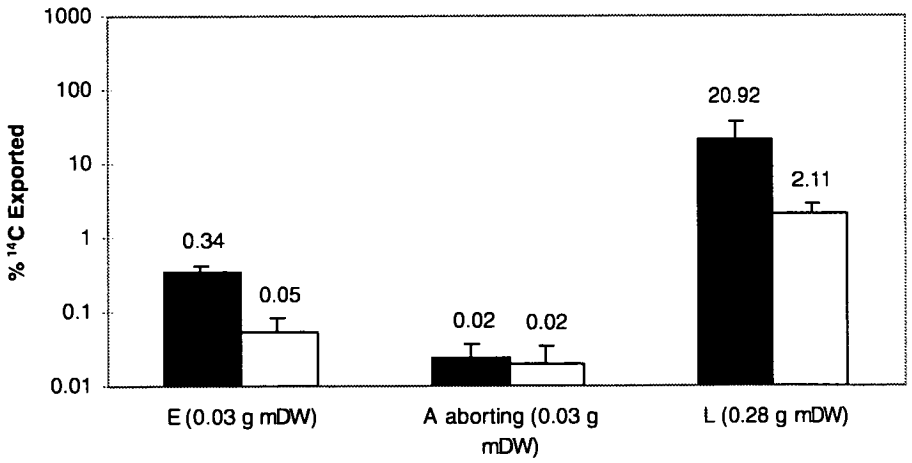

Figure VI.3: Soluble (■) and insoluble (□) fractions containing ^{14}C partitioned to the inflorescence from the outer leaf bases during inflorescence development in *N. bowdenii* 'Line 63'. Categories are E: inflorescences between 0.027 - 0.038 g mean DW (n = 4), A: aborting inflorescences between 0.02 - 0.38 g mean DW (n = 4) and L: inflorescences between 0.26 - 0.29 g mean DW (n = 3). Data are shown as means (value above column). Standard errors are shown in Table 1 below. Note that the Y-axis scale is logarithmic.

	Early (0.032 g mean DW)	**Aborting (0.028 g mean DW)**	**Later (0.275 g mean DW)**
Soluble fraction	0.087 g	0.013 g	16.910 g
Insoluble	0.030 g	0.014 g	0.695 g

Table V.1: Standard error values for means shown in the previous figure (Figure 3).

Though highly variable between individual plants, radioactivity was ubiquitous in the ethanol-soluble extracts from all bulb components (Figure VI.1). The highest mean values were recorded in the roots of all categories, followed by the inflorescence of L

bulbs (inflorescences with DW 0.26 - 0.29 g). The outer leaf bases (3 and 4 from the outside of the bulb), leaf base 15 (and other inner leaf bases where present), E (bulbs with inflorescence DW 0.020 - 0.038 g) and A (bulbs with aborting inflorescences DW 0.020 - 0.038 g) base plates and bulblets also showed relatively high concentrations of ethanol soluble radioactively labelled compounds. N+2 leaf primordia (incorporating N+1 inflorescence) exhibited relatively little imported soluble label. The least amount of variation was found in the basal plate, leaf primordia, and inner leaf bases 13 and 14 from the outside in L bulbs.

The only significant variation between categories occurred between the inflorescences of L bulbs and both other groups. Distributions were similar to that found in the soluble fractions, with leaf primordia again containing the lowest percentage of exported insoluble radioactivity outside of the leaf bases. Levels of insoluble ^{14}C in the leaf bases was consistently low with the exception of some third and fourth L leaf bases.

Examination of the soluble and insoluble radioactivity accumulated in the inflorescences of the various categories shows that soluble label increases in orders of magnitude from A to E to L. While this is also true of insoluble radioactivity in inflorescences of E and L bulbs, the difference between A and E is less substantial and is not statistically significant (Table VI.1). The two most significant aspects of partitioning to the aborting inflorescence (A) is the low ratio of soluble:insoluble label (1.2) compared to inflorescences in the early (E) stages of elongation (6.35) and later stages(L) (9.90), and the lower mean ^{14}carbon found in aborting

buds compared to early and later elongation stages. The mean radioactivity found in later developing inflorescences was one and two orders of magnitude greater than that found in early elongating scapes and florets respectively.

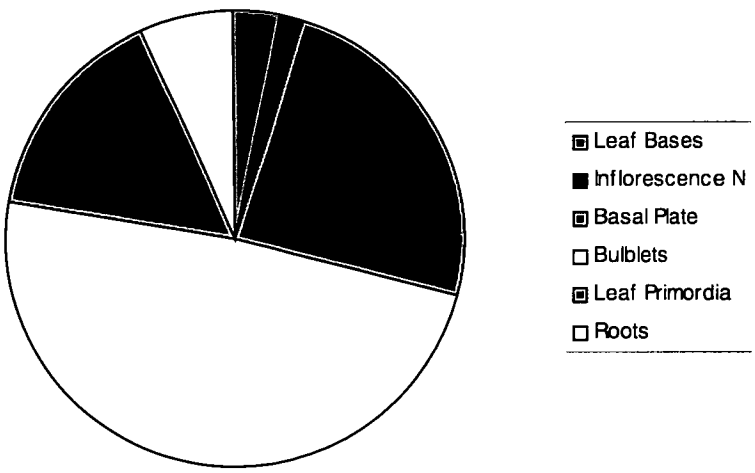

Figure VI.4: Relative specific activity (RSA) of *N. bowdenii* 'Line 63' organs in bulbs with normally developing inflorescences between 0.027-0.038 g DW (E). ^{14}Carbon originated from the outermost leaf bases.

RSA values of organs immediately prior to rapid scape elongation (E) show that the strongest sink are the bulblets (Figure VI.4). The basal plate and leaf primordia are also strong sinks. The current season inflorescence, N, is, in contrast, a very weak sink at this stage. According to their respective RSA values, the hierarchy of sinks investigated is as follows:

bulblets > basal plate > leaf primordia > roots > leaf bases > inflorescence

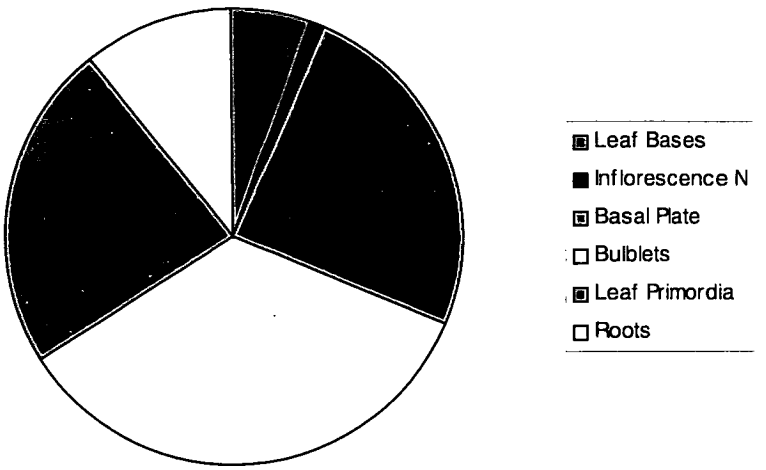

Figure VI.5: Relative specific activity (RSA) of *N. bowdenii* 'Line 63' organs in bulbs with aborting inflorescences between 0.02-0.038 g DW (A). ^{14}Carbon originated from the outermost leaf bases.

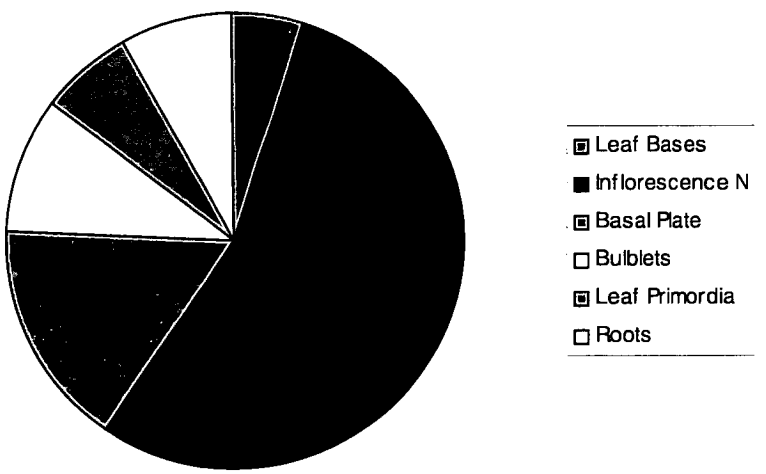

Figure VI.6: Relative specific activity (RSA) of *N. bowdenii* 'Line 63' organs in bulbs with normally developing inflorescences between 0.26-0.29 g DW (L). ^{14}Carbon originated from the outermost leaf bases.

Bulbs containing an inflorescence that is aborting (Stage A) at this time showed a markedly different proportional distribution of carbon from the outer leaf bases (Figure VI.5). The proportion of radioactive label exported to bulblets decreased. Increased partitioning was observed to the leaf primordia, roots, basal plate and leaf bases. However, the relative sink hierarchy remained unchanged compared to bulbs containing normally developing inflorescences.

Bulbs containing a flower bud with a dry weight of 0.26-0.29 g (L) showed substantially different patterns of ^{14}carbon partitioned from the outer leaf bases (Figure VI.6). The RSA of the inflorescence was more than double that of any other organ. The relative sink strength of the roots and leaf bases was similar to that observed during earlier inflorescence development. The relative activity of bulblets and leaf primordia was substantially decreased. The sink hierarchy within the bulb at stage L according to RSA values was as follows:

Inflorescence N > basal plate > bulblets > roots > leaf primordia > leaf bases

DISCUSSION

From the ubiquitous distribution of both soluble and insoluble ^{14}C-label, it is apparent that *N. bowdenii* exhibits a highly integrated pattern of assimilate partitioning similar to that found in

monopodial orchids (*Aranda* hybrids), with leaf bases acting simultaneously as sources and sinks (Neo *et al.*, 1991). Although net accumulation of carbon is not necessarily representative of the true sink strength of an organ, which must take into account carbon loss through respiration (Ho, 1988), respiratory loss of carbon in this experiment, over the time scales involved, was considered to be relatively minor. Most of ^{14}C labelled sucrose partitioned from the outer leaf bases during the early stages (E) of rapid scape elongation went to the roots. However, when the relatively large weight of these structures is taken into account, sink strength is comparatively low. The rapid rise in the percentage of recovered exported soluble radioactivity in the inflorescence during later (L) elongation, and the dramatic rise in its RSA suggests that this sink strength in this organ increases significantly during the later stages of inflorescence and scape development. Bulblets and the N+2 leaf primordia/N+1 inflorescence import relatively small amounts of assimilate from the outer leaf bases. However, these organs are strong sinks until the Stage L, when the inflorescence N is able to attract more ^{14}carbon relative to organ weight. While the N+2 leaf primordia/N+1 inflorescence complex shows a high apparent sink activity relative to its size, carbon demand may not be substantial enough to affect assimilate distribution to other organs. Bulbs containing an aborting inflorescence showed increased relative sink activities to leaf/inflorescence primordia, roots, and leaf bases. One of the most significant findings was that the basal plate, or compressed stem, of *N. bowdenii* is acting as a strong sink during all stages of rapid inflorescence growth. It is possible that carbohydrate accumulates at the basal plate as a result of the

presence of high amounts of abscisic acid (ABA). ABA is a known growth inhibitor in *Tulipa gesneriana* which may be synthesised in the basal plate (Terry *et al.*, 1982). High concentrations of ABA are also found in non-elongating *T. gesneriana* shoots. A significant decrease in exported label was found in this tissue as inflorescence growth accelerated. These results suggest that the basal plate may be acting as an intermediary storage organ for supplying assimilate to rapidly growing organs such as the inflorescence, and presumably also leaf growth. The relationship between ABA, scape growth and sucrose fluxes is an area which requires further study.

It is interesting to note that a substantial proportion of radioactivity was partitioned to other leaf bases. Although there was no discernible differences in labelled carbon accumulation between different stages of development, higher amounts were generally partitioned to nearby leaf bases. This may be due to a continuous centripetal movement of carbohydrate into the bulb as new leaf bases are initiated and caused by enhanced mobilisation in the outermost leaf bases. However, the observed response may also have been caused by the application of substantial amounts of exogenous sucrose which have disturbed assimilate bulk flow in the bulb. It is interesting to note that the RSA of leaf bases and roots remain at a similar level following rapid elongation of the scape. All other organs, excepting the inflorescence, have diminished sink activities.

Partitioning to the bulblets was not substantially altered in bulbs with an aborting inflorescence in contrast to findings of studies investigating the impact of low light intensities in bulbous *Iris*

(Elphinstone *et al.*, 1987). This may be a consequence of the perennial/annual growth habits. Bulblets were, however, greater importers of carbon from the outer scales than inflorescences early in development or aborting inflorescences, as were every other bulb organ.

The low proportion of insoluble radioactivity accumulated in all organs over the 36 h period excepting the roots, suggests that the initial storage or utilisation form is soluble fructan. This may be due to the presence of the enzymes responsible for catalysing fructan synthesis in the cell vacuole and this form of carbohydrate may be synthesised from sucrose preferentially over starch in some instances (van der Meer *et al.*, 1994). The relatively high amount of insoluble ^{14}C-label found in root tissue may be a result of the large sucrose flux into the organs.

The logarithmic increase in soluble radioactivity in the developing inflorescences may indicate a role for soluble matter, such as soluble NSC, in lowering vacuolar water potential to facilitate cell elongation. It has previously been demonstrated that rapid tissue expansion can be achieved as a result of hydrolysis of soluble fructan polymers leading to an increase in hexose moieties in the vacuole (Bieleski, 1993). Since no distinction can be made as to the cellular location of soluble radioactivity, soluble NSC utilised in the vacuole is indistinguishable from that in the apoplast. However, levels in the apoplast may be low if there is a turgor regulated phloem unloading mechanism through which reduction of cell turgor increases apoplastic sucrose uptake (Wyse *et al.*, 1986; Oparka & Wright, 1988), although this does not always

occur case (Pomper & Breen, 1996). Lowered water potential in the apoplastic region leads to an increase in phloem unloading (Schulz, 1994), and differences between turgor potentials largely determines the flux of carbohydrates into the sink (Kuiper, 1993)

It is evident that aborting inflorescences act as sink organs. The major difference expressed in aborting buds compared with non-aborting buds is the level of soluble radioactivity, which also may be expressed as a low ratio of soluble:insoluble ^{14}C-label. There are some hypotheses which may be drawn based on these findings as to possible factors which may be involved in the inflorescence abortion process. If consumption of sucrose falls (which is certainly occurring at some stage of the abortion process) the following events are plausible. Rate of delivery of sucrose would fall leading to an accumulation of sucrose in recovery cells, as supply exceeds its metabolism (Farrar, 1993). Less transport of sucrose from the phloem occurs, which alters water relations in the phloem at the point of unloading, either as a result of a rise in internal solute or as a decline of solute in the apoplast. In either event, turgor within the phloem is increased affecting the Münch-type pressure gradient, reducing sucrose import into the organ. Such an occurrence presumably alters turgor gradients between other sinks and sources and may result in increased partitioning to other sinks and altered sugar-regulated gene expression in sink and/or source organs (Farrar, 1993).

The possibility of competition between the current season inflorescence and leaf/floral primordia, as raised by Theron & Jacobs (1996a), is supported by the RSA data. Although there are

stronger sinks (daughter bulbs and basal plate) than these organs, their position in the bulb relative to the current inflorescence means there may be a case for a role of apical dominance in inflorescence abortion. However, it also possible that these structures receive increased assimilates as a result of a failure by the N inflorescence to increase its sink activity under certain conditions.

In this chapter, issues relating to the partitioning of carbohydrate from the outer leaf bases during early stages of rapid inflorescence development have been discussed. However, another issue arises once this stage has been successfully completed in the bulb. This is the process of scape elongation which ultimately determines the height of the inflorescence.

VII

Scape and Foliage Development

INTRODUCTION

In preceding chapters, successful development of the inflorescence has been cited as the primary horticultural consideration for growers. While this is true, a secondary attribute that has a significant effect on financial success is cut flower length. The regulation of flower stem lengths is a primary consideration in most floriculture crops (Okubo & Uemoto, 1984a).

Previous studies have identified agronomic practices leading to improved flowering percentages (Shillo *et al.*, 1997). In the warm, dry climate of the Negev desert region of Israel, shading and mulching of *N. bowdenii* led to improved rates of flowering (Shillo *et al.*, 1997). Presumably this was due to moderated soil temperatures (Groen, 1997). Shading also has a role in maximising flower stem lengths in some other bulbous species (Okubo & Uemoto, 1984a; Okubo & Uemoto, 1984b), making this

agronomically interesting. The fact that Tasmanian-grown *N. bowdenii* exhibit a hysteranthous habit also poses some horticultural conundrums. Does the fact that the bulb has no, or very little, aerial foliage at the time rapid inflorescence elongation impact on the success or quality of the crop? This is indeed presents an interesting issue in the light of South African findings that successful growth of foliage was related the ability of the inflorescence to develop (Theron & Jacobs, 1996a).

The results presented in the previous chapter, as well as observational evidence, suggests that the time of inflorescence abortion was during the season that emergence occurred. Thus, it was concluded that an experiment could be conducted over a single growing season to address the above issues. The possibility remains, however, that events prior to the season of inflorescence emergence predispose the organ to abort. Initially, mulching and shade treatments were imposed in a field environment to examine the effect of these treatments on flowering percentage and scape length. Secondly, bulbs were transferred to a temperature controlled glasshouse to examine the effect of shade and defoliation on leaf number and scape length.

MATERIALS AND METHODS

Field trial

Flowering-size bulbs were lifted from the Woodbridge field planting during the winter dormancy period and replanted at Kettering, some 10 km to the north. Climatic conditions for Woodbridge are

given in the Appendix, and conditions at Kettering were very similar. Bulbs were planted in a randomised complete block design consisting of three replicates of treatments randomly distributed in three plots. Each replicate treatment contained 40 bulbs. The treatments, applied at planting, were: shade, mulch and controls (no treatment) was applied. Shade was applied by using 80% shadecloth attached to cube-shaped wooden frames, each frame covering 40 bulbs. Mulching was applied in the form of 5-10 cm of pea straw covering the ground around bulbs. At anthesis, percentage flowering was recorded and scape length was measured from the point of emergence at the bulb neck to the base of the spathe. The scape and inflorescences were then removed and taken back to the laboratory where scape cell lengths were measured to determine if cell division and/or cell elongation was altered by treatments. Longitudinal sections were taken from tissue at the base, middle and top of the scape and the parenchymatous cells immediately posterior to collenchyma and fibre rings were examined using a Zeiss stereomicroscope. Cells were stained with toluidine blue by immersion in the stain for approximately 40 seconds followed by rinsing with deionised water and mounting.

Glasshouse trial

In order to examine the role of current season foliage on current season inflorescence development, bulbs were lifted and planted in 20 cm diameter pots at the Horticultural Research Centre glasshouse (University of Tasmania, Hobart). Glasshouse temperatures were maintained at 15°C (night) and 25°C (day). Incident light in the glasshouse was reduced with a heavy

application of glasshouse whitewash during spring and summer. Light intensity varied between 200 to 500 μmol photons $m^{-2}s^{-1}$. Daylengths vary between 9.04 hr (winter) and 15.3 hr (summer). Plants were arranged in a split-plot design containing three treatments; shaded, defoliated and control (no treatments). Three plots each contained a replicate of each treatment, each replicate comprising of 42 bulbs (control and defoliated treatments) or 34 bulbs (shade treatment). Shaded plants were heavily shaded by enclosing the replicates within two layers of 80% shadecloth. Bulbs subjected to the defoliation treatment had foliage removed as soon as leaves reached 50% of their estimated total length, at which time they were deemed net exporters of photoassimilate (Theron & Jacobs, 1996a). Allowing foliage to develop until this stage presumably resulted in the leaves acting as a net drain on bulb carbohydrate reserves. The number of leaves removed and rate of foliage emergence in shade and control treatments was recorded. At anthesis scape height was measured and cell lengths determined as described above.

RESULTS

Field trial

N. bowdenii planted in the outdoor field trial reached anthesis between 28 April and 10 May; the leaves of plants in all trials were fully senescent at this time. Flowering percentages were low for all treatments (9.2, 8.3, and 9.2% for shaded, mulched and controls respectively), differences in flowering percentage were not statistically significant. The low flowering percentages observed

are believed to be related to the replanting of bulbs during the rest period of the bulb. Although these flowering percentages are lower, a similar observation was made in the Negev (Shillo *et al.*, 1997). It underlines the requirement for *Nerine* bulbs to be left *in situ* for as long as possible until overcrowding occurs (Shillo *et al.*, 1997). Results of scape height measurements are presented graphically in Figure VII.1 (below). Shaded plants possessed a higher mean scape length than control or mulched treatments. Analysis of variance demonstrated that these differences were statistically significant, both returning P values less than 0.001. However, there was no statistically discernible differences between mulched and control treatments. There was no statistical difference between replicate plots of the same treatment.

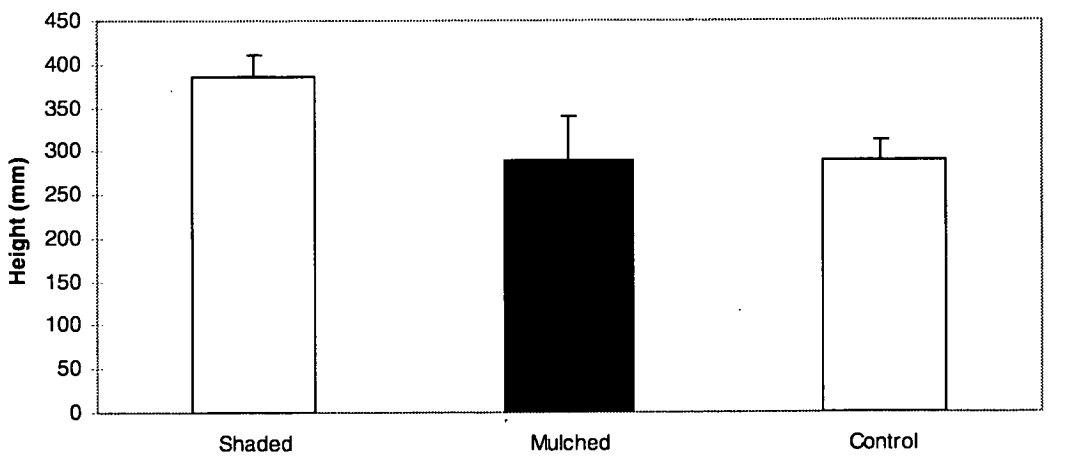

Figure VII.1: Differences in scape height of field-grown *N. bowdenii* 'Line 63', comparing shaded (n = 11), mulched (n = 10) and control (n = 11) plants. Error bars represent SE.

Glasshouse trial

Scape lengths of glasshouse-grown plants were consistently greater than those of plants grown in the field. Flowering percentages were again low, shaded, defoliated and controls resulting in 10, 10, and 7% flowering respectively. Shaded plants produced consistently longer scapes than defoliated or control treatments. Control scapes were, in turn, longer than those of defoliated plants (Figure VII.2). The difference in scape height between all treatments was statistically significant ($P < 0.001$).

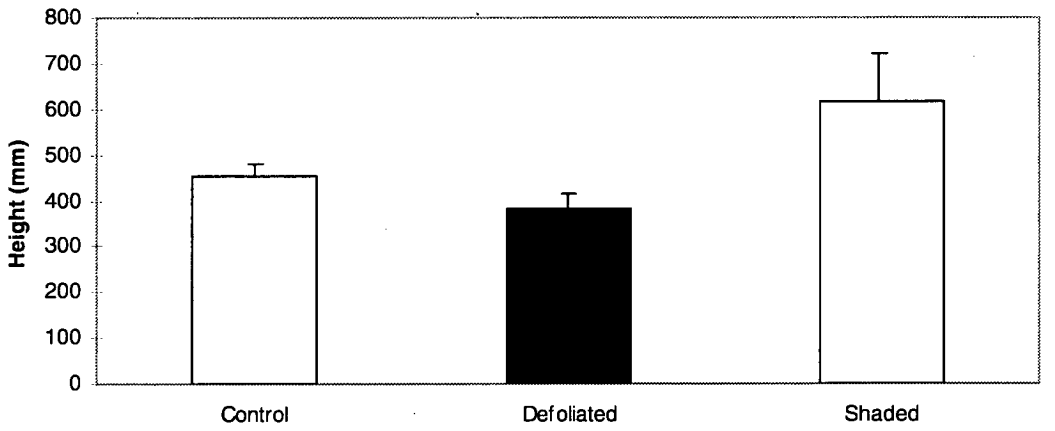

Figure VII.2: Differences in scape height of glasshouse-grown *N. bowdenii* 'Line 63', comparing control (n = 9), defoliated (n = 11) and shaded (n = 13) plants. Error bars represent standard error.

The pattern of leaf emergence in shaded plants differed dramatically to that observed for other treatments (Figures VII.3 & VII.4). The total number of leaves produced by shaded bulbs was significantly less than that produced by defoliated and control bulbs ($P < 0.001$) (Figure VII.3). Generally, the leaves produced at the onset of leaf emergence were present throughout the trial,

contrasting with the control plants, which displayed a constant turnover of foliage (Figure VII.4). The foliage of control bulbs became completely senescent while many shaded plants showed no signs of senescing. There was no significant difference between the numbers of leaves produced by defoliated and control plants. It is interesting to note that all glasshouse-grown plants exhibited a synanthous habit in contrast to those grown in the field.

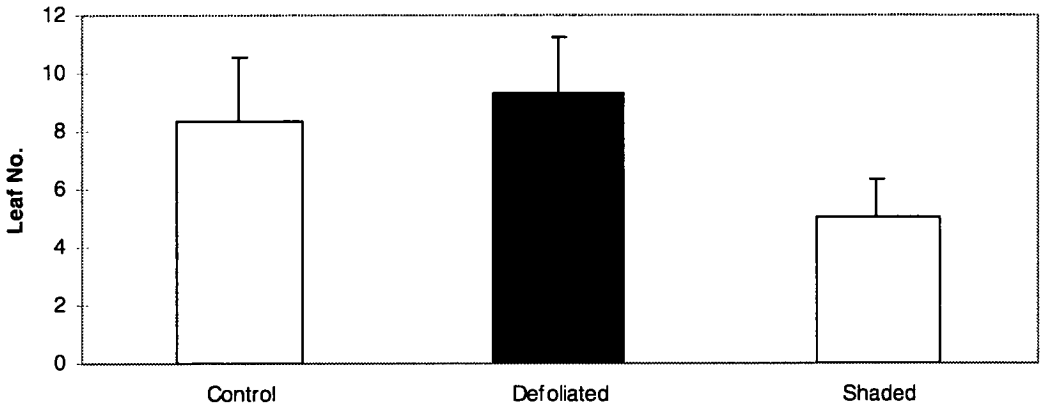

Figure VII.3: Total number of leaves produced by glasshouse grown *N. bowdenii* 'Line 63', comparing control (n = 128), defoliated (n = 110) and shaded (n = 130) treatments. Error bars represent standard error.

Scape growth

Examination of parenchymatous cell lengths revealed that the smallest cells were most likely to be found at the base of the scape (Figures VII.5 & VII.6). These cells showed relatively less variability in size and also appeared much less differentiated in terms of cell wall and chloroplast development than those in the middle and at the top of the scape. Scape cell lengths of shaded and control plants grown under field conditions were generally very similar (Figure VII.5).

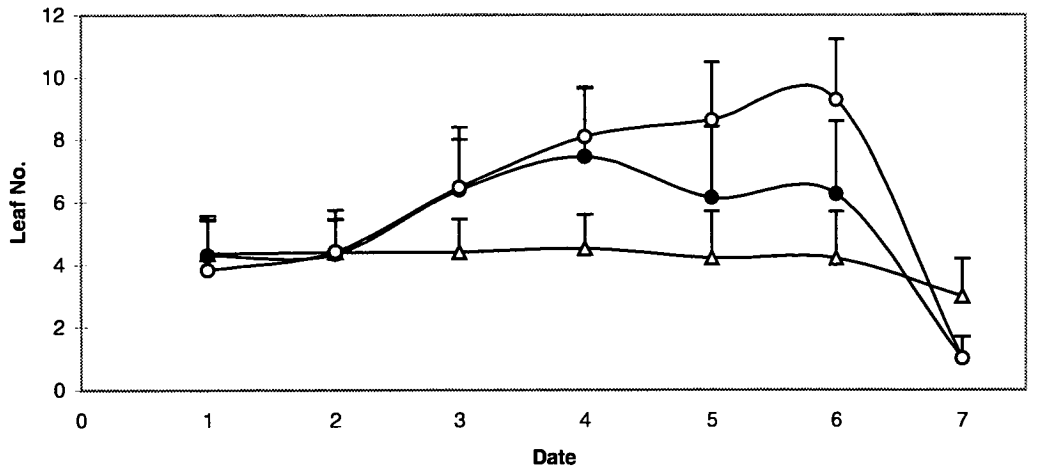

Figure VII.4: Patterns of leaf emergence in glasshouse-grown *N. bowdenii* 'Line 63', comparing control (•), defoliated (o) and shaded (Δ) treatments. Dates: 1 = 17/10, 2 = 10/11, 3= 15/12, 4 = 25/1, 5 = 9/3, 6 = 11/4, 7 = 5/5. Error bars represent standard error.

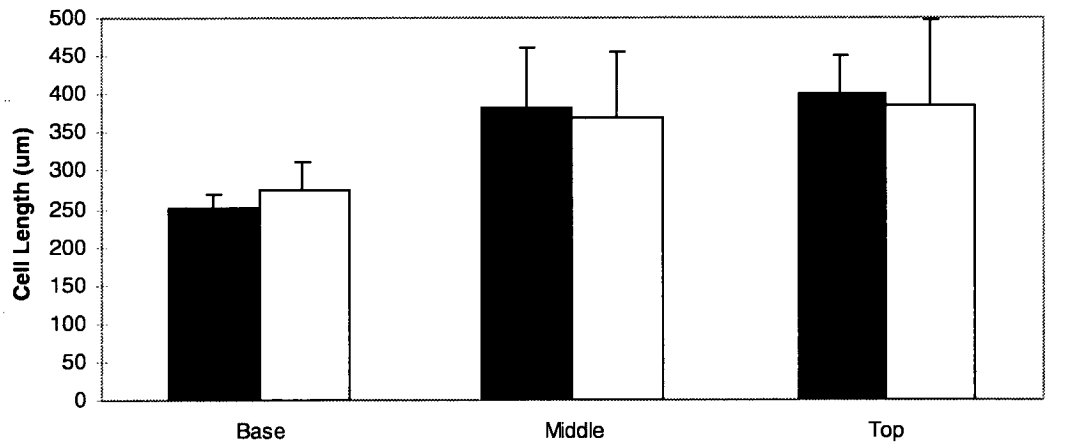

Figure VII.5: Parenchymatous cell sizes in the base, middle and top of inflorescence scapes from field-grown *N. bowdenii* 'Line 63'. Dark bars represent shaded plants, light bars are the controls. Error bars represent standard error.

Glasshouse grown inflorescence scapes showed a marked size increase between parenchyma cells found in the middle of the scape and those at the top. Cells at the top of the scape were generally longer than those at the same position from scapes grown in the field. Plants grown under shade in the glasshouse generally had longer sized cells than glasshouse-grown control bulbs at the same position in the scape. Calculations of scape length based on mean cell sizes of scapes showed that the number of cells in shaded and control plants were similar, differences being in the order of 50 to 70 cell lengths. Therefore, it appears that a large part of the difference in scape height can be attributed to differences in cell length.

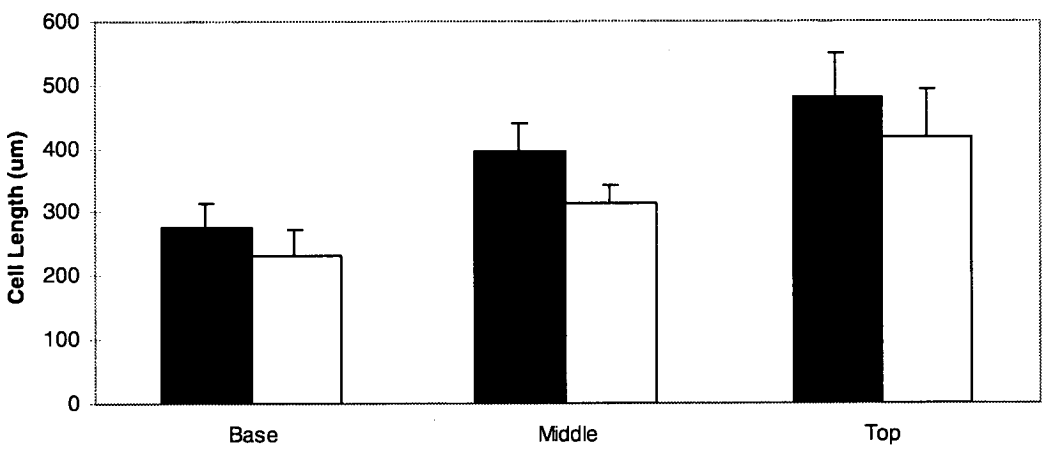

Figure VII.6: Parenchymatous cell sizes in the base, middle and top of inflorescence scapes from glasshouse-grown *N. bowdenii* 'Line 63'. Dark bars represent shaded plants, light bars represent controls. Error bars represent standard error.

DISCUSSION

The flowering percentages of *N. bowdenii* in these experiments was lower than expected. This was attributed to the effect of transplanting of the bulbs prior to commencement of the trial. Similar behaviour of transplanted bulbs has been recorded previously, with marked improvements in flowering percentages in the following years (Shillo *et al.*, 1997). That similar flowering percentages were found in both field and glasshouse environments suggests that the effect was probably due to the lifting process itself rather than climatic variables or treatments imposed on transplanted bulbs. Such a marked response to handling may be a result of root damage during lifting/replanting and/or moisture changes (van Brenk & Benschop, 1993). If this is the case, the roots may play an important role in maintaining bulb processes during the "rest" period. The importance of the root system as an integral component of the metabolic processes occurring within the bulb have been illustrated by the amount of carbon partitioned to these organs from the outer leaf bases. Alternatively, it may also be possible that inflorescence development is adversely affected by wound responses occurring as a result of root damage. Shading and mulching caused no appreciable improvement in flowering percentage when applied before emergence of the current season inflorescence, however, exceptionally low flowering percentages were obtained in all treatments.

Leaf emergence in *N. bowdenii* is more plastic than previously reported (Rees, 1985). Annual foliage units apparently are dependant to some degree on prevailing climatic conditions rather

than the species or cultivar. Recently, it has been found that *N. bowdenii* can exhibit an evergreen habit similar to that found in *Hippeastrum* (Shillo *et al.*, 1997). Shading bulbs under greenhouse conditions had the effect of prolonging aerial foliage relative to glasshouse control plants, although the total number of leaves that emerged was substantially reduced. Plasticity was also observed in regard to the hysteranthous habit normally observed in Tasmanian-grown *N. bowdenii* and displayed by field grown plants in this trial. Glasshouse plants were synanthous in their flowering behaviour (inflorescence and foliage coinciding), demonstrating that the periodicity of the bulb may be manipulated to a large extent.

Current season foliage was not important with regard to successful inflorescence development under glasshouse conditions, although the scapes produced were significantly shorter than those of control or shaded bulbs. This finding is in agreement with a previous study (Theron & Jacobs, 1996b) which found a low percentage of bulbs reached anthesis when defoliated around the time of initial leaf emergence. That inflorescences are able to flower at the same percentages as control bulbs suggests that photosynthetically derived photoassimilate is not required for elongation of the inflorescence scape under the conditions in which the trial was conducted. However, the fact that defoliated bulbs produced a significantly shorter scape than control bulbs may indicate that photoassimilate is utilised by the elongating scape to some degree. *Tulipa* placed in total darkness during stem elongation displays a similar non-requirement for photoassimilate for stem growth (Lambrechts *et al.*, 1994; Suh, 1997). Similarly

shading did not reduce flowering percentage under field or glasshouse conditions, although extreme shading has been reported to cause abortion in *N. bowdenii* (Theron & Jacobs, 1996b).

Scape length was increased by shading in both field and glasshouse environments. This suggested that shading may be used commercially to maximise scape height and, therefore, financial returns. Similarly, *Tulipa* grown in conditions of total darkness during defined periods exhibit increased internode lengths (Okubo & Uemoto, 1984a; 1984b; Suh, 1997). There is evidence to suggest that shading may also improve flowering percentages in *N. bowdenii* when applied over a longer term (Shillo *et al.*, 1997). Furthermore, scape heights were greatest under glasshouse conditions, perhaps suggesting that other variables such as temperature or watering can be optimised during the scape elongation period, although the condition of the following season inflorescence must also be considered.

Scapes appear to elongate via cell division at a meristem at the base of the scape as was indicated by the presence of small undifferentiated cells in this region. Cell elongation also occurs in the lower section of the scape, cells reaching a maximum length at the middle of scapes from field-grown bulbs and at the top of scapes produced in the glasshouse. Cell length differences could be observed between shaded and control bulbs from the glasshouse. This accounted for much of the substantial increase in scape height from control to shaded bulbs. The observed

increase in scape height is, therefore, a product of enhanced cell elongation and, to a lesser extent, increased cell divisions.

VIII

General Discussion

Despite a recent increase in the literature being published on *Nerine* physiology and agronomy, many questions remain. Year round forcing of *N. bowdenii* has been found to be possible in the Netherlands using precise temperature regimes. However, the practice is not economically viable during the period from February to June due to low flowering percentages (Groen, 1997). Similar problems have been reported from field crops in South Africa (Theron & Jacobs, 1992), Israel (Shillo *et al.*, 1997) and Australia (Blake *et al.*, 1997).

Altered patterns of carbohydrate partitioning within the bulb has been implicated in the abortion of the inflorescence (Theron & Jacobs, 1996a). Patterns of dry weight distribution and carbohydrate mobilisation suggest that the source of carbohydrate for the development of aerial organs, including the inflorescence, is principally from the outer leaf bases of the bulb. This evidence was supported by experiments involving defoliation of bulbs as foliage emerged. This treatment did not significantly retard flowering in glasshouse-grown bulbs (although flowering percentage was too low to allow definite conclusions). However, it may cause source

limitations in the following seasons. Analysis of dry matter data suggests that photoassimilate was transported to the middle leaf bases in hysteranthous *N. bowdenii*. Subsequently, it then acts as a secondary source during current season inflorescence development and primary source in the following year.

The major non-structural carbohydrates identified in *N. bowdenii* 'Line 63' were: ethanol-soluble and insoluble fructan, starch, sucrose, fructose and glucose (Figures V.3-V.7). The transport form of carbon in leaf assimilate was identified as sucrose. In contrast to previous studies (Theron & Jacobs, 1996b), the major form of non-structural carbohydrate was ethanol-soluble fructan, or relatively short chain fructosylsucrose polymers. The bulbs used in this studies were hysteranthous under field conditions, unlike those from bulbs used by Theron & Jacobs (1996b). However, the hysteranthous habit of Tasmanian-grown *N. bowdenii* was shown to be dependant on environmental conditions. The bulbs became synanthous under glasshouse conditions. A high degree of plasticity in terms of foliage production has also been found in the Negev region of Israel (Shillo *et al.*, 1997). Analyses of other species and cultivars grown under contrasting environmental conditions indicate that the high fructan levels are widespread in Tasmanian grown *Nerine* species and are not particular to the *N. bowdenii* 'Line 63' population sampled (Table V.1). The role of fructan in bulbous plants is poorly understood (Ohyama *et al.*, 1984). Why fructan polymers are present in these bulbs yet not in those grown elsewhere is problematic. A number of roles have been implicated in other species including: resistance to chilling and freezing, low oxygen tolerance, osmoregulation and interim

storage of carbohydrate, although none of these roles are clearly established (Pollock & Cairns, 1991). Fructan often plays a key role in avoiding the down-regulation of sucrose production in the mesophyll of photosynthesising tissue by avoiding the negative feedback mechanism controlling sucrose flux (Jang & Sheen, 1994). The interconversion of starch, sucrose and low DP soluble fructan occurs readily in tulip (Haaland & Wickstrom, 1975). Fructans may, therefore, be playing a similar role in bulb tissue as it does in photosynthesising organs, i.e., avoiding build up of sucrose which may interfere with starch hydrolysis. The fact that fructan does not occur in some *N. bowdenii* bulbs appears to preclude the possibility that these polymers are playing a pivotal role distinct from that of the other carbohydrates present in the physiology of the bulb. Some effect on osmoregulation is inevitable, however, as the presence of soluble fructan in the vacuole will lower the osmotic potential of the cell to a large extent (e.g., Bieleski, 1993). Fructan accumulated as a result of oxygen deficiency in some species (Albrecht *et al.*, 1993; 1997), while starch concentrations, however, remained relatively constant (Albrecht *et al.*, 1997). Fructan is also known to accumulate as a result of nutrient deficiency in temperate grasses (Wang & Tillberg, 1996; 1997). The response of hydroponically-grown barley (*Hordeum vulgare*) to low nitrogen levels was a decrease in invertase activity in sink organs and an increase in the activity of sucrose:sucrose frutosyltransferase (SST) in source tissue (Wang & Tillberg, 1996). This resulted in up to a 200% increase in starch and a 700% increase in fructan. Phosphorous deficiency in the same species resulted in increased fructan concentrations without significantly affecting starch levels (Wang & Tillberg, 1997). These

higher fructan levels occurred as a result of increased activity of the sucrose synthesis pathway (Wang & Tillberg, 1997). Chilling is known to increase fructan levels in a wide range of species and families and has been linked to possible roles in increasing cold hardiness (Pollock, 1986; Pontis, 1989; Tognetti *et al.*, 1989; Machado de Carvalho & Dietrich, 1993; Puebla *et al.*, 1997). Cold-induced fructan synthesis in barley (*Hordeum vulgare*) has the effect of elevating total carbohydrate content above normal levels (Koroleva *et al.*, 1997). A case study of the North American genus *Bromus*, showed that species grown in cold desert areas were constitutive fructan accumulators, while those growing in milder climates only accumulated fructan when subjected to cold stress (Puebla *et al.*, 1997). Plants not accumulating fructan demonstrated higher activities of sucrose metabolising enzymes such as invertase and sucrose synthase (SS). Many of the conclusions drawn in these studies relate to restricted growth and oversupply of photosynthates from foliage (Pollock, 1984; Pollock & Cairns, 1991). Fructan synthesis will also protect plant tissue to some extent from freezing due to colligative effects in the vacuole (Pollock, 1986). The decrease in cell water potential generated by the increase in vacuolar solutes may also reduce freezing-induced dehydration (Levitt, 1980 cited in Pollock, 1986).

Along with increased fructan content, total NSC concentration was also generally greater in the bulbs studied (Figure V.1) than in a previous report (Theron & Jacobs, 1996b). Exposure of barley (*Hordeum vulgare*) to chilling resulted in increased fructan synthesis and an overall increase in total NSC concentration (Koroleva *et al.*, 1997). It may, therefore, be postulated that

increased NSC levels found in *N. bowdenii* 'Line 63' are a result of fructan synthesis, despite reduced starch levels. Furthermore, in leaves of grasses, it has been noted that the levels of soluble NSC in the vacuole can far exceed those of starch (Pollock & Cairns, 1991). It has been hypothesised that the use of the vacuole as a storage compartment is suitable for plants exploiting marginal environments, where mobilisation of reserves are required to sustain growth between periods of positive carbon balance. While this applies to *Nerine*, it also applies to many other geophytic species that do not contain fructan. It has often been noted that fructan accumulation is only one of a number of mechanisms to overcome various environmental stress. Fructan accumulating species have been reported growing alongside species which do not accumulate fructan (Pollock & Cairns, 1991), or even with species capable of producing fructan but which do not produce large amounts (Tertuliano & Figueiredo-Ribeiro, 1993).

Patterns of storage carbohydrate mobilisation were observed (Figure IV.2) that were similar to those recorded in tulip (Moe & Wickstrom, 1973; Lambrechts *et al.*, 1994). Evidence of mobilisation tended to occurred in a centripetal fashion, as previously described in onion (Henry & Darbyshire, 1979; Pak *et al.*, 1995). Although these changes in carbohydrate composition in outer part of the bulb occurred prior to rapid elongation or the inflorescence, source mediation of the process has been discounted in tulip (Thompson & Rutherford, 1979). However, reserve mobilisation probably enhances stem elongation in the tulip (Lambrechts *et al.*, 1994). Mobilisation of carbohydrate in N-1/-2 and N units prior to rapid scape elongation indicates that

mobilisation of reserve carbohydrate probably is not controlled by assimilate demand from the sink, unless it is catalysed by an intermediate sink. The results demonstrate that the dry weight of reserve leaf bases may be composed of up to 90% non-structural carbohydrate. Considering that photosynthetically derived assimilate is directed towards these leaf bases, ensuring that reserves remain high following foliage expansion, there is apparently a large amount of carbohydrate available to the developing scape and any other potential sinks. This would suggest that any processes occurring in the bulb at this time would not be source-limited unless the demands were extreme.

Glucose levels in N-1/-2 leaf bases are generally analogous with those of fructose, albeit on a much reduced scale (Figures V.3). This pattern is not repeated in N leaf bases where glucose distributions appeared to be erratic. Glucose concentrations were often variable and not considered to be indicative of physiological state in storage organs using starch exclusively a reserve carbohydrate (Merlo *et al.*, 1993). However, disparities between glucose concentrations in the units may be indicative of slightly different non-structural carbohydrate metabolic processes. If this was the case, it could be postulated that the inner leaf bases are thermally insulated to some extent by those exterior to them, resulting in altered enzyme activities.

It has been hypothesised that, once mobilised, a number of organs in the bulb may compete for stored carbon (Theron & Jacobs, 1996a). Partitioning of ^{14}C-sucrose has demonstrated that while the roots imported a large amount of radioactive carbon, the

strongest sinks relative to organ weight were initially the daughter bulbs, basal plate, and leaf/floral primordia. It was found that the inflorescence was able to develop normally despite the removal of all foliage; effectively denying the bulb of photosynthetically derived sucrose. The total pool of reserves in the bulb must, therefore, be quite large. Furthermore, the requirement of the inflorescence for assimilate was low at the time abortion occurs. Relative sink activity of the roots and leaf/floral primordia rose as a result of abortion. This data supports the hypothesis of Theron & Jacobs (1996a), who suggested that inflorescence N may fail to compete for assimilate with primordial structures if few florets reach "Late G" by mid January. The hierarchy of sinks (according to their relative specific activity) within the bulb was the same in bulbs with inflorescences that were aborting and those with healthy inflorescences prior to very rapid scape elongation. This was as follows:

bulblets > basal plate > leaf/floral primordia > roots > leaf bases > inflorescence N

Once rapid elongation of the scape and florets had commenced the hierarchy became:

inflorescence N > basal plate > daughter bulbs > roots > leaf/floral primordia > leaf bases

Partitioning of radioactive-labelled sucrose from the outer leaf bases has shown that the basal plate is a major carbon sink for assimilate. If this is also the case during early mobilisation of

reserves in the outer bulb, it may explain the dry weight loss observed at this time. Whilst the observation that the basal plate is a major sink has not been previously considered in *Nerine*, it is recognised as an important sink in onion (*Allium cepa*) (Pak *et al.*, 1995). The predominant carbohydrate stored by the basal plate of onions is fructan, which is believed to be stored as a result of assimilate supply exceeding demand (Pak *et al.*, 1995). The results found with *N. bowdenii* 'Line 63' support this theory (Figures VI.1-2). The high levels of radioactivity found in the basal plate of aborting *N. bowdenii* indicated that competition between sinks may not be the principal cause of inflorescence abortion in this species. RSA of the base plate is reduced when demand is high (during rapid elongation of inflorescence N). However, in bulbs containing aborting inflorescences no such decrease was found, rather, increased partitioning to the primordia appeared to be at the expense of peripheral bulblets. Lack of a decrease in the RSA of the basal plate may indicate that supply of carbohydrates are not limiting.

The hypothesis the role of the basal plate as temporary storage organ could be extended to the roots which could possibly provide more carbohydrate as growth continued to accelerate and demand exceeded the short-term storage capacity of the basal plate. Such a mechanism would be advantageous to the plant as the carbohydrate accumulated may assist in the maintenance of the pressure gradients between the roots and other organs. It may not be necessary only for carbon partitioning, but also for other assimilate components moving through the plant via the bulk flow mechanism.

Failure of the inflorescence to attract sufficient carbon for accelerated growth may be due to an inability of the sink to maintain its own assimilate supply (Walker *et al.*, 1978; Ho, 1988). There are a number of possible reasons this may occur. The most common, and most often cited in the literature, is a failure of the sink to metabolise and compartmentalise sucrose (e.g., into amyloplasts in the form of starch) so that turgor and solute concentration gradients are interrupted. This is usually a result of either reduced sucrose synthase (SS) or ADP-glucose pyrophophorylase activity, which result in the inability of a sink to synthesise starch and is characterised by a high ratio of soluble:insoluble NSC (Doehlert & Kuo, 1990; Doehlert, 1993; Wang *et al.*, 1993; Cheikh & Jones, 1995).

Alternatively, there are three possible sites for invertase activities to hydrolyse imported sucrose. These are cell wall acid invertase, cytosolic neutral invertase, and vacuolar acid invertase (Hawker *et al.*, 1991). These enzymes are responsible for hydrolysing sucrose into fructose and glucose; thereby, maintaining a sucrose gradient between the phloem and the apoplast (Miller & Chourey, 1992). Activities of these invertases are reduced by heating to different degrees. Cell wall invertases are usually more robust in relation to heat stress (Cheikh & Jones, 1995).

The fifth known mechanism for hydrolysing sucrose arriving in the sink are the fructan synthesising enzymes sucrose:sucrose fructosyltransferase (SST) and fructan:fructan fructosyltransferase (FFT) or sucrose:fructan fructosyltransferase (SFT) (Nelson &

Spollen, 1987; Roth *et al.*, 1997). Activities of these enzymes increase after chilling (Koroleva *et al.*, 1997). These five pathways are thought to be exclusive to various tissues (Hawker *et al.*, 1991), however, it is possible that both fructan and starch are accumulated in the *N. bowdenii* inflorescences as fructan was only found in very low levels previously (Theron & Jacobs, 1996b). However, fructan plays a major role in expanding tissue of fructan accumulators (Roth *et al.*, 1997). As these enzymes occur in the cell vacuole (Pollock, 1986; Darwen & John, 1989) and not at the site of either symplastic or apoplastic unloading, it is more likely that fructan synthesising enzymes may play only a secondary role in remetabolising non-structural carbohydrate (NSC) in the cell.

The role of plant growth regulators in originating in the inflorescence or other plant organs can not be discounted in relation to their influence on sink activity. Cytokinins are well established to increase sink activity by stimulation of cell division (Kuiper, 1993; Miedema, 1994). In mature tissue, cytokinins promote anabolic processes such as protein synthesis. Accelerated protein production implies high turnover of enzymes and structural proteins. Thus, slowing senescence by minimising the amount of reducing and energy-rich compounds available for tissue degrading processes (Kuiper, 1993) However, the role of cytokinins in regard to sink activity has been refuted in some species (Halevy, 1988). Gibberellins also effect sink activity by partially hydrolysing cell walls and promoting cell elongation. Turgor potential and, thus, sink activity are effected. Gibberellins may also slightly increase pH in the apoplast increasing invertase activity in this region, an important process as monosaccharide

uptake from the apoplast occurs in sink tissue (Kuiper, 1993). Ethylene appears to play a role in the senescence and/or abortion of some species (van Meeteren & de Proft, 1982; Halevy, 1988; Whitehead & Vasiljevic, 1993), but not in others (Bieleski & Reid, 1992). Stimulation of growth in *Narcissus* and tulip scapes can be controlled by removal of floral parts (gynoecium) and addition of pastes to the cut scape containing growth regulators (Hanks & Rees, 1975; 1977; op den Kelder *et al.*, 1971).

It is also known that heat stress and other physiological stresses will not only alter metabolic activity at the time but also may have long-term effects on the organs future ability to maintain sink activity (Kuiper, 1993, Novoplansky, 1996). This may be the result of inadequate vascular orientation (Murfet, 1985; Sachs *et al.*, 1993), or damage to cell wall invertase (Cheikh & Jones, 1995). It is possible, therefore, that reduced sink activity in the inflorescence is related to past events and is not necessarily related to conditions at the time that abortion occurs.

Following abortion and/or desiccation of flower buds, senescence of the inflorescence apparently occurs, and a physical disjuncture forms at the base of the scape (Plate VI.1). The two contemporary theories relating to the initiation of senescence at the whole plant level are: (1) a senescence factor (possibly hormonal) is exported from flowers or fruits and (2) developing seeds or fruits act as a nutrient drain, directly or indirectly triggering senescence (Sklensky & Davies, 1993). Senescence of the scape associated with aborting umbels may therefore be considered to be either a function of inadequate supply of nutritional substances or

production of a senescence factor by aborting florets. Another possibility, of course, is the cessation of production of an agent (possibly hormonal) that is responsible for maintaining the organs ability to attract assimilate. Interestingly, an invertase inhibitor has been associated with senescence in the inflorescence of gladioli (Halaba & Rudnicki, 1989), resulting in an increased sucrose:hexose ratio the senescing tissue (Bieleski & Reid, 1992; Yamane *et al.*, 1995). However, to what extent this NSC ratio is a cause or effect of senescence is unknown.

If rate of development of the primordia was greater than that of inflorescence N at the time of rapid scape elongation, and carbohydrates are not limiting, it may be the lack of ability of the inflorescence to attract assimilate (at this time or at an earlier stage) that causes its eventual abortion. The presence of insoluble radioactivity in aborting inflorescences indicates that some hydrolysis of sucrose is occurring, the levels of soluble radioactivity are, however, relatively low. It is more likely that import into a utilisation sink such as an inflorescence is controlled by metabolic activity rather than the transport processes themselves (Ho, 1988). As has been discussed, reduced hydrolysis of sucrose leads to a reduction in sink activity, which in turn is manifested by a decreased rate of import to the sink. Decline in sink activity has been linked to a number of enzymes, plant growth regulators and/or past physiological or environmental stress conditions. Such conditions include heat stress which has been suggested as a possible factor in inflorescence abortion in *Nerine* (Shillo *et al.*, 1997; Groen, 1997). The final outcome of inflorescence abortion is an apparent senescence of the organ, which, in gladioli, has been

associated with the inhibition of invertase activity. This suggests that the reduction of sink activity may produce physiological conditions within the inflorescence that are responsible for initiating senescence following anthesis.

Patterns of leaf emergence in *N. bowdenii* 'Line 63' in terms of foliage turnover and duration of aerial foliage were shown to be highly plastic. Whilst local field-grown bulbs are hysteranthous in habit (including plants grown under shade), bulbs growing in a temperature controlled glasshouse were observed to grow in a synanthous manner. Shaded glasshouse plants demonstrated a much lower rate of foliage turnover than non-shaded plants. Whether the hysteranthous habit is beneficial in terms of flowering is impossible to state at this time. However, the ability of manipulating foliage turnover may have benefits in terms of optimising periodicity of bulbs in terms of the inflorescence position in the bulb relative to leaf bases. It may be possible that a high or variable rate of leaf turnover could adversely effect the inflorescence by effectively moving the bud further to the outside of the bulb. This may not be optimal with regards to the transfer of assimilate. Although purely speculative, would the ability of a bud to attract assimilate be hampered by its position away from other sinks such as new leaf primordia, leaf bases of aerial foliage and the meristem? Aside from such mechanisms controlling assimilate flow, regulated patterns of foliage initiation/emergence would presumably be beneficial to the long-term flowering success of the bulb, providing a pattern could be determined that favoured the flowering response. Such a regime appears to be the basis of controlled environment forcing in the Netherlands (Groen, 1997).

Scape elongation was significantly increased by the covering of plants with shadecloth under glasshouse conditions (Figure VII. 2). This also occurred under field conditions, where shaded plants produced stem heights greater than those from mulched and non-shaded/non-mulched treatments (Figure VII.1). Increased scape height under shade in the glasshouse was shown to be primarily related to increased cell sizes and secondarily to increases in cell division. Cell division in scapes appears to occur at a plate-type meristem located at the base of the scape. The issue of increased and/or uniform scape heights is an important one from the growers perspective in order to maximise the returns from the crop. Currently minimum scape heights are imposed on *Nerine* stems to be exported to the United States (R. Crowden, pers. comm.). Shading, even the application of total dark periods, has been recognised as a method for enhancing stem elongation in other geophyte species (Okubo & Uemoto, 1984a; 1984b). No adverse effects of shading were found with regard to flowering percentage, when compared to non-shaded bulbs in the field or in the glasshouse. However, flowering rates were low in all experiments using transplanted bulbs. Such a phenomenon has been reported previously (Shillo *et al.*, 1997), with flowering percentages gradually increasing over time until overcrowding occurred. A purely conjectural reason for this observed response may be a sensitivity of roots to disturbance during the "rest" period, when bulbs are lifted. This root disturbance may then have an adverse effect on the roots either directly or indirectly.

Therefore, a number of conclusions have been made. They are:

Bulbs of *N. bowdenii* use carbohydrate reserves from leaf bases in a centripetal fashion.

Carbon is primarily transported within the plant as sucrose.

Fructan can constitute a major form of non-structural carbohydrate in *N. bowdenii* 'Line 63.'

The roots and basal plate of the bulbs are the primary carbon sinks prior to, and during, the early stages of rapid inflorescence elongation.

The relative sink hierarchy as determined by relative specific activity is as follows prior to rapid expansion of the scape:

bulblets > basal plate > leaf/floral primordia > roots > leaf bases > inflorescence N

This hierarchy remains unchanged in bulbs containing aborting inflorescences. Once rapid elongation of the scape begins to occur, the following sink hierarchy becomes established:

inflorescence N > basal plate > bulblets > roots > leaf/floral primordia > leaf bases

Scape elongation can be increased by the application of shading.

Scape elongation occurs as a result of cell division at the base of the scape and also through elongation of these cells. Cell elongation is increased as a result of shading.

The scape elongation processes and the mechanism of inflorescence abortion require further study. Inflorescence abortion appears to occur as a result of an inability of the organ to maintain or to accelerate the rate of assimilate import into the organ. A number of mechanisms have been proposed which could cause this to occur. There is a need to establish the enzyme activities in the inflorescence and monitor their levels in aborting inflorescences and following the application of temperature extremes to healthy organs. If low enzyme activities do appear to be the problem and temperatures do impact upon these activities, it may then be possible find methods of maintaining suitable growing condition for the bulbs. Continued study of the physiological basis of the *Nerine* flowering process will contribute to the development of improved management practises for the crop. These studies are also likely to elucidate processes occurring during important developmental stages such as inflorescence abortion and scape elongation which will contribute to our understanding of these key flowering events in other geophytic crop species.

IX

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X

Appendix

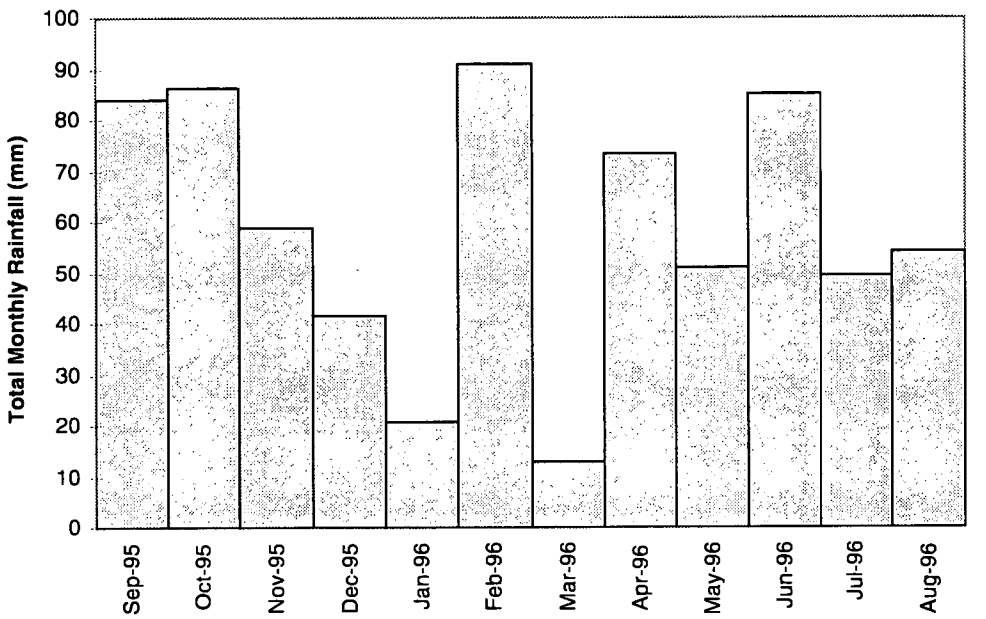

Figure A.1: Total monthly precipitation (mm) at Kettering, southern Tasmania, during 1995/1996.

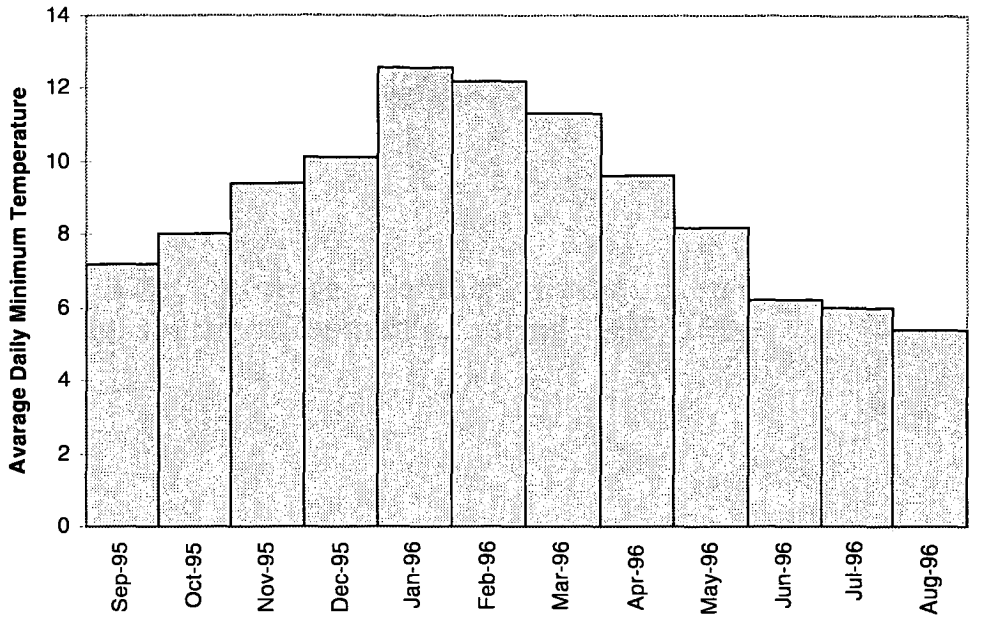

Figure A.2: Average monthly minimum temperatures (°C) at Kettering, southern Tasmania, during 1995/1996.

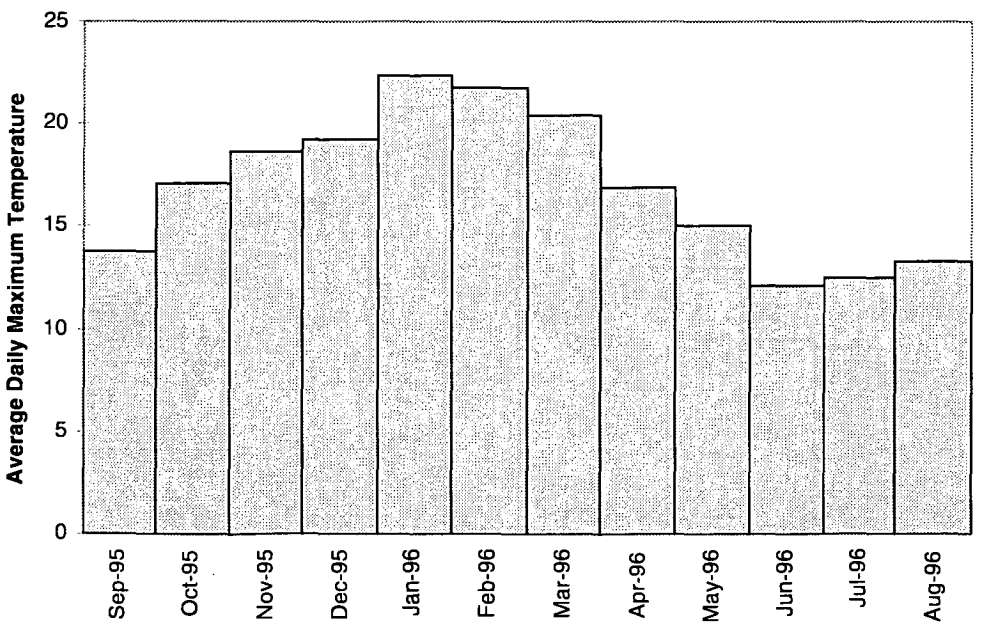

Figure A.3: Average monthly maximum temperature (°C) at Kettering, southern Tasmania, during 1995/1996.