ROLE OF LIGHT AND TEMPERATURE IN THE FLOWERING OF *WATSONIA* SPECIES

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by

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"Nothing in the world can take the place of persistence.
Talent will not: nothing is more common than unsuccessful men with talent.
Genius will not: un-rewarded genius is almost a proverb.
Education will not: The world is full of derelicts
Persistence and determination alone are omnipotent".

...borrowed from President Coolidge

DECLARATION

The experimental work described in this thesis was conducted in the Research Centre for Plant Growth and Development, School of Biological and Conservation Science, University of KwaZulu-Natal, Pietermaritzburg Campus, from February 2004 to April 2006, under the supervision of Professor J. van Staden and co-supervision of Dr D.I. Thompson and Dr J. Erwin.

These studies represent original work by the author and have not been submitted in any other form to another institution. Where use was made of the work of others, it has been duly acknowledge in the text.

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We declare the above statement to be true.

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ABSTRACT

The role of light and temperature on flowering of South African *Watsonia* species were evaluated to asses the potential for this genus as a commercial flower crop. Species were selected that represent different climatic regions of South Africa, with the aim of understanding how ecologically distinct species perform under cultivation. The four selected species were *W. borbonica* and *W. tabularis* (winter-rainfall area), *W. angusta* (shared rainfall) and *W. pillansii* (summer-rainfall area).

In order to establish the optimum temperature required for flowering, plants were exposed for 12 weeks to three temperature regimes (12/7 °C, 21/15 °C and 29/21 °C) after attaining their first and/or second leaves. A temperature shift of 12/7 °C was used to assess if the plants had a vernalisation requirement. Controls were maintained under 25 % shade under natural conditions, with an average temperature of 24/7 °C. An elevated temperature of 29/21 °C was detrimental to plant growth. Moderate temperatures of 21/15 °C significantly ($P<0.001$) increased the height and the number of leaves produced per plant relative to the 12/7 °C treatment. These temperatures significantly ($P<0.001$) increased the total number of flowers produced per plant compared to low temperatures. However, flowering percentage and quality of flowers were reduced.

A low temperature regime of 12/7 °C was efficient in satisfying vernalisation requirements and inducing flowering in four selected species. However, the total number of leaves produced per plant was signifcantly reduced. The summer-rainfall species, *W. pillansii*, displayed a qualitative response to vernalisation, as no flowering was observed in non-vernalised plants. Two winter-rainfall species, *W. borbonica* and *W. tabularis*, demonstrated a quantitative response to vernalisation. These species flowered at non-vernalising temperatures. *W. angusta* behaved like the winter-rainfall species in terms of flowering. Overall, a vernalisaton treatment marginally reduced days to flower while flowering percentage was increased compared to other temperature regimes. However, there was no increase in the total number of flowers

produced per plant. Low temperatures were not only effective for flower induction, but also for releasing corm dormancy, thus synchronising growth. Storing corms at either 4 or 10 °C resulted in 100 % sprouting within 4-6 weeks.

The role of daylength in flowering of *Watsonia* plants was established by subjecting plants to long days (LD) of 16 h light and 8 h dark and to short days (SD) of 8 h light and 16 h dark. The number of leaves and flowering were significantly (*P<0.01*) promoted under the LD regime. However, there was strong temperature and daylength interaction in terms of flowering potential, as at low temperatures flowering was induced irrespective of daylength. In *W. pillansii,* flowering was obtained under both regimes (LD and SD) applied at the second leaf stage. Flowering in *W. borbonica* and *W. tabularis* was only observed under the LD regime at the second leaf stage. In both species, flowering was also obtained in SD-treated plants, provided treatment occurred after the formation of the third leaf. However, the total number and quality of flowers were reduced.

To examine the effect of light intensity on flowering, plants at different developmental stages (first and/or second or beyond the third leaf stage) were exposed to photosynthetically active radiation (PAR) of 150 µmol $m^{-2}s^{-1}$ or 39.5 µmol $m^{-2}s^{-1}$ for 7 weeks. Exposure to low light intensity at either developmental stage compromised leaf quality. No flowering was observed following low light intensity treatment during the first to third leaf stages, even though plants were exposed to low temperature and LD regimes, both of which promoted flowering. Observation of the shoot apical meristem revealed that the second leaf stage was critical as the anatomical transition to flowering occurred at this level. When beyond the third leaf stage, low light intensity did not prevent flowering. However, the number of flowers produced per plant was reduced compared to plants maintained at 150 µmol $m^{-2}s^{-1}$. Thus, light intensity played a role in both plant morphogenesis and flowering. LDs were effective in promoting vegetative growth whereas high light intensity and low temperature regimes played pivotal roles in flower induction. This makes them useful horticulture tools to produce desirable *Watsonia* plants for commercialisation.

CONFERENCE CONTRIBUTIONS FROM THIS THESIS

I) ORAL PAPERS

MTSHALI N.P., THOMPSON D.I. and VAN STADEN J. 2004. The effect of light and temperature on the flowering of *Watsonia* species. 6th Annual Meeting of the Research Centre for Plant Growth and Development. School of Biological and Conservation Sciences, Piermaritzburg.

MTSHALI N.P., THOMPSON D.I. and VAN STADEN J. 2005. Vernalisation and the competence to flower: An overview of two Iridaceae genera. 7th Annual Meeting of the Research Centre for Plant Growth and Development. School of Biological and Conservation Sciences, Piermaritzburg.

II) POSTER

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LIST OF ABBREVIATIONS

ABA:	Abscisic Acid
°C:	Degrees Celcius
DIF:	Difference between day (DT) and night temperature (NT)-(DT-NT=DIF)
DNP:	Day Neutral Plant
FLDP:	Facultative Long Day Plant
FR:	Far-red
FSDP :	Facultative Short Day Plant
GA:	Gibberellic Acid
LDP:	Long Day Plant
OLDP:	Obligate Long Day Plant
PAR:	Photosynthetically Active Radiation
PPF:	Photosynthetic Photon Flux Densities
SAM:	Shoot Apical Meristem
SDP:	Short Day Plant

Chapter 1:

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION TO THE GENUS

South Africa is truly a land of uniqueness and diversity. Gold and diamonds are not the only endowments but the exceptional diversity of indigenous flora makes South Africa exceptionally verdant. The Cape Floral Kingdom alone has approximately 8550 species of flowering plants in 957 genera, with 73 % of species being endemic to the region (DOUTT, 1994). In this group, geophytes are in abundance. Geophytes encompass plants that produce underground storage organs, viz. corms, rhizomes, tubers and bulbs (DU PLESSIS and DUNCAN, 1989).

Many of South African geophytes are propagated all over the world. Some were collected to Europe by early explorers, but most by the wealthy and privileged who maintained them in large greenhouses (GOLDBLATT, 1996). Subsequent breeding of these plants resulted in the numerous cultivars that are in existence and commercially exploited today. African indigenous geophytes, such as *Gladiolus, Freesia* and *Sandersonia* have already made an important contribution to horticulture world wide (GOLDBLATT, 1996). However, relatively few species were used to produce the modern cultivars (DU PLESSIS and DUNCAN, 1989).

Traditionally materials such as roses and tulips have dominated the floriculture market. However, over the last few years, changes in consumer behaviour have stimulated an increasing interest in new floricultural subjects (KARAGUZEL *et al.*, 2005). South Africa has an abundance of geophytes many with unsurpassed beauty and unique horticultural characters. In order for these species to be fully domesticated, growing conditions that will optimise performance need to be thoroughly examined and understood. Knowledge of the flowering process is also a prerequisite.

The climate varies substantially in the different provinces of South Africa. In the coastlands of KwaZulu-Natal and the lowlands below the Great Escarpment up to the border with Mozambique, the climate becomes almost tropical. Winters are warm, with summer-rainfall dominating. In the interior of South Africa total rainfall is greatest in the east and gradually decreases westwards (TALJAARD, 1996).

The Western Cape Province has the distinction of housing the Cape Floristic Region. It harbours Mediterranean vegetation. However, in South Africa, only a very small region enjoys such a Mediterranean climate. This winter-rainfall area is in the south westernmost part of the country, extending from the Cape of Good Hope approximately 483 km northward along the Atlantic coast, and inland for 80 to 113 km (DOUTT, 1994) (Figure 1.1.1A-C). The Mediterranean climate produces vegetation adapted to survive a summer drought period and is genetically programmed for active growth during the winter when the rains occur (DOUTT, 1994). In South Africa, this kind of vegetation is called fynbos, a term derived from Dutch and Afrikaans words meaning "bush with fine leaves." Fynbos typically grows on fairly nutrient-depleted, mostly sandstone-derived acidic soils and is dominated by sclerophyllous, flowering shrubs, many of which are *Proteas, Ericas* and geophytes (GOLDBLATT, 1989).

It is not coincidental that such a drastic variation in topography and climatic conditions has resulted in the distinct exceptional floristic diversity and endemism that exists in South African flora today. The wide distribution of geophytes throughout southern Africa shows the ability of these plants to evolve with different and specific climates (Figures 1.1.1A-C and 1.1.2)

This study focuses on the geophytic genus *Watsonia,* which shows extensive diversity in terms of distribution. Distribution patterns indicate that *Watsonia* is predominantly a mountain genus (GOLDBLATT, 1989) (Figure 1.1.2). *Watsonia* species fall into two broad ecological groups: winter-rainfall and summer-rainfall species (Figure 1.1.2). Understanding the characteristics of their indigenous habitat is

the key to growing these plants successfully *ex situ* and characterising the physiological cues that induce flowering. In addition, in order for this genus to be successfully commercialised, ways to manipulate growth and flowering need to be investigated extensively. This includes understanding the environmental cues influencing flowering as well as maximizing yield potential.

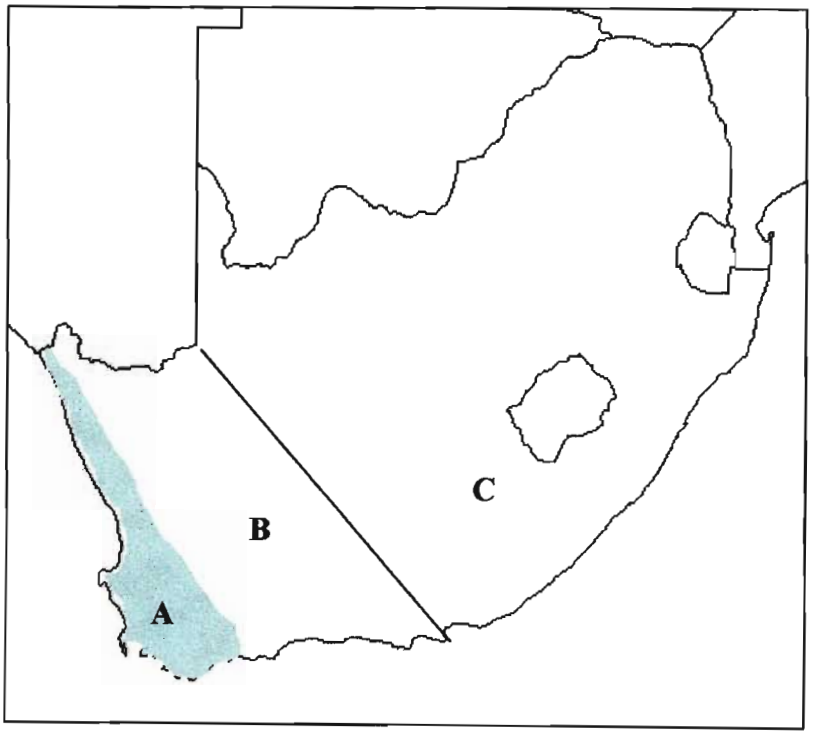

Figure 1.1.1 (A-C). Annual rainfall patterns in the Republic of South Africa (DOUTT, 1994).
A: winter-rainfall area with Mediterranean climate
B: rainfall throughout the year.
C: summer-rainfall area.

1.1.1 GENERAL MORPHOLOGY OF *WATSONIA* SPECIES

The petaloid monocotyledonous, Iridaceae has a worldwide distribution. However, the family is particularly diverse in Africa where there are some 1000 species, most of which are restricted to southern Africa (REEVES *et al.*, 2001). *Watsonia* Miller is one of the larger genera of African Iridaceae, comprising 52 species, restricted to southern Africa. In South Africa, *Watsonia* is distributed from the mountains and

coastal belt of the Western Cape Province to the Drakensberg escarpment of KwaZulu-Natal up to Mpumalanga Province and Swaziland (GOLDBLATT, 1989).

Winter-rainfall *Watsonia* species are concentrated in the Western Cape Province, where species grow under a winter-rainfall, Mediterranean regime on nutrient-poor sandstone soils (GOLDBLATT, 1989; DOUTT, 1994) (Figure 1.1.1A). Typically they are members of the fynbos community. Summer-rainfall species are found in the inland areas of South and tropical Africa (Figure 1.1.1 B and C). In eastern southern Africa rainfall peaks in the summer. Here, the species grow variably, but generally in richer soils than their winter-rainfall counterparts (GOLDBLATT, 1989).

Watsonia species are perennial, corm-bearing geophytes which can be robust, with some species reaching 2 m in height. The primary patterns of variation in *Watsonia* involve the structure of the perianth, symmetry of the androecium (correlated style orientation), degree of branching of the inflorescence and capsule and seed structure (GOLDBLATT, 1989). Variation in corm and leaf shape, number and degree of marginal thickenings and overall plant size provide useful characteristics for defining species or more exclusive taxa (GOLDBLATT, 1989). The number of flowers varies from 40–60 in some of the taller species, to only 3-5 in some dwarf species like *W. occulta* (L. Bolus). The *Watsonia* perianth consists of a well-developed dimorphic tube and large lanceolate to ovate tepals, either symmetrically disposed or held apart. The tube has a slender, erect more or less cylindrical lower part, which widens abruptly curving outwards into either a short or long cylindrical funnel (GOLDBLATT, 1989).

Watsonia has a cormous rootstock with several equitant, linear to lanceolate leaves and a spicate inflorescence (GOLDBLATT, 1989). Many species are evergreen, with persistent older leaves surviving for over a year and still present on the plant when the new season's leaves are produced. A significant proportion of the species are perennials. Leaf number ranges from several (usually 4-6), the presumed ancestral state, to few, sometimes consistently only two. The stems of most species are thick,

rigid and usually more or less erect. The *Watsonia* spike is distichious during and after flowering and is one of the features that distinguishes the genus (GOLDBLATT, 1989).

1.1.2 DISTRIBUTION AND DIAGNOSTIC FEATURES OF SELECTED *WATSONIA* SPECIES

1.1.2.1 Watsonia angusta

Watsonia angusta (Ker Gawl) occurs over almost the entire winter-rainfall area, extending from the Piketberg in the west to the Eastern Cape, an area that is part of southern African summer-rainfall region (Figure 1.1.2). It grows in more or less permanent wet places such as marshes and seeps, along stream banks and sometimes even in gently flowing streams. This species produces red to orange flowers (Figure 1.1.3 A).

1.1.2.2 Watsonia borbonica

Watsonia borbonica (Pourret) Goldblatt, is restricted to the extreme south-western Cape. It extends from the Du Toits Kloof mountain and Breede River valley south to the Cape Peninsula and east through the mountains of the Caledon district to Bredasdorp (Figure 1.1.2). Plants grow on rocky sandstone soils, usually on well-drained slopes. Occasionally they are also found in deep sand at the foot of mountains. The diagnostic feature of this species is that it is an exceptionally tall plant with a branched stem reaching 2 m in height. The flowers are pale to deep pink (rarely white) and relatively large (Figure 1.1.3 B). Tepals are 26-36 mm long and the upper perianth tube is flared and 8-20 mm long. The capsule is large (20-30 mm long) and ovoid to obvoid-truncate. The large seeds have two opposed wings. The leaves are broad (2-4 cm wide) and glossy and the margins are usually only lightly thickened.

1.1.2.3 Watsonia pillansii

Watsonia pillansii (L. Bolus) is widely distributed in the eastern half of southern Africa (Figure 1.1.2). It extends from the Langeberg above Swellendam and the Outeniqua mountains in the west through the southern Cape coastal ranges to the Eastern Cape and the KwaZulu-Natal Drakensberg. It occurs both along the coast and at lower and middle elevations, usually in rich mixed grassland but sometimes in more exposed sites among rocks. In this species, flowers are orange (Figure 1.1.3 C).

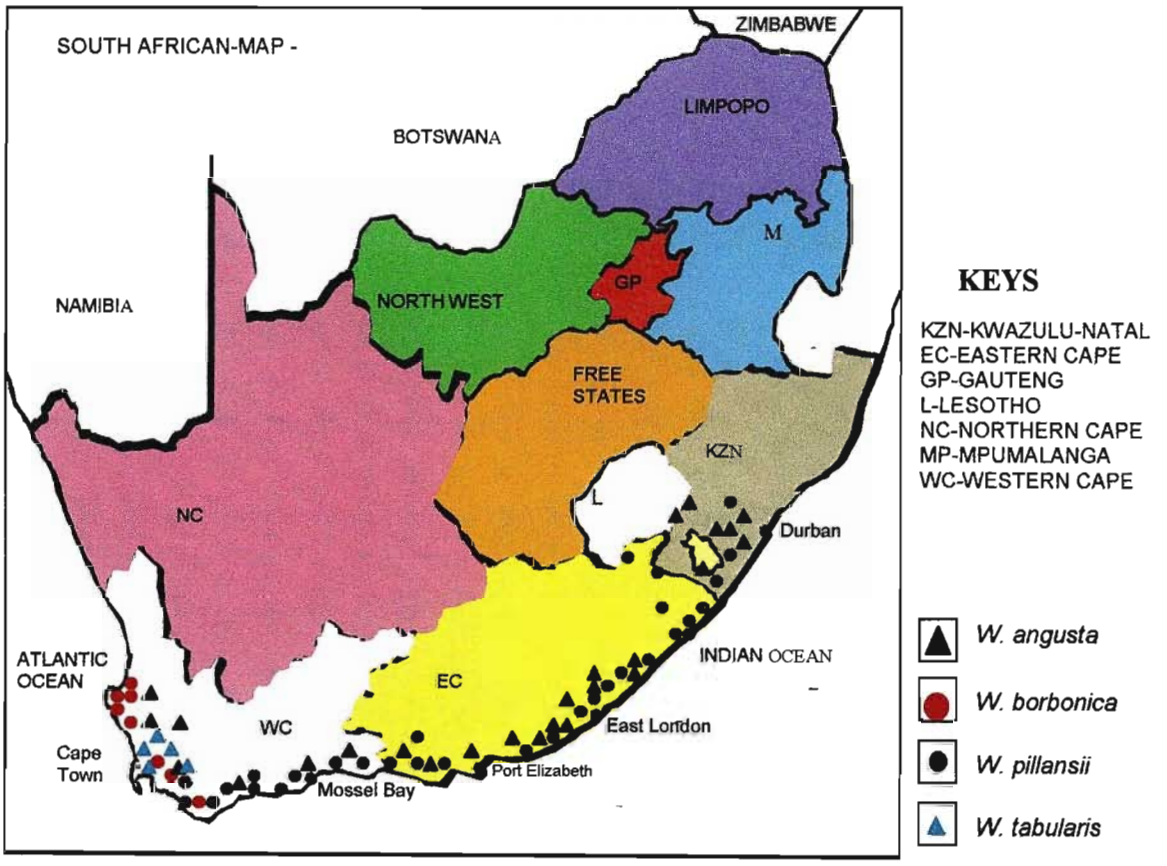

Figure 1.1.2. Schematic diagram depicting the distribution pattern of four *Watsonia* species. The genus is found in both summer and winter-rainfall areas (GOLDBLATT, 1989).

1.1.2.4 Watsonia tabularis

Watsonia tabularis (J.W. Mattews and L. Bolus) is endemic to a small area of the Cape Peninsula and adjacent Cape Flats (Figure 1.1.2). It occurs on rocky sandstone flats, slopes and plateaus. Flowering is strongly influenced by fire especially in the

southern Peninsula. However, in areas of higher rainfall, as on the upper plateau of Table Mountain, this species flowers even when there has been no burning for several years. Plants from the Table Mountain range have deep to pale pink flowers, in which the inner tepals are lighter in colour then the outer, and sometimes nearly white. To the south, flower colour is a uniform orange-red, with a slightly lighter shade in the inside of the tube (Figure 1.1.3 D).

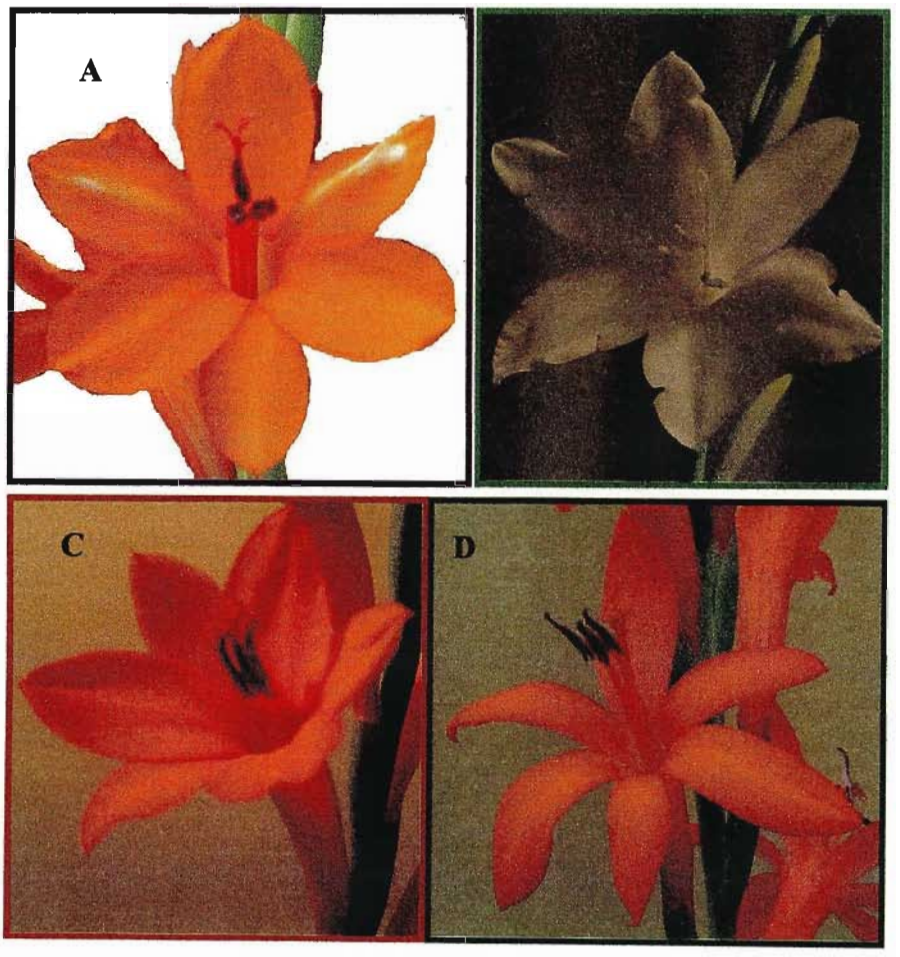

Figure 1.1.3 (A-D). Species of *Watsonia* from different climatic regions of South Africa selected for this study.
A: *W. angusta,* grows in both winter and the summer-rainfall areas, with orange-red flowers.
B: *W. borbonica,* which comes from winter-rainfall area, with white flowers.
C: *W. pillansii* with a distinctive orange flower, comes from the summer-rainfall area.
D: *W. tabularis* with an orange flower, comes from the winter-rainfall area.

1.2 LITERATURE REVIEW

1.2.1 CHARACTERISTICS OF GEOPHYTES

Geophytes can be divided in several ways as to their growth habit and flowering cycles: annual or perennial, deciduous or evergreen, synanthous or hysteranthous (HALEVY, 1990). In their native habitats, geophytes are subjected to a wide range of climatic conditions, characterized by a marked seasonal change in temperature, rainfall and photoperiod (REES, 1992; BOROCHOV *et al.*, 1997; DOLE and WILKINS, 1999). Low or high temperatures, a water deficiency or short days all result in plant stress, which initiates a cessation of growth or a temporary inactive state in most plants (LANG, 1987). To cope with such adverse conditions, many geophytes have developed survival mechanisms.

1.2.1.1 Geophyte dormancy

Dormancy is defined as 'the temporary suspension of visible growth of any plant structure containing a meristem' (LANG, 1987). Consequently plant dormancy is considered an aspect of growth cessation characterized by partial metabolic arrest. The induction and termination of dormancy is under hormonal and environmental control (RUDNICKI, 1974). In bulbs the onset of dormancy results in the suspension of growth and the inhibition of metabolic activities, whereas dormancy release is associated with a gradual mobilization of storage materials, an increase in activity of several enzymes and the initiation of growth processes (RUDNICKI, 1974).

Dormancy in bulbous plants can be likened to that of seeds. The food reserves in seeds as well as bulbous plants allow the plant to survive unfavourable periods when growth is not possible. It has been shown that dormancy in some bulbs and corms is comparable to true physiological dormancy, as found in seeds and buds, whereas in others it is less pronounced or it may be completely absent (RUDNICKI, 1974).

Despite the absence of visible organogenesis, the corms or bulbs are never physiologically or biochemically at complete rest and therefore, exhibit continuous

physiological activity (LANG, 1987; DE HERTOGH and LE NARD, 1993). The fact that most bulbs or corms contain fairly high moisture levels and have dry matter content of about 30 % probably constitutes one of the major factors, which prevent true physiological rest during storage (DE HERTOGH and LE NARD, 1993).

The differences in the degree of dormancy exhibited by different species seem to indicate an adaptive character arising from different necessities of plant adaptation to certain habitats (RUDNICKI, 1974). Geophytes that exhibit active growth and flowering in spring generally exhibit a dormancy period in summer when temperatures are high and the soil is dry (DOLE and WILKINS, 1999). Their growth resumes in autumn and this group of geophytes may require a warm-cold-warm sequence to express active growth and complete their growth cycle. Examples include *Tulipa*, *Freesia*, *Narcissus* and *Hyacinthus* spp. (HALEVY, 1990; DOLE and WILKINS, 1999). The summer-flowering bulbs have active growth during spring and summer. Their dormancy period generally occurs in winter, when the temperatures are low (REES, 1992; DOLE and WILKINS, 1999). They need a cold-warm-cold temperature sequence to express active growth and complete their growth cycle. Species from the genera *Allium*, *Gladiolus* and *Lilium* belong to this group (DOLE and WILKINS, 1999).

Dormancy results from (i) an environmental or endogenous signal perceived by the organ itself, (ii) a response to a biochemical signal from another organ, or (iii) is the result of one or more unsuitable environmental factors (REES, 1992; BOONEKAMP, 1997). There are three subdivisions of dormancy: (i) endodormancy, (ii) paradormancy and (iii) ecodormancy. Inputs such as water potential, nutrient status, oxygen levels, temperature, photoperiod and endogenous signals can induce one of these states of dormancy (*endo*, *eco* and *para*) (LANG, 1987). Collectively, geophytes exhibit all three types of dormancy (BOONEKAMP, 1997).

The prefix *endo* means 'within'. Endodormancy is used to describe dormancy where the initial reaction leading to growth control is the specific perception of an environmental or endogenous signal in the affected structure alone (LANG, 1987). Endodormancy is therefore, synonymous with 'winter dormancy', 'innate dormancy'

and 'deep dormancy'. Examples of this type of dormancy include the bulbs of lilies, onions, and *Gladiolus* corms, which exhibit a relatively long period during which the differentiation of new organs or their elongation is completely arrested (RUDNICKI, 1974). Dormancy is released slowly over several months, following a period of low temperatures (RUDNICKI, 1974). Endodormancy may be compared to the dormancy of many seeds of temperate-growing species (RUDNICKI, 1974).

Ecodormancy is synonymous with quiescence and 'imposed dormancy' and is regulated by environmental factors (FUCHIGAMI and NEE, 1987; LANG, 1987). The prefix *eco* (i.e. environment) is used to describe dormancy when one or more environmental factors in the basic growth environment are unsuitable for overall growth and metabolism (LANG, 1987). This type of dormancy is imposed directly by environmental factors, which include temperature, nutrient deficiencies and humidity (LANG, 1987). The growth of the plant is resumed as soon as the environment is once again favourable for plant development (RUDNICKI, 1974).

Paradormancy describes dormancy when the initial reaction leading to growth control involves a specific signal originating in, a different structure from the one in which dormancy is manifested (LANG, 1987). For example, the specific signal may be the perception of an environmental cue or the continuous production of inhibitory factors, as in apical dominance (LANG, 1987). Bulbs such as tulips, daffodils and hyacinths exhibit paradormancy, which is less pronounced. It starts after flower formation and prevents the growth of the stem (RUDNICKI, 1974; FUCHIGAMI and NEE, 1987). Usually these bulbs have a well-defined temperature requirement and need an exactly defined period of low temperature for normal development of floral stalks (RUDNICKI, 1974).

1.2.1.2 Breaking dormancy

For horticultural purposes, plants are required all year-round. Breaking dormancy using chemicals is therefore a common practise. Employing chemicals such as

cynamide, mineral oils and plant growth regulators have all been used to break dormancy.

As the dormancy process is regulated by plant hormones, it is not surprising that several plant hormones are used to break dormancy. Gibberellins (GA) have been reported to play a role in breaking dormancy and have a flower-inducing ability in some species (RUDNICKI, 1974). For example, GA is effective in breaking dormancy and inducing sprouting in *Laportea bulbifera*, *Elastostema involucratum* and *Sedum bulbiferum* (OKAGAMI, 1979).

A study by BOONEKAMP (1997) showed that the induction of dormancy and induction of new bulbs operate by the same mechanism, one that is controlled by abscisic acid (ABA). In lily the addition of ABA blocked leaf formation and was required for the induction of dormancy, whereas fluoridine (an inhibitor of ABA-synthesis) arrested bulb formation and prevented the development of dormancy (BOONEKAMP, 1997).

Ethylene and smoke are well documented as being involved in rest-breaking. Numerous studies conducted on plants such as *Freesia* (UYEMURA and IMANISHI, 1983; IMANISHI and BERGHOEF, 1986; BERGHOEF *et al.*, 1986a) and Dutch iris (*Iris hollandica*) (IMANISHI and FORTANIER, 1983) using smoke and ethylene, demonstrated that these agents are very effective in breaking dormancy. In addition to breaking dormancy, exposure to ethylene also promoted early flower initiation and induced flowering even in the smallest corms that generally do not initiate flowering. Furthermore, ethylene shortened the dormancy period in *Iris* bulbs (UHRING, 1973).

Even though ethylene has been reported as effective in shortening or terminating dormancy and in turn inducing flowering in several plants, this is not universal. For example, IMANISHI and FORTANIER (1983) reported that ethylene treatment had a marginal effect on flowering of Dutch iris and was ineffective in promoting flowering in *Crocus sativus* (MOLINA *et al.*, 2004). In *Freesia*, *Gladiolus* and *Liatris*, ethylene has been reported to break dormancy but not to promote flowering (IMANISHI and FORTANIER, 1983; MOE and BERLAND, 1986; HALEVY, 1990). Interestingly, the

results obtained by IMANISHI and FORTANIER (1983), showed that smoke was more effective in inducing flowering in three-leaved plants and in earlier sprouting and flowering.

In addition to chemicals being used to break dormancy, the manipulation of environmental factors in breaking dormancy cannot be neglected. Dormancy can be broken by modifying the growing conditions, especially temperature. The role of light in the dormancy process is unclear and appears to be minimal. In contrast, temperature appears to be the dominating environmental factor regulating dormancy. This is supported by numerous studies. In *Gladiolus*, freshly harvested corms and cormels planted under favourable growing conditions do not sprout immediately (DE HERTOGH and LE NARD, 1993). This dormancy, due to internal factors, is broken more rapidly at low (<10 °C) rather than high (> 20 °C) temperature treatments (DE HERTOGH and LE NARD, 1993). Dormancy in *Gladiolus* is overcome by 2-5 months of cold storage treatment at 2-10 °C (DE HERTOGH and LE NARD, 1993; DOLE and WILKINS, 1999). A high temperature treatment of 38 °C for a few days, followed by the prescribed cold treatment is more effective than cold alone in breaking dormancy (DOLE and WILKINS, 1999). In geophytes like *Sandersonia aurantiaca* it has become a general practise to chill the tubers at 4 °C for at least 12 weeks to overcome dormancy (CATLEY *et al.*, 2002b).

Warm temperatures are generally not as effective as low temperatures in overcoming dormancy. However, in some plants low temperatures induce dormancy whereas high temperatures break the dormancy period. For example, in the corms of *Freesia* and *Zephyra elegans*, 30 °C and 25 °C respectively were effective in breaking dormancy (BERGHOEF *et al.*, 1986a; YANEZ *et al.*, 2005). A temperature above 10 °C such as 13 °C was reported to induce pupation in some cultivars and species of *Freesia* (GILBERTSON-FERRISS *et al.*, 1981a).

Corms of *Crocus* are typically cured for 20-30 days at a temperature of 30 °C. This temperature effectively shortens bud dormancy and accelerates bud growth and

flower formation (MOLINA *et al.*, 2005). However, a longer exposure to 30 °C was deleterious for bud growth and flower formation (MOLINA *et al.*, 2005). Other high temperature treatments effective in breaking dormancy include steam or hot water (RUDNICKI, 1974; FUCHIGAMI and NEE, 1987). In *Gladiolus* hot water (used for eliminating diseases) was effective in breaking dormancy (SIMCHON *et al.*, 1972).

1.2.2 THE ROLE LIGHT AND TEMPERATURE ON PLANT MORPHOGENESIS

Plant morphogenesis is influenced by several environmental factors such as temperature, light (both intensity and quality), carbon dioxide, nutrients and air humidity (MOE and HEINS, 1990; CARVALHO *et al.*, 2002). Typically these environmental factors do not operate alone but interact with one or several other factors to produce the overall effect. Temperature and light quality influence important morphogenic processes such as the elongation of stems, flower stalks and leaf petioles, lateral branching, shoot and leaf orientation and leaf and flower pigmentation (MOE and HEINS, 1990). Morphological responses to light and temperature are referred to as photomorphogenesis and thermomorphogenesis, respectively (MOE and HEINS, 1990).

In general, cut flowers with long stems are desirable. Plant regulators are used to promote stem elongation. However, pot flowers need to be short and compact, and growth retardants are used to dwarf them. In recent years, there has been a growing trend to opt for natural methods of controlling plant height, which include the additional benefits of promoting healthy soil and reduced pollution.

One of the most researched thermomorphogenic processes is the difference between night and day temperatures. The difference between day temperature (DT) and night temperature (NT) is defined as DT-NT=DIF. This is based on the use of temperature to regulate stem length. Furthermore, DIF is used as an alternative to chemical growth regulators to control stem growth in greenhouse crops (NEILY *et al.*, 2000) The observation that stem elongation responds to differences in day and night temperatures was first noted in tomato plants by WENT (1944). Since then it has

been reported for a wide range of plant species, especially ornamental flowers. ERWIN *et al.* (1989) introduced the DIF concept when they found that *Lilium longiflorum* had the same final height when grown at the same DIF (using 25 combinations of DT and NT ranging from 14 to 30 °C), regardless of the mean temperature. According to ERWIN *et al.* (1989), DT and NT influenced plant height in opposite ways. For example, increasing DT increased plant height, whereas increasing NT decreased plant height. Therefore, temperature combinations resulting in a negative DIF produced plants that were shorter than those grown under positive DIF (ERWIN *et al.*, 1989). Further discussion on the DIF are beyond the scope of this study.

Studies by MOE and HEINS (1990) showed that in many instances thermomorphogenesis is similar to photomorphogenesis in the following manner: (i) red (R) light (high R/FR ratio) is equivalent to a negative DIF, (ii) far-red light (FR) (low R/FR ratio) is equivalent to a positive DIF. In *Campanula isophylla*, red light or light with a high R/FR ratio (e.g. fluorescent lamps) applied as day-extension or night–interruption (NI) suppressed stem elongation and promoted lateral branching. Far-red light or light with a low R/FR ratio (e.g. incandescent lamps) strongly enhanced stem elongation and inhibited lateral branching (MOE and HEINS, 1990).

It is well established that temperature influences growth, tuber development, responses to photoperiod and flowering of many species (SCHIAVINATO and VALIO, 1996). Many ornamental flowers are geophytes, meaning that they have a storage organ. Usually these storage organs are important source of reserves. For example, developing storage organs (lily and tulip bulbs, and *Gladiolus* corms) are capable of moving photosynthates and storing them as reserves (WANG and ROBERTS, 1983). Subsequently, the sink organs of the previous growing season become the source for meeting the carbohydrate demands of the new expanding shoots the following season (WANG and ROBERTS, 1983). These activities are affected by light, especially daylength. In several species the formation of these storage organs depends on, or is accelerated by exposure of leaves to specific photoperiods.

Frequently, tuber and tuberous root formation is promoted by short days (SCHIAVINATO and VALIO, 1996). Warm temperatures increase the growth rate and thus, prompt an increase in number of storage bulbs. In Easter lily, warm temperatures in spring were conducive to early secondary bulb initiation whereas warm temperatures in winter favoured early flowering (ROBERTS and TOMASOVIC, 1975).

In summary, plant morphogenesis is influenced by different environmental factors, which may ultimately influence the reproductive stage. This stage entails the plant integrating external and internal signals to coordinate the remarkable transition to flowering.

1.2.3 OVERVIEW ON FLOWERING CONCEPTS

For a long time, researchers were in pursuit of a chemical substance that was responsible for integrating external and internal flowering signals in plants. Through the use of grafting experiments, CHAILAKHYAN (1936) proposed that flowering was induced by a substance produced in the leaves and which was transmissible from the donor plant to the grafted recipients. This remarkable flowering factor was referred to as florigen. According to the author 'florigen' regulates flowering and has the same nature in different plants.

The identification of a graft-transmissible inhibitor also led to the concept of a competing antiflorigen (LANG, 1965; EVANS, 1971). Many attempts have been made to extract florigen from the phloem sap, but its chemical nature has remained elusive. Failed attempts to isolate florigen led researchers to channel their focus elsewhere for a flowering hormone(s). SACHS and HACKETT (1983) postulated the nutrient diversion hypothesis, which proposed that inductive treatments result in an increased amount of assimilates moving to the apical meristem, which in turn, induces flowering. Later, additional studies led to new discoveries, which questioned the nature of florigen: is it a single substance or does it consist of multiple components?

The mounting evidence that the flowering stimulus is not a single component led to the multi-factorial control model, which was proposed by BERNIER (1988). It proposes that a number of promoters and inhibitors, including phytohormones and assimilates, are involved in controlling the developmental transition. According to this model, flowering can only occur when the repressive and inductive factors are present at the shoot apex in the suitable concentrations and at the appropriate time (BERNIER, 1988; BERNIER *et al.,* 1993; BERNIER *et al.*, 2002). This model attempted to account for the diversity of flowering responses by proposing that different factors could be limiting for flowering due to different genetic backgrounds and under particular environmental conditions (BERNIER *et al.*, 1993).

Plant hormones are capable of inducing flowering in some plants. GAs are capable of inducing flowering in most long day plants. For example, at the onset of long day-induced floral evocation of the grass *Lolium temulentum,* levels of GA_5 and GA_6 double at the shoot apex (EVANS, 1971; BERNIER, 1988; KING, 2003). However, later it was reported that applied GAs are rarely effective at inducing flowering in short day plants (EVANS, 1971; ZEEVAART, 1983). These findings diminished the hope of GAs being florigenic factors. At present, the role of GAs in the transition to flowering is difficult to assess.

Other hormones such as cytokinins have also been implicated as part of the flower induction stimulus. Studies with *Sinapis alba* showed that cytokinins may be involved in long distance signalling and may play a role in the transition to flowering in response to inductive photoperiods (BERNIER *et al.*, 1993). In addition, the role of sugars has been evaluated (BERNIER, 1988; BERNIER *et al.,* 1993; LEVY and DEAN, 1998). In the long-day plant *Fuchsia x hybrida* sucrose supply regulates flowering, as apex sucrose content and flowering increase in parallel with an increase in light intensity in non-inductive short days (KING, 2003). Studies conducted in *Sinapis alba* have also shown that sucrose may function in long distance signalling during floral induction (BERNIER *et al.,* 1993; LEVY and DEAN, 1998). This is due to the observation that after flower induction in *Sinapis alba* by either long day or

displaced short day treatment, the concentration of sucrose in the phloem reaching the apex increases rapidly and transiently (BERNIER *et al.,* 1993).

In summary, although controversy continues to surround the existence of florigen (s), the original idea of its existence cannot be dismissed because the perception of photosynthetic activities (photoperiod) occurs in the leaves and it therefore, makes sense that florigenic factors will be transported from the leaf to the shoot apex (EVANS, 1971; BERNIER *et al.*, 1993). Obviously, these results indicate that there are several groups of compounds whose concentration in leaves are influenced by daylength and which can intervene to tip the balance from vegetative growth to flowering under some conditions (EVANS, 1971; KING, 2003). The fact that different compounds are capable of inducing flowering in different plant species under various photoperiodic conditions suggests that florigen could consist of multiple components; one part of which may be either GAs, cytokinins and sugar. Genetic analysis of flowering time conducted in plants such as pea, cereals and *Arabidopsis* support the idea that the transition to flowering is under multi-factorial control (LEVY and DEAN, 1998; KING, 2003).

1.2.4 THE PROCESS OF FLOWER FORMATION

The shoot apical meristem (SAM) is the source of all the above ground parts in plants (YONG *et al.*, 2000). Therefore, in order for a plant to flower the SAM must be competent to respond to the flowering stimulus such as cold exposure or certain daylength periods. The vegetative meristem is thought to pass through a juvenile phase during which it is incompetent to respond to internal or external factors that would trigger flowering in an adult meristem (LEVY and DEAN, 1998). In general, the juvenile growth phase is characterized by the most rapid rate of growth of plants. The acquisition of reproductive competence is marked by changes in the morphology and/or physiology of vegetative structures. Leaf shape offers one example in a process known as vegetative phase change (LEVY and DEAN, 1998).

The flowering process involves the transition of the different developmental patterns, the development of the flower organs and the communication and interaction of signals under the control of environmental and endogenous factors (YONG *et al.*, 2000). Plant morphology plays a part in determining floral initiation, in the sense that the apex must produce a fixed or minimum number of leaves before a flower is initiated (REES, 1992). Flowering in geophytes and other plants involves five successive stages: (i) flowering initiation or induction (ii) evocation (iii) organogenesis (differentiation of floral parts), (iv) maturation and growth of floral parts), (v) anthesis (REES, 1992; DE HERTOGH and LE NARD, 1993; YONG *et al.*, 2000). Induction is fundamental and provides the foundation for others stages, ultimately determining flowering time (YONG *et al.*, 2000). During this stage there are no morphological changes at the shoot apex meristem (SAM), but changes in physiology, biochemistry and gene expression occur (YONG *et al.*, 2000).

According to EVANS (1971), the term induction should be reserved for the arrival of the floral stimulus from the leaves. Prior to induction the shoot apical meristem has been initiating leaves, but at this stage it undergoes the transition to reproductive development (SCHIAVINATO and VALIO, 1996; LEVY and DEAN, 1998). This change in the developmental fate of the SAM primordia is controlled by environmental and endogenous signals (BERNIER, 1998). Following the arrival of the inductive stimulus at the shoot apex many genes must be called into play to determine floral characteristics (EVANS, 1971). The processes leading to the initiation of flower primordia can be referred to as evocation. Evocation can be viewed as resulting from a general activation of the shoot apex, leading to an increase in size and the elimination of vegetative activity, thereby allowing a new floral geometry to be established (EVANS, 1971).

One of the most striking early events of floral evocation in many plants is that the shoot apex swells and becomes turgid, assuming a dome-like shape (EVANS, 1971). However, the SAM of the plant is not irreversibly committed to reproductive development once flower initiation commences (LEVY and DEAN, 1998). Exposure to

additional inductive cycles such as photoperiod (refer to Section 1.2.5.1) often cause development to proceed more rapidly. Lack of sufficient inductive cycles for full flower development may cause floral morphogenesis to simply stop at some point. In some cases there may be actual reversion to a vegetative pattern of growth at the shoot apex (EVANS, 1971).

In geophytes the timing of flower initiation seems to be related to whether floral differentiation is supported by (i) continuous leaf activity, (ii) by the previous year's photosynthate or (iii) is delayed until the start of the next season's leaves (REES, 1992).

The control of flowering in geophytes in horticulture can have up to four major objectives: (i) promotion of flowering (ii) retardation of flowering (iii) prevention of flowering and (iv) induction of flower abortion (DE HERTOGH and LE NARD, 1993). The first two objectives allow out-of-season or year-round flower production. Hastening or retardation of flowering is generally obtained by applying specific treatments (temperature, moisture, light or hormone) to the propagules (REES, 1992). Prevention of flowering in bulbs such Dutch iris can be obtained by proper control of the physiological processes and this can be achieved through the manipulation of temperature (REES, 1992).

In most geophytes, there are two main times of flower initiation relative to development. Firstly, initiation occurs a short time after flowering, whilst the storage organ is not growing actively. In plants like *Narcissus*, initiation occurs before the leaves have wilted whereas in *Tulipa* initiation occurs when there are no above-ground organs. Secondly, in commercial practices, flower initiation occurs during storage or is delayed until the organ has been replanted and a shoot has emerged (as in *Freesia, Iris* and, *Gladiolus*). Flowers here are initiated after the start of shoot elongation and after the initiation of a fixed number of leaves (REES, 1992).

Not all plant species flower in response to environmental cues (such as temperature, light quality, nutrients and water availability). Some plants appear to flower in response to internal cues such as plant size or the number of vegetative nodes or leaves. Flowering occurs via an autonomous promotion pathway and is monitored by the endogenous developmental state of the plant (LEVY and DEAN, 1998; DIELEN *et al.,* 2001). This floral repression (pathway) may be a built-in mechanism that prevents flowering until the plant has reached a certain age or size (LEVY and DEAN, 1998). Furthermore, flowering can also be induced by stresses such as nutrient deficiency, drought and overcrowding. This stress response enables the plant to produce seeds, which are much more likely to survive the stress than the plant itself (LEVY and DEAN, 1998). In most plant species, floral transition is promoted by light (photoperiod) and temperature.

1.2.5 FACTORS THAT EFFECT FLOWERING IN GEOPHYTES

Seasonal flowering of plants involves responses to many environmental signals including changes in irradiance, temperature and daylength. Genetic studies of flowering in *Arabidopsis* highlight a network of interacting pathways involving plant responses to daylength, vernalisation, photosynthetic input, GA and a fifth autonomous pathway, whereby the plant progresses to flowering despite a lack of external signals (LEVY and DEAN, 1998; KING, 2003). Whether this general phenomenon is applicable to all plants is still not clear.

1.2.5.1 Light

When defining light, it is difficult to separate daylength and light intensity effects because the overall light effect may be due to the intergration of both factors and is therefore the result of the sum of solar irradiance intercepted (SHILLO and HALEVY, 1976b; OYAERT *et al.*, 2003). The common term for day and night length control is photoperiodism, a term defined by JULIEN TORNOIS (1914) from hops. Later, it was explained in greater detail by GARNER and ALLARD (1920) (reviewed by EVANS;

1971; THOMAS and VINCE-PRUE, 1997). Photoperiod is a reliable environmental signal for flower induction with respect to calendar date at a given latitude, where light intensity flunctuates both daily and seasonally (RUNKLE *et al.*, 1998).

In plants there are two main functions of light: The use of light through chemical reducing or oxidising power, photosynthesis, and the release of stored energy (photo-light perception) (ERWIN, 1993). There are four main light receptors: chlorophyll (a/b), carotenoids, phytochrome and cryptochrome (ERWIN, 1993; THOMAS, 1993). Plants use phytochrome pigments to perceive information about their surroundings and it exists in two forms, namely P_r and P_{fr}. It is obvious that phytochromes are involved in both daylength perception and promotion of flowering by inductive photoperiods (ERWIN, 1993; THOMAS, 1993).

Physiological experiments suggest that an endogenous circadian rhythm provide the timer (COUPLAND, 1997), thus a plant can distinguish between long days (LDs) and short days (SDs). The transition from light to darkness sets the phase of the rhythm, and detection of the point during the circadian rhythm that plants are again exposed to darkness enables them to measure the duration of the photoperiod (COUPLAND, 1997). Three parameters determine daylength: (i) length of light, (ii) length of dark and (iii) the relative length of light and dark.

Photoperiodic responses of plants can be divided into three distinct groups: short day (SD), long day (LD) and day neutral (DN) (ERWIN and WARNER, 2002). Short day plants require a night length longer than a specific number of hours for flower induction to occur. Alternatively, the critical daylength to induce flowering must be less than some maximum number of hours. Critical photoperiod was defined by THOMAS and VINCE-PRUE (1997) as the daylength that marks the transition between vegetative growth and flowering during an experiment. In contrast, long day plants require a night length shorter than a specific number of hours for flower induction to occur. The critical daylength must be longer than a minimum number of hours (ERWIN and WARNER, 2002).

ROBERTS and SUMMERFIELD (1987) proposed several definitions for the critical photoperiod of a long day plant (LDP), including that photoperiod above which time to flower is minimal and not affected by further increases in photoperiod and below which flowering is delayed. From this definition, a LDP that flowers most rapidly under 24 h of continuous light would have a critical photoperiod of 24 h. RUNKLE *et al.* (1998) defined the critical photoperiod of LDP as the photoperiod if met, or exceeded, which induces a population of plants to flower completely, rapidly and uniformly. Daylengths shorter than the critical daylength may induce incomplete or delayed flowering. Transitional photoperiods, those that induce only part of a population to flower, are horticulturally least desirable (RUNKLE *et al.*, 1998). In day neutral plants (DNP) flower induction is unaffected by daylength but is often affected by the total irradiance a plant is exposed to during a 24 h period (BERNIER *et al.,* 1993; ERWIN and WARNER, 2002). SD plants perceive the dark period. They require long, uninterrupted dark periods during which P_{fr} decays. A night break from light resets the P_{fr}. Alternatively, long day plants perceive the length of light.

SD and LD plants can exhibit a facultative (quantitative) or an obligate (qualitative) response (ERWIN and WARNER, 2002). The flowering of plants with a facultative response is hastened by the required photoperiod. In contrast, plants with an obligate response must be exposed to the required photoperiod to flower (ERWIN and WARNER, 2002; SOLTANI *et al.*, 2004). Where species are photoperiod sensitive, increasing the daylength will delay flowering time in SDPs, but hasten flowering in LDPs as observed in *Osteospermum jacundum* (PEARSON *et al.,* 1995).

Research has shown that LDs increase flowering percentage (by reducing flower blasting), floret number per spike and spike length, but delays flower development and anthesis (REES, 1992). However, LDs reduce the number of flower initials (REES, 1992), which seems to be promoted by SDs (REES, 1992). In *Gladiolus*, SDs hasten anthesis but decrease the percentage of flowering plants, the number of florets per spike and the height of the plants (SHILLO and HALEVY, 1976b).

Under poor light intensity conditions, flower abortion can affect the entire inflorescence and even the youngest leaves (SHILLO and HALEVY, 1976a; DE HERTOGH and LE NARD, 1993). In *Gladiolus*, low light intensity experienced during winter was reported to be the major cause of blindness (failure to flower), and also for a reduction of plant quality (SHILLO and HALEVY 1976b; IMANISHI and IMAE, 1990). Furthermore , the authors observed that in summer, shortening the length of the daylight period (SD) caused a decrease in either the flowering percentage in *Gladiolus* or the number of florets produced per spike. Studies of light intensity in *Gladiolus* plants demonstrated that insufficient illumination especially at different stages of develoment decrease flowering percentage and number of florets per spike (SHILLO and HALEVY, 1976a).

To counteract the effect of poor lighting, photoperiodism can be regulated by light of very low intensity. Long photoperiods can be supplied during natural SDs by extending the daylength with an electric lamp or shortening the daily dark period by interruption with a period of low intensity light (LAURIE *et al.*, 1979; DE HERTOGH and LE NARD, 1993).

Added artificial LD lighting was reported as very effective in preventing the incidence of blasting and improving flower quality (IMANISHI and IMAE, 1990). Furthermore, supplementary light on *Chrysanthemum* significantly increased the number of flower buds compared to control plants under natural light (ANDERSSON, 1990). At the highest irradiance (60-50 µmol $m^{-2}s^{-1}$) and longest duration, the number of *Chrysanthemum* flower buds was approximately double compared to plants grown under natural light and lowest irradiance conditions (35 µmol $m^{-2}s^{-1}$) (ANDERSSON, 1990).

Studies by MATTSON and ERWIN (2005) demonstrated that photoperiod interacted with light intensity or irradiance to affect mean dry weight gain per day of 11 species tested. Irradiance can effect earliness of flowering. For example, ZHANG *et al.* (1996) demonstrated that flowering in *Achillea millefolium* was advanced to 57, 45 and 37

days under LDs (16 h) with an irradiance of 100, 200 and 300 µmol $m^{-2}s^{-1}$, respectively. ERWIN and WARNER (2002) showed that amongst 18 species tested, 11 ornamental species flowered earlier when plants were exposed to ambient daylength (10-14 h) photoperiod plus 25-50 µmol $m^{-2}s^{-1}$ high pressure sodium lighting for up to 18 h daily compared to ambient daylength alone.

Irradiance (light intensity plays a crucial role in advancing or retarding flowering processes. Due to this observation, ERWIN and WARNER (2002) classified the effect of irradiance into three groups: (i) facultative irradiance responses which reduce leaf number below the first flower as irradiance increases under inductive conditions for flowering, (ii) irradiance indifferent responses where increasing irradiance under inductive conditions for flowering does not reduce leaf number below the first flower, and (iii) detrimental irradiance responses, are plants that increase leaf number under inductive conditions as irradiance increases.

In conclusion, 'light' encompasses the effect of photoperiod and light intensity or irradiance resulting in overall light integral (photoperiod x light intensity) (SHILLO and HALEVY, 1976b; OYAERT *et al.*, 2003; MATTSON and ERWIN, 2005). Interestingly, the effect of a SD treatment in *Gladiolus* was similar to a reduction in light intensity and is apparently due to a reduction in total solar irradiance (SHILLO and HALEVY, 1976b and c). Furthermore, in *Spathiphyllum*, flower initiation was hastened by SDs but the effect of photoperiod was influenced by light intensity (OYAERT *et al.*, 2003). Photoperiodic induction of flowering should proceed most satisfactorily if all other environmental factors are suitable (LAURIE *et al.*, 1979). However, photoperiodism is not the only factor capable of inducing flowering. Another environmental factor influencing flowering is temperature.

1.2.5.2. Temperature: Vernalisation

Winter traits and biennial plants require a period of low temperature in order to flower. The promotion of flowering in response to prolonged exposure to cold temperatures (i.e. winter) is a useful adaptation for plant species that flower in spring (LEVY and

DEAN, 1998; MICHAELS and AMASINO, 2000). This process is known as vernalisation (CHOUARD, 1960). It was defined by CHOUARD (1960) as, "the acquisition or acceleration of the ability to flower by chilling treatment." Importantly, vernalisation does not refer to the breaking of dormancy by cold, such as the release of pre-formed floral buds after chilling or the promotion of seed germination by cold (stratification) (MICHAELS and AMASINO, 2000).

The vernalisation response is important in fitting the plant lifecycle to the environment in which it is grown, so it can make the best use of the seasonal opportunities for growth and avoid adverse climatic conditions (ROBERTSON *et al.,* 1996). The major effect of vernalisation is to shorten the duration of the phase of leaf primordia production. It does this by bringing forward the time of initiation of the collar primordium and as a consequence reduces the number of leaves initiated on the main shoot (ROBERTSON *et al.,* 1996). As a result final leaf number is reduced, given the assumption that the rate of leaf primordia production is dependent on temperature alone (ROBERTSON *et al.,* 1996). Vernalisation thus affects not only the timing of floral initiation but also the leaf number and the timing of other growth stages up until the emergence of flowering stage (ROBERTSON *et al.,* 1996).

It has been demonstrated that vernalisation given to an imbibed seed or young plant promotes flowering at subsequent higher temperature (THOMAS and VINCE-PRUE, 1997). Unlike photoperiodic induction, vernalisation differs remarkably and is characterised by the following: (i) the site of perception of vernalisation is the shoot apex, but all actively dividing cells, not only those at the shoot apex may be capable of responding to vernalisation (LEVY and DEAN, 1998), (ii) unlike photoperiodic induction, vernalisation prepares the plant to flower but does not itself evoke flowering (LEVY and DEAN, 1998), and (iii) vernalisation does not initiate flower primordia, but creates the capacity for subsequent flowering. For example, there is a clear temporal separation between cold treatment and flowering, which commonly occurs after a period of growth at warmer temperatures or photoperiod (BERNIER *et al.,* 1998; MICHAELS and AMASINO, 2000; HENDERSON and DEAN, 2004).

Vernalisation is slow and quantitative and requires active metabolism (LEVY and DEAN, 1998; YONG *et al.,* 2000). Dry seeds therefore, cannot be vernalised, although imbibed seeds of many species are responsive (MICHAELS and AMASINO, 2000). Other plants, however, cannot be vernalised as seeds or seedlings and must reach a critical age or developmental stage before vernalisation can occur (MICHAELS and AMASINO, 2000).

The duration of cold treatment required to elicit vernalisation varies depending on the plant species. Typically 2-12 weeks at 1-7 °C is used to induce flowering (LEVY and DEAN, 1998; RUNKLE *et al.,* 1998; MICHAELS and AMASINO, 2000). Lower temperatures (viz. 0 and - 6 °C) have been reported to be effective for cereals (BERRY *et al.*, 1980; BERNIER *et al.,* 1998). However, it must be stated that a cold-temperature treatment, not necessarily the one that vernalises the plant, could enhance flowering in any of the following ways: (i) higher flowering percentage, (ii) faster flowering, (iii) increased flower production, (iv) improved flowering uniformity, and (v) increased vigour (RUNKLE *et al.,* 1998). Thus a clear distinction must be made between flower induction temperature and vernalisation temperature.

Vernalisation requires gene expression. Vernalisation is required in each generation for winter annuals and biennials and each growth year for perennials, which suggest that meiosis or some other aspect of reproductive growth resets the requirement for vernalisation (LEVY and DEAN, 1998). Once the "vernalised state" has been achieved, it is stable throughout subsequent mitotic division, thus, is not passed to the next generation, which suggests an epigenetic basis (LEVY and DEAN, 1998; MICHAELS and AMASINO, 2000; BOSS *et al.,* 2004; HENDERSON and DEAN, 2004).

Vernalisation can be facultative or obligate. The time of flowering can be postponed up to several weeks, even to several months, if the plants are not treated with a low temperature (ROBERTSON *et al.,* 1996). For example, winter annuals have a facultative vernalisation response, i.e. cold exposure is not required for flowering, but

flowering will occur more rapidly after cold exposure (MICHAELS and AMASINO, 2000). Biennials in contrast have an obligate requirement for cold treatment and thus cannot flower without prior cold exposure (MICHAELS and AMASINO, 2000).

In most species the effect of a vernalising cold treatment can be partially or totally eliminated by several days of heat treatment, typically 30-40 °C. The process is referred to as de-vernalisation (MICHAELS and AMASINO, 2000). It means that the effect of vernalisation will disappear after incubating at approximately 33 ±2 °C for several days (YONG *et al.,* 2000). The consistency of the cold treatment can also impact the effectiveness of vernalisation and as with temperature and duration of cold treatment, the optimum is species-dependent (MICHAELS and AMASINO, 2000). However, the longer the duration of the low temperatures (e.g. 2 °C), the more insensitive the plant becomes to subsequent de-vernalisation temperatures, until vernalisation is irreversible (ROBERTSON *et al.,* 1996).

In summary, vernalisation works in concert with photoperiod. Vernalisation is required to make plants sensitive to photoperiod. This acts as a 'fail-safe' system to ensure flowering at the appropriate time of year.

1.2.5.3 Propagule size and age

The ability to flower in many geophyte species is related to propagule size. Even under inductive conditions, propagules below a critical size normally fail to flower (HALEVY, 1990; REES, 1992; DE HERTOGH and LE NARD, 1993). However, they will eventually reach a critical size and flowering is then initiated. The critical size is genus or species dependent (DE HERTOGH and LE NARD, 1993). For example, *Liatris* may produce high quality flowers from very small corms and above the critical size of 0.6 g (HALEVY, 1990).

The inability of small propagules to flower has been suggested to be caused by the internal source–sink relationship between the inflorescence and other organs (ROH,

2005). For example, in tulips, the sink strength of the flower is superior to that of the daughter bulb (BOONEKAMP, 1997). In smaller sized bulbs almost all reserve material from the scales is transported to the flower, whereas in larger bulbs, excess carbohydrates were also transported to the daughter bulbs. In general, the sink strength of flowers is strongly dominant over that of daughter bulbs (BOONEKAMP, 1997).

There are varying views on whether the ability to flower also depends on the size of the shoot apical meristem or the storage organ size. HALEVY (1990) suggested that in some geophytes, flower competence may be controlled, not just by storage organ size, but also by the size of the apical meristem. In Dutch iris and lily, bulb size was positively correlated with the apex diameter (KOHL, 1967; DOSS and CHRISTIAN, 1979). These results suggest that there is a critical meristem size below which flower induction will not take place in these species.

In other plants, apex dimensions increase with plant age or maturity. Thus, apex size alone cannot be used as an indicator of potential floral behaviour in bulbs. Studies conducted by CLARK and BURGE (2002) showed that the performance of tubers was influenced by tuber weight rather than the size of the growing point. Results from three species of *Brodiaea* indicated that the corm size for 100 % flowering varies within species and the source of the corms (HAN, 2001). The number of flowers per inflorescence was correlated with the size of the mother corm, where the larger the corm, the more flowers per inflorescence were produced (HAN, 2001). Propagule age is directly related to maturity but not with propagule size (WAITHAKA, 1986). Therefore, small propagules are capable of flowering, if they are mature and they are above a certain critical size. Environmental conditions during which propagules are produced may play a significant role in the flowering potential of species (HAN, 2001).

1.3 OBJECTIVES OF THIS STUDY

As indicated earlier the flowering requirements of Watsonia are not known yet these plants can make effective horticulture subjects. Investigation of the cues that induce flowering at the physiological level in Watsonia species is therefore a valuable exercise: attempts were made to:

(i) establish the critical corm size capable of flowering;

(ii) to clarify the influence of cold treatments on subsequent flowering and to determine the relationship between temperature and time to flowering; and

(iii) to investigate the influence of light on flowering by (a) establishing the critical photoperiod necessary to induce flowering and (b) the role of light intensity on flowering.

Chapter 2:

EFFECTS OF TEMPERATURE ON FLOWERING OF *WATSONIA* SPECIES

2.1 INTRODUCTION

The growth and development of plants is dependent on temperature. In most geophytes the major factor controlling flowering and dormancy is seasonal thermoroperiodicity, i.e. the change in mean temperature over various seasons and plant growth stages (HARTSEMA, 1961; HALEVY, 1990; WHITMAN *et al.*, 1997; RUNKLE *et al.*, 1998). Temperature also plays an important role in the production of high quality plants, which are required for the successful international bulb/corm trade (DU TOIT *et al.*, 2002). In addition, temperature manipulation can be used to hasten or delay flowering in the floriculture industry (REES, 1992).

Physiological abnormalities such as inflorescence abortion or blindness and flower blasting are common phenomena in bulbs or corms that have been subjected to low or high temperature extremes (SHILLO and HALEVY, 1976d; DE HERTOGH and LE NARD, 1993; DU TOIT *et al.*, 2002; ROH, 2005). Understanding the influence of temperature on plant morphology and flowering would therefore be of benefit in the successful commercialisation of *Watsonia* species.

In geophytes there are two processes which are tightly regulated by temperature - dormancy and vernalisation. These two processes differ considerably, although in some cases, they have been used interchangeably (DENNIS, 1987). However, their similarities led to the suggestion that these two phenomena might be different manifestations of the same metabolic conditions (DENNIS, 1987).

Dormancy can occur intrinsically as part of the developmental process but can also be instigated by adverse environmental conditions. The dormancy process is a useful adaptation because it enables plants to survive adverse conditions such as hot and dry summers. In some geophytes such as *Gladiolus* and *Freesia,* dormancy usually

occurs after flowering and production of seed. In others such as *Brodiaea* and *Crocus*, it occurs after leaf senescence and flower bud initiation (HAN *et al.*, 1991; MOLINA *et al.*, 2005). In most instances, temperature regulates the initiation and cessation of the dormant period. Both high and low temperature can be used to break dormancy depending on the plant species (DENNIS, 1987).

Exposure to cool temperatures (vernalisation) has been reported to promote flowering in many cultivated ornamentals and commercially valuable monocotyledons such as wheat and rice (CHOUARD, 1960; WANG *et al.*, 1970; BERRY, 1980; CHANDLER and DEAN, 1994; ROBERTSON *et al.*, 1996). As originally described, vernalisation refers to a response in which treatment of plants with cool temperatures ultimately leads to flowering (CHOUARD, 1960; BERNIER *et al.,* 1981). The treatment either accelerates progress towards flowering (quantitative), or may be a pre-requisite (obligate or qualitative) (MICHAELS and AMASINO, 2000). In many cases, the response may be conditional upon cool temperatures being followed by long days or high temperatures (BERNIER *et al.,* 1981).

One of the most difficult issues to resolve in the literature is the characterisation of vernalisation across a range of low temperatures. It has been shown that the duration of cold treatment suitable for promoting flowering varies, with 1 to 3 months being common (BERNIER *et al.,* 1981; MICHAELS and AMASINO, 2000). Temperatures between 0-10 °C are most effective for flower induction but also lower leaf number (ROBERTSON *et al.*, 1996). However, precaution must be taken, as prolonged low temperature exposure may result in over-cooling and may alter plant morphology and architecture, as observed in Easter lily (DOLE and WILKINS, 1999).

Another issue surrounding vernalisation is accurately defining the response of leaf number to low temperature, i.e. the interaction of plant age with responsiveness. During the vegetative stage, many plants go through a juvenile phase in which the plant shoot apical meristem (SAM) is unresponsive to internal or external factors that would trigger flowering in an adult meristem (LEVY and DEAN, 1998). Some plants can only be vernalised at a certain developmental stage. For example, wheat is

capable of being vernalised up until the six leaf stage and the older the plant within this developmental window, the shorter the period of cold required for reducing final leaf number (ROBERTSON *et al.*, 1996). Thus, it is also important to identify when a plant becomes competent for vernalisation.

Furthermore, ROBERTSON *et al.* (1996) reported that if a plant receives periods of cold that are less than the requirement for saturation of vernalisation, then several further leaves must be initiated before the apex becomes floral (ROBERTSON *et al.*, 1996). Vernalisation is said to have been saturated in those treatments where the number of leaves initiated at the end of the treatment equals the final number of leaves produced, so transition of the apex to a reproductive state occurred immediately after the treatment ends (ROBERTSON *et al.*, 1996).

Vernalisation treatments does not only induce flowering but it also reduces the leaf number (ROBERTSON *et al.*, 1996). In nature, plants are exposed to a spread of temperatures outside the range commonly regarded as being vernalising (above 12-18 °C) but still capable of reducing leaf number thus forwarding flowering time. Such low temperatures have been shown to be favourable for flower initiation in a number of species such as onion (*Allium cepa*) and *Lachenalia* cultivars (HARTSEMA, 1961; DU TOIT *et al.*, 2002; ROH, 2005). In some plants such as *Freesia*, low temperature (12-15 °C) is usually applied after the formation of the seventh visible leaf for 6-9 weeks (BERGHOEF *et al.*, 1986b). This temperature stimulates floral induction and initiation (GILBERTSON-FERRIS and WILKINS, 1978). However, this direct effect of low temperature on flower initiation is strictly distinct from vernalisation. With a direct low temperature response, floral initials differentiate during the cold and so can be distinguished from vernalisation, which is an inductive phenomenon (ROBERTSON *et al.*, 1996). After vernalisation is complete, flower initials are not yet present and differentiate only later when the plant is returned to a higher temperature and in many cases also to a particular photoperiod regime (BERNIER, 1988; THOMAS and VINCE-PRUE, 1997; ADAMS *et al.*, 1998).

The concept can be extended to encompass flower commitment. After plants have been exposed to conditions inductive for flowering, even though no visible change may have occurred at the apex, the fate of the meristem becomes irreversible. Therefore, exposure to a non–inductive environment will not prevent the evocation process, instead the shoot apical meristem (SAM) will continue toward inflorescence initiation (BERNIER *et al.*, 1998; ADAMS *et al.*, 1998). Consequently, if commitment to flowering occurs as the direct result of a cold treatment, the response cannot be strictly classified as vernalisation (ADAMS *et al.*, 1998).

One commonly used technique in vernalisation studies is reciprocal transfer, whereby plants are transferred from inductive to non-inductive temperatures at regular intervals throughout growth. The advantage of this experimental procedure is that the onset of sensitivity to cold (end juvenile phase) and the duration of floral induction can be determined.

2.1.1 OBJECTIVES

- To determine at which temperature ranges dormancy of *Watsonia* plants can be broken; and
- To clarify the influence of temperature on plant morphogenesis and on subsequent flowering of selected *Watsonia* species.

2.2 MATERIALS AND METHODS

2.2.1 EXPERIMENTAL SITE

The experimented site was located in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg (altitude 762 m; 29° 37'S: 30° 24'E). The study was conducted in the Botanic garden, in the 25 % shade house and greenhouse.

2.2.2 PLANT MATERIAL

Corms of *Watsonia* species were sourced from commercial growers between March–April 2004 and January 2005. The four species were *Watsonia angusta, W. borbonica, W. pillansii* and *W. tabularis.*

2.2.3 ESTABLISHING TEMPERATURE TREATMENT SUITABLE TO BREAK DORMANCY IN *WATSONIA* CORMS

Corms of *W. borbonica* and *W. tabularis* were harvested at the end of 2004, following flowering and leaf senescence. The lifted corms were cleaned, dried and graded according to size. They were subsequently stored in brown paper bags at 4 °C, 10 °C and ambient temperature ±25 °C. Corms were observed weekly for morphological changes. Each treatment consisted of 20 replicates. The dormancy of a particular corm was deemed to have ended when contractile roots and the leaf sheath emerged.

2.2.4 EFFECT OF A RANGE OF TEMPERATURES ON GROWTH AND FLOWERING OF *WATSONIA* PLANTS

Corms of *W. borbonica, W. pillansii* and *W. tabularis* were planted in 12 cm pots, containing a sterile soil mixture of loamy soil, compost and sand (3:1:1, v/v/v). Plants

were watered twice weekly and fertilised with a hydroponics nutrient (Chemilcult) solution, fortnightly.

The plants were maintained in a 25 % shade-house until the emergence of the first leaf and/or second leaf. These plants were then transferred to temperature-controlled growth cabinets (Convirons), which were set to low (LTR) (12/7 °C), moderate (MTR) (21/15 °C) and high temperature (HTR) (29/21 °C) (day and night regimes) with 16 h illumination at ± 150 $\mu mol^{-2}ms^{-1}$. These temperature regimes were chosen to represent prevalent South African climates. In addition, low temperatures of 12/7 °C were used to assess if the plants had a vernalisation requirement. Untreated plants (control plants) were maintained in the 25 % shade-house and greenhouse throughout the study. At the end of the treatment (12-14 weeks), plants were returned to the 25 % shade-house. Temperature data were recorded using Hobo® data loggers, with measurements at 5h00, 8h00 and 12h30, as 'minimum' and 'maximum' temperatures, respectively.

In addition to a temperature regime of 12/7 °C being used to determine vernalisation requirements in 2004, this temperature regime was used in a reciprocal experiment conducted in 2005, with *W. borbonica*, *W. angusta* and *W. tabularis*. The aim was to quantify the nature of vernalisation: (I) when were plants sensitive to low temperatures? and (II) the time required for the vernalisation treatment to be reciprocated in plants.

The reciprocal experiment was conducted in two Conviron growth cabinets at constant day and night temperatures of either 12/7 °C or 21/18 °C for 12 weeks. Light intensity was maintained at 150 $\mu mol\ m^{-2}s^{-1}$, provided by white fluorescent tubes, with 16 h daylength. Plants were exposed to either growth cabinet after attaining two leaves. Each chamber contained 10 plants. Untreated plants (control) were maintained at either temperature regime throughout the study. After 6 weeks, plants were transferred to and from each cabinet. In total, the plants were maintained under both of these treatments for 12 weeks. After 12 weeks, plants were returned to the green house.

2.3 RESULTS AND DISCUSSION

2.3.1 TEMPERATURE IN RELEASING DORMANCY

In the initial study (2004) corms of *Watsonia: viz borbonica*, *tabularis* and *pillansii* displayed non-synchronised sprouting, varying by 4 to 6 weeks. As a result, it was hard to control the experiment. Such data suggested that at least some corms were dormant when planted. In an attempt to break corm dormancy in *Watsonia* species, the effectiveness of various temperatures (4 °C, 10 °C and 25 °C) were assessed. The results indicated that low temperatures of 4 °C and 10 °C were more effective than 25 °C in releasing corm dormancy; 4 °C was marginally more effective than 10 °C in this regard. The two temperatures resulted in 100 % sprouting, irrespective of the corm size in both species (Figure 2.3.1 A-B).

Dormancy was also broken under ambient temperatures with 60 and 40 % in *W. tabularis* and *W. borbonica* corms, respectively. However, the dormancy duration was prolonged (> 6 weeks). In addition to low temperature being effective for dormancy release, it also promoted secondary shoots, which resulted in a leaf number increase (Figure 2.3.1 B-D). The dormancy observed in selected *Watsonia* corms under high temperatures was consistent with the summer dormancy growth habitat observed in other geophytes such as *Nerine sarniensis, Gladiolus* corms and *Iris* bulbs, which do not grow when maintained above 30 °C (SIMCHON *et al.*, 1972; HALEVY, 1990).

The practise of using low temperatures to break corm dormancy has been well established in the horticultural industry. Commercial *Sandersonia aurantiaca* tubers are chilled to 4 °C for at least 12 weeks to overcome dormancy (CLARK, 1995; CATLEY *et al.*, 2002b). SIMCHON *et al.* (1972) found that the storage of *Gladiolus* corms at 10 °C was more effective than storage at 5 °C in breaking dormancy, this despite 5 °C being widely used to overcome dormancy in *Gladiolus* corms.

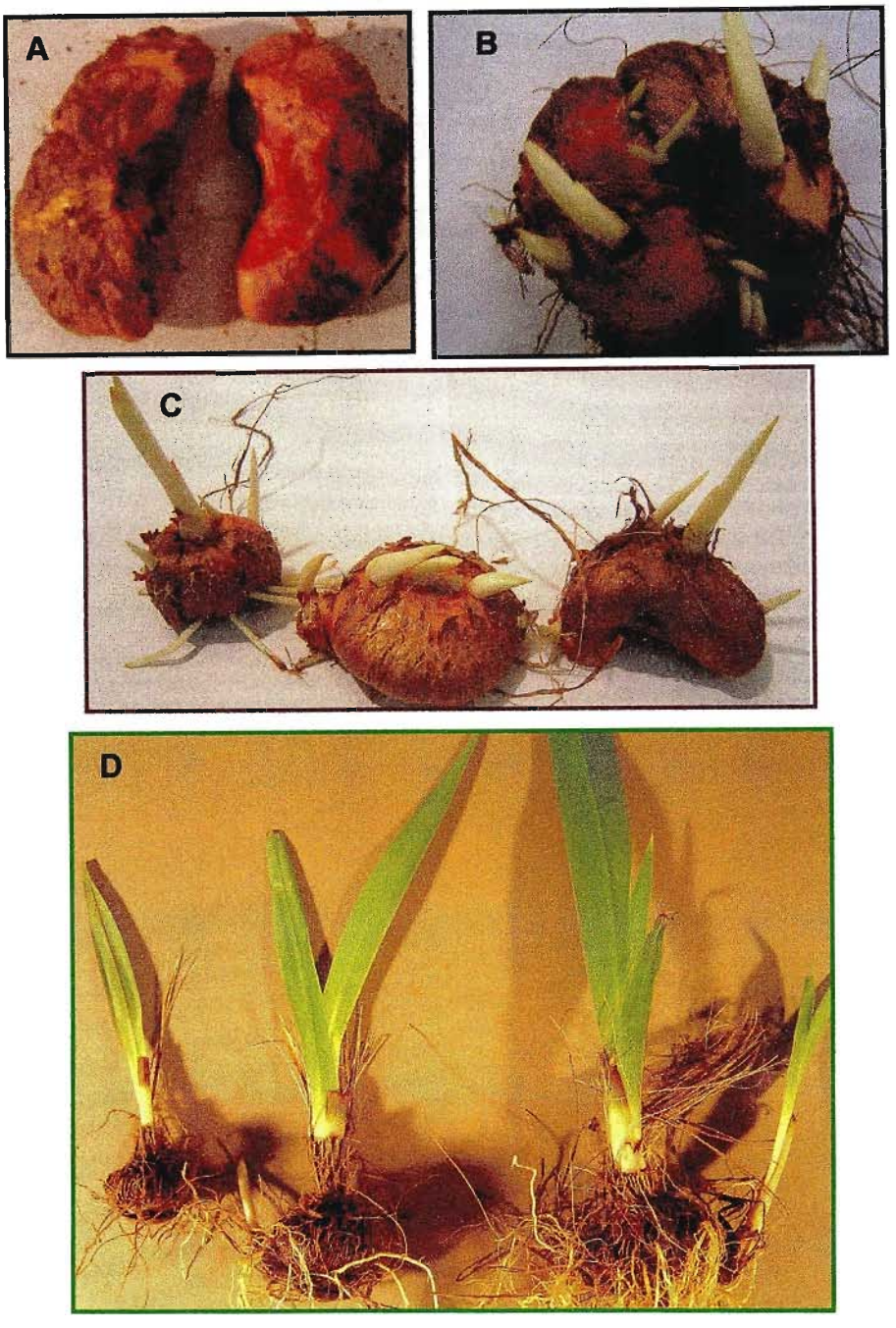

Figure 2.3.1 (A-D). The influence of temperature on dormancy in *Watsonia* corms.
A: Dormant corm of *W. tabularis* after harvesting. Note the absence of growth.
B: Cormel of *W. tabularis* after exposure to 10 °C. Note the emerged contractile roots and leaf sheath indicating release from dormancy.
C: Different sized-cormels of *W. tabularis* after exposure to 4 °C. Note synchronize sprouting irrespective of corm size.
D: Corms of *W. borbonica* after exposure to 4 °C. Even sprouting and growth was observed irrespective of corm size. Note lateral shoots emerging.

prior to dormancy initiation, have been reported to have a shorter subsequent dormancy period. Similar results were reported for *Freesia* corms. For example, 93 % of *Freesia* corms grown at 28/23 °C sprouted after 45 days, while the cooler-grown (18/13 °C) corms needed 75 days to reach the same level (LEE *et al.*, 2003). In this study, temperature in which corms were planted before dormancy initiation was not recorded because corms were commercially sourced immediately prior to experimentation, pre-dormancy temperature exposure was unknown and therefore, logical conclusions could not be drawn.

In summary, low temperatures are effective in releasing corm dormancy in *Watsonia* corms. In addition, results show promise that low temperatures can be used to synchronise the sprouting rate of corms to ensure even growth. Under favourable conditions this could lead to even flowering. Moreover, low temperatures ensured uniform sprouting in all corm sizes and also promoted side shoots, making such a treatment a useful horticultural tool. This could be used to increase the leaf number of a plant.

2.3.2 THE EFFECT OF TEMPERATURE ON GROWTH AND FLOWERING OF *WATSONIA* SPECIES

The results indicated that plant morphogenesis; flowering process and flowering time were highly influenced ($P<0.001$) by temperature (Table 2.3.1). However, higher temperature regimes (HTR) of 29/21°C resulted in a higher degree of corm rot and were detrimental to plants in general, resulting in tall, collapsed and deformed plants (Figure 2.3.2 A-C). Consequently, data from the 29/21°C treatment were not included in the analysis.

Table 2.3.1. Effect of different temperatures on three selected *Watsonia* species. In each species, there were 10-15 plants. Each species was analysed separately by ANOVA, MINITAB. Treatment means with letters in common are not significantly different at 5 % (*P<0.05*), separated by Fisher's test.

Species	Treatments	Leaf number	Leaf height (cm)	Days to flowering	Inflorescence length (cm)	Flower number per plant
W. borbonica	LTR (12/7 °C)	4.60c	58.00c	170b	98.30c	16.00b
	MTR (21/15 °C)	6.00a	70.00a	182b	115a	18.00a
	Control (23/14°C)	5.20b	67.90b	189b	110.8b	18.60a
	Control (24/7 °C)	6.00a	49.60d	185b	85.00d	15.40b
W. pillansii	LTR (12/7 °C)	4.50b	35.00c	285a	58.30a	12.00a
	MTR (21/15 °C)	5.40a	49.00a	0.00b	0.00b	0.00b
	Control (23/14°C)	5.70a	45.30b	0.00b	0.00b	0.00b
	Control (24/7 °C)	5.50a	29.30d	0.00b	0.00b	0.00b
W. tabularis	LTR (12/7 °C)	5.40c	45.20d	166b	82.00c	11.00b
	MTR (21/15 °C)	7.00b	73.20a	179a	120a	14.00a
	Control (23/14°C)	7.00b	69.00b	164b	98.30b	13.20a
	Control (24/7 °C)	8.00a	52.20c	176a	79.00c	11.30b

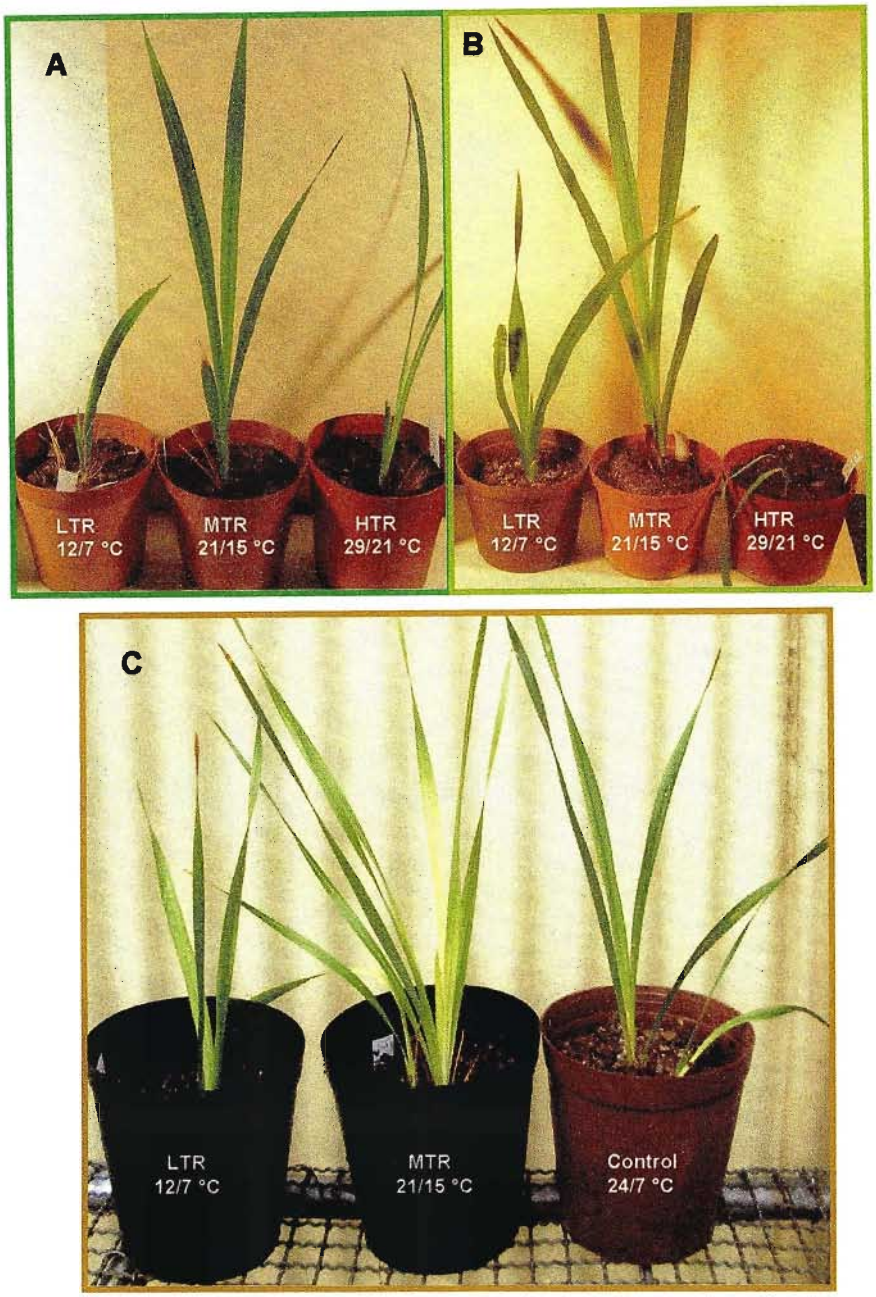

Figure 2.3.2 (A-C). The influence of temperature treatments on leaves of three *Watsonia* species.
A: *W. tabularis* plants maintained under LTR, MTR and HTR. Note the number and the length of the leaves.
B: *W. borbonica* plants also maintained in LTR, MTR and HTR. Note the height and the number of leaves. HTR were detrimental to plants. Note thin and collapsing plant on the right in both A and B.
C: *W. pillansii* plants. Note the total number of leaves in LTR compared to MTR.

Moderate temperature regimes of 23/14°C and 21/15 °C significantly increased (*P<0.001*) the height of the plants compared to plants exposed to 12/7 °C and 24/7 °C (Table 2.3.1). In addition, to being taller, shoots maintained at 21/15 °C were also undesirable as the plants tended to be lodged and could not support the shoot and the inflorescence (Figure 2.3.2 A-C). These plants were slightly etiolated, which was caused by a light imbalance (red to far-red ratio (R: FR)) (MOE and HEINS, 1990). Exposure to LTR resulted in compact plants similar to the control plants, maintained at moderate temperatures but with higher light intensity. It was obvious that the light ratio needed to be increased as the temperature increased as observed by CATLEY *et al.* (2002a and b) and DAVIES *et al.* (2002) for *Sandersonia aurantiaca.* In this species, the strongest-self supporting stems were produced under a combination of lower temperature and high light intensity (CATLEY *et al.*, 2002a).

In all three *Watsonia* species, the number of leaves was significantly (*P<0.001*) influenced by temperature (Table 2.3.1). However, the total number of leaves produced was species-specific (Table 2.3.1). *W. tabularis* produced a higher number of leaves than *W. borbonica* and *W. pillansii* (Table 2.3.1). In MTR, the leaf number was significantly increased compared to low LTR (Table 2.3.1) (Figure 2.3.2 C). This result demonstrated that elevated temperatures were effective at increasing leaf number (Figure 2.3.2 A-C).

The total flower number produced per plant was significantly increased (P<0.001) by MTR compared to LTR (Table 2.3.1). Generally, temperatures of 23/14°C and 21/15 °C resulted in more flowers per plant compared to 12/7 °C (Table 2.3.1). This result indicated that moderate to higher temperatures promoted flower development. The results were in agreement with those of MOLINA *et al.* (2005), where flower formation in saffron was enhanced by elevated temperatures (23-27 °C).

Although MTR was efficient in increasing the total number of flowers produced per plant, flowering percentage was poor, with the exception of *W. tabularis* (Figure 2.3.3 A-B). Control temperatures (24/7 °C) resulted in a higher flowering % in *W. borbonica* and *W. tabularis* (Figure 2.3.3). This could be attributed to the fact that

control plants experienced fluctuating cold winter temperatures which were inductive for flower initiation, while subsequent warm summer days promoted flower differentiation. Similar observations were reported by ROH (2005) in the flowering of *Lachenalia aloides* where exposure to 17/15 °C accelerated flowering compared to 21/29 °C. Work done on *Freesia* species also showed that floral initiation was enhanced by exposure to low temperatures of 15/17 °C (GILBERTSON-FERRISS *et al.*, 1981b). DU TOIT *et al.* (2004) reported an increase of *Lachenalia* flowering at low temperature regimes.

Higher and moderate temperature regimes were detrimental for spike emergence and differentiation (Figure 2.3.4 A-C). Either the spike was "burnt" or the inflorescence was aborted. As a result further floret differentiation was inhibited (Figure 2.3.4 B). This was more pronounced in the control plants exposed to natural temperatures in the 25 % shade house (2005 experiment). This was attributed to slightly warmer temperature regimes experienced in the 2005 planting year as compared to those observed in 2004. To increase flower quality and longevity, it seems necessary that the temperature be lowered.

Even though the total flower number produced per plant was low under LTR, flowering was induced in all three species (Table 2.3.1). Plants that were only capable of flowering under these temperature regimes, demonstrated that they had a vernalisation requirement. Interestingly, plants were capable of flowering under this regime with a minimum number of leaves (Figure 2.3.5 A-B). Although no significant difference was observed in the number of days to flower, LTR marginally reduced the time taken to flower compared to MTR (Table 2.3.1). Results in *W. pillansii* demonstrated that this species had an obligate response to vernalisation, as no flowering was observed in non-vernalised and control plants (Table 2.3.1) (Figure 2.3.5). However, in order for plants to flower, exposure to higher temperatures was necessary.

This behaviour seems to fit the classic vernalisation response where once plants are committed to flowering, exposure to non-inductive conditions will not hamper flowering (CHOUARD, 1960; ROBERTSON *et al.*, 1996; ADAMS *et al.*, 1998;

BERNIER *et al.*, 1998). Furthermore, vernalised plants needed long days or/and warm temperatures for further flower development. This is in accordance with the reports of CHOUARD (1960) and ADAMS *et al.* (1998).

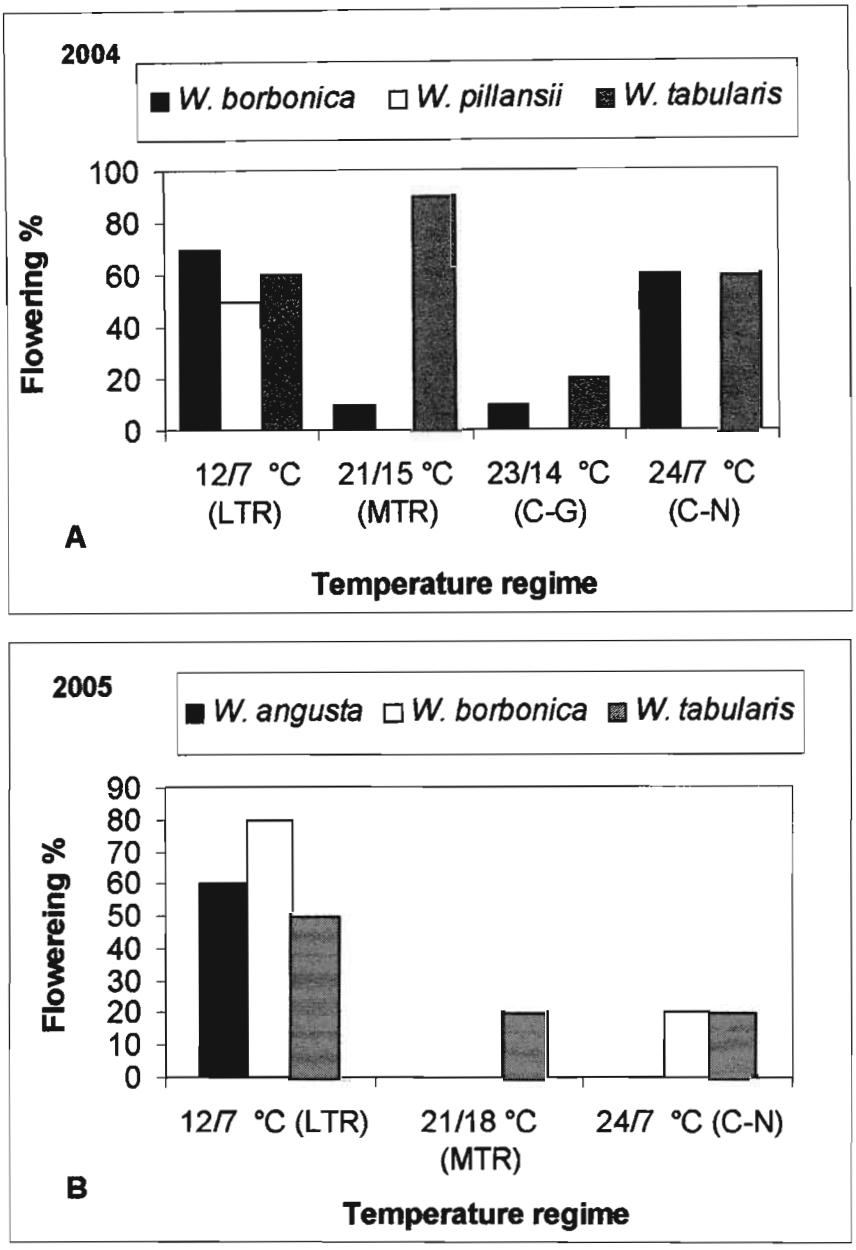

Figure 2.3.3 (A-B). Flowering of *Watsonia* species at different temperature regimes. Data were collected in 2004 and 2005 flowering seasons. Each treatment consisted of 10-15 plants.
A: In the experiment conducted in 2004, plants were exposed to temperature regimes after attaining first and/or second leaves.
B: In the experiment conducted in 2005, plants were exposed to three temperature regimes after attaining 3-4 leaves. Note the reduced flowering % under MTR.

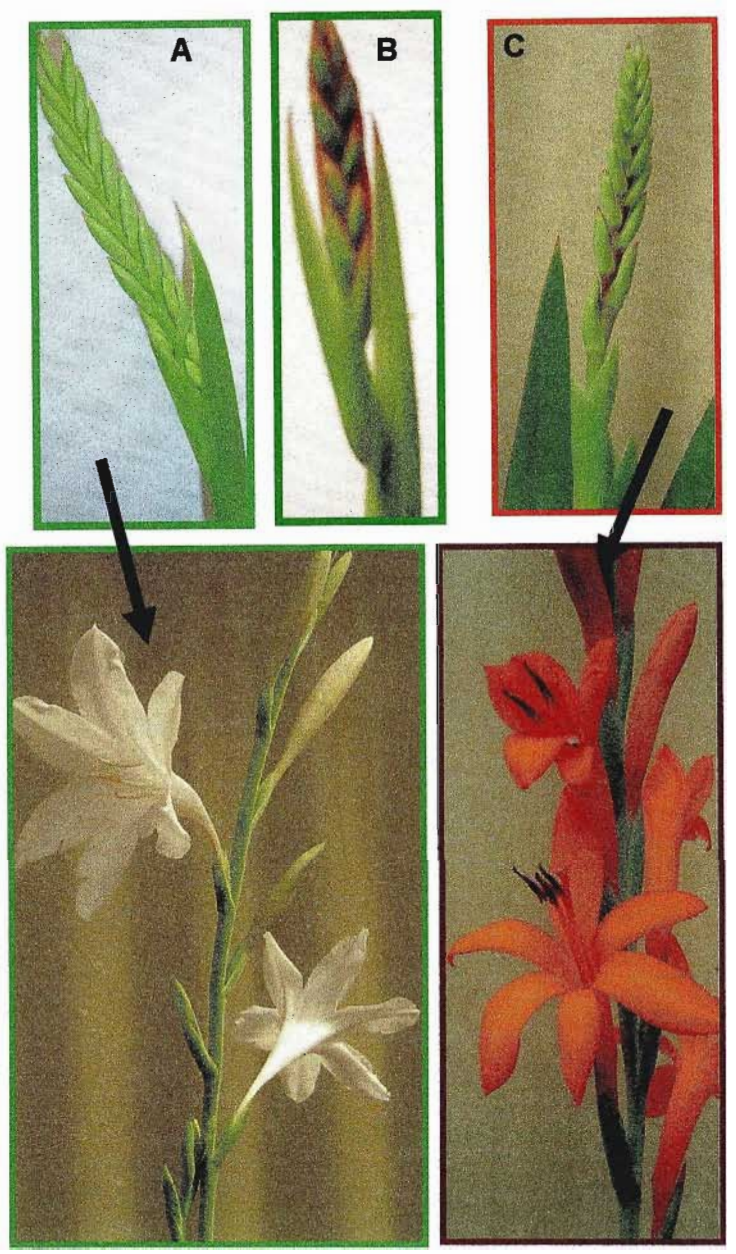

Figure 2.3.4 (A-C). The effect of temperature on spike and flower development of *Watsonia* species.
A: Normal spike of *W. borbonica*, which differentiated into a normal inflorescence with attractive flowers.
B: Burnt spike of *W. angusta* under elevated temperature/s. No further flower differentiation occurred in this plant.
C: Normal spike emerging in *W. tabularis*, which differentiated into normal flowers.

Plants that were not "cooled" did not flower under this photoperiod. Vernalising *Lavandula angustifolia* at 5 °C, for 5 weeks resulted in visible buds and flowering approximately 30 days sooner than non-vernalised plants (WHITMAN *et al.*, 1996). WANG *et al.* (1970) reported a similar effect of vernalisation in *Lilium longiflorum* after 6 weeks of vernalisation at 4.4 °C. This reduced the number of leaves. However, the internode length was increased so that vernalised plants were taller than the controls.

Results from reciprocal transfers conducted in 2005, further comfirmed that flowering in the three species was mainly controlled by temperature (Figure 2.3.6 A-B). However, there were technical problems experienced with the Convirons, as they were subjected to heating and as a result a lot of plants died. It was therefore, hard to interpret the results due to uncontrolled temperature effects.

Plants with three to four leaves, maintained in the warm temperatures in a 25 % shadehouse were used as substitute material. The results indicated that exposing plants to 21/18 °C inhibited flowering and increased flower abortion (Figure 2.3.6 C). The results also showed that flowers were already initiated at this stage but continuous exposure to higher temperatures was detrimental to their development. Interestingly, in plants that failed to flower or aborted their inflorescences, a higher number of cormels were present (Figure 2.3.6 C). It is possible that floret differentiation is more sensitive to stressful conditions than to cormel initiation and differentiation. As a result carbohydrate reserves could have been directed to cormel initiation and differentiation. Inflorescence blast (which occurred after three days of high temperature treatment) in *Lachenalia* species was suggested to be due to the limited supply of cabohydrates to developing florets, which could act as strong sinks (ROH, 2005).

Exposing plants to 12/7 °C (after attaining three to four leaves) for 6 weeks increased the leaf number and induced flowering (Figure 2.3.6 A and B). However, transient flowering was observed, indicating that 6 weeks were not sufficient to produce an uniform flowering time. This result indicated that plants were capable of receiving cold treatment even after the first and/or second leaf stages. Taken together, the results indicated that these three species are quantitative with regard

to their vernalisation response. It is possible that any low temperature not necessarily one that vernalises the plant would be capable of inducing flowering. IVERSEN and WEILER (1994) reported that cold treatment was not a requirement for complete flowering in *Phlox paniculata*. However, plants exposed for 12 weeks at 4.5 ° C flowered more uniformly and 1 to 15 days sooner. This behaviour, where flower initiation occurs as a direct consequence to vernalisation does not fit the description given to vernalisation, which is an inductive process (ROBERTSON *et al.*, 1996; ADAMS *et al.*, 1998).

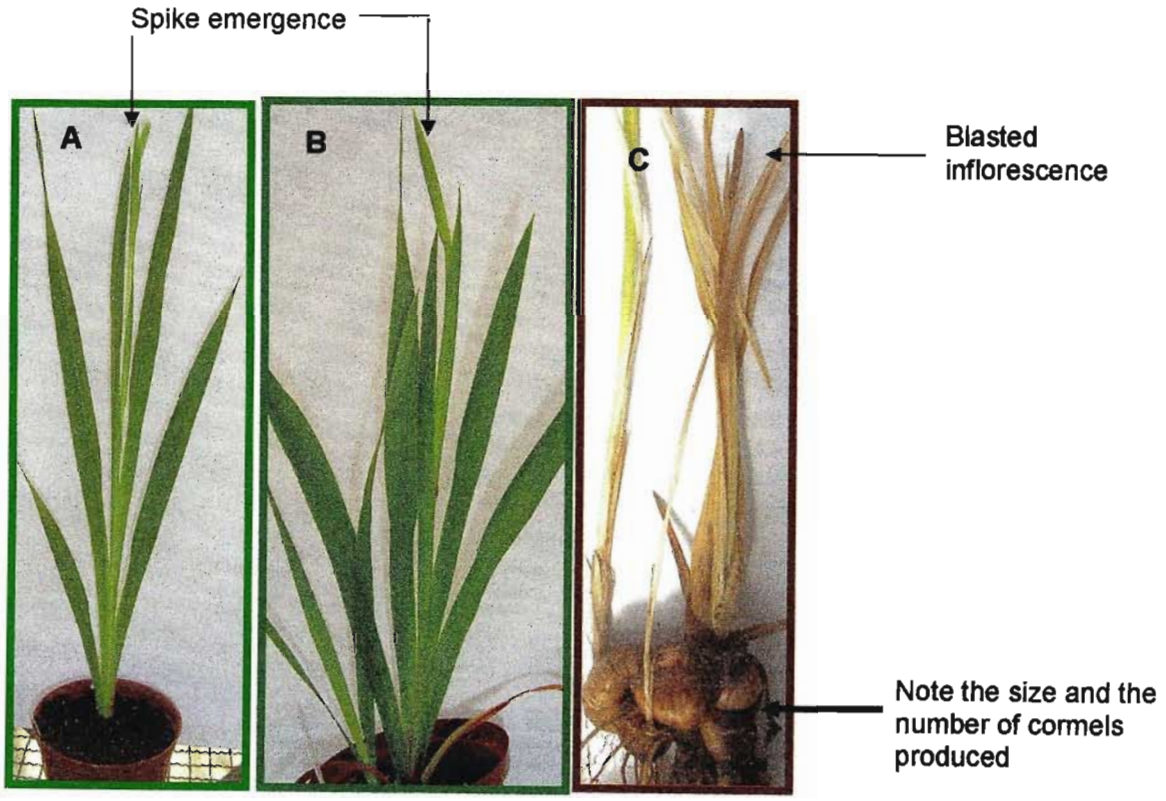

Figure 2.3.6 (A-C). *Watsonia* plants exposed to two reciprocal temperatures (12/7 °C and 21/18 °C) after attaining three to four leaves.
A: *W. borbonica*, with 6 leaves. Note the spike emerging.
B: *W. angusta* plant with a spike emerging. Note the increase in the number of leaves
C: *W. tabularis* with blasted inflorescence. Note the size and the increase in the number of cormels initiated.

Interestingly, *W. angusta* and *W. tabularis* were more sensitive to higher temperatures at the spike emergence stage compared to *W. borbonica*. Although these three species responded well to 'warm-cold-warm' temperature sequences, it was obvious that for keeping quality, temperature needed to be lowered.

In summary, flowering variation among species is likely due to differences in the environment from which they originate. The results from two winter-rainfall species (*W. borbonica* and *W. tabularis*) and shared rainfall area (*W. angusta*), suggested that vernalisation was quantitative to these species. In contrast, data from the summer-rainfall species (*W. pillansii*), showed a qualitative response to vernalisation. A suggestion would be to expose plants (quantitative–vernalisation) to moderate temperature regimes to increase vegetative growth until the fourth leaf stage. Lowering temperature will induce and synchronise flowering. A subsequent temperature increase should be applied just before spike emergence. In plants with a qualitative vernalisation response, such as *W. pillansii*, low temperature should be used to make them competent to flower. These plants need 'cold-warm-cold' sequences as oppossed to 'warm-cold-warm' sequence as their winter-rainfall counterpart.

Chapter 3:

EFFECTS OF LIGHT ON FLOWERING OF *WATSONIA* SPECIES

3.1 INTRODUCTION

Light controls diverse processes in plant growth and development (MOHR and SHROPSHIRE, 1983; THOMAS, 1993). Light has three main characteristics that affect plant morphogenesis and flowering: quality, quantity and duration (ERWIN, 1993). Light quality refers to the wavelength reaching the plant surface, whereas, light quantity refers to the intensity or concentration of sunlight and varies with the season of the year. Photoperiod refers to the amount of time that a plant is exposed to sunlight. When discussing the effect of light on flowering all three characteristics should be considered.

Plants perceive light by using photoreceptors (phytochromes and cryptochromes) to obtain information about their immediate environment and changing seasons (BUTLER *et al.*, 1959; THOMAS, 1993). Different phytochromes regulate either distinct light responses or similar responses under different light conditions (light quantity, quality and timing) (LIN, 2000). Furthermore, phytochromes have important roles in developmental events such as the switch to flowering, the timing of which can be crucial for the reproductive success of the plant (HALLIDAY and WHITELAM, 2003).

Many angiosperms flower in response to photoperiodism as the year progresses (THOMAS and VINCE-PRUE, 1997; HENDERSON and DEAN, 2004). Photoperiod functions according to certain latitudes, whereas light intensity fluctuates daily. It is therefore, difficult to control light intensity in nature. The changes in solar radiation occurring in summer and winter are due to changes in both light intensity and daylength which contribute to light sum or total solar irradiance (SHILLO and HALEVY, 1976c). Thus, it is difficult to separate these two factors as they produce similiar effects. The light requirements for *Watsonia* species are not known, therefore, attempts were made to address two characteristics of light: i.e. intensity and daylength. The response to light quality was beyond the scope of this study.

3.1.1 OBJECTIVES

-To determine the critical daylength required for optimal flowering of *Watsonia* species; and

-To investigate the impact of light intensity on flowering of *Watsonia* species.

3.2 MATERIALS AND METHODS

3.2.1 DETERMINING THE EFFECT OF DAYLENGTH ON PLANT MORPHOGENESIS AND FLOWERING OF *WATSONIA* SPECIES.

This experiment was conducted using the following *Watsonia* species: *borbonica*, *tabularis* and *pillansii* in April 2004. After emergence of the second leaf, plants were transferred from the shadehouse to Conviron growth chambers. A comparison was made between long day (16 h light/ 8 h dark) and short day (8 h light/16 h dark). In both regimes, temperatures were maintained constant at 12/7 °C by programming the microprocessor installed in the growth chambers. Under LD, daylengths were extended from 0400h to 16h00, whereas in the SD regime, lamps were switched on automatically at 08h00 am until 16h00. The light intensity was recorded by the radiation sensor, where instantaneous reading was 150 µmol $m^{-2}s^{-1}$, using cool white fluorescent tubes and incandescent globes. Light intensity was similar in both regimes.

A similar study was conducted concurrently in the greenhouse, however, the temperature was not controlled, and it was influenced by day and night temperatures prevalent on the particular day. In LD treatment, daylength was extended from 04h00 by illuminating the plants with low light intensity of ± 10 µmol $m^{-2}s^{-1}$ until 08h00 (using white fluorescent tubes), when natural light was used. In the SD treatment, natural light was used from 08h00 to16h00. Daylength was shortened by covering the plants with black plastic sheets.

The study was repeated in February 2005 but the growth conditions were slightly modified. Plants were left under day neutral conditions in a 25 % shadehouse until they had developed three or more leaves. Plants were then exposed to either LD or SD at 12/7 °C. After 7 weeks, plants were returned to the greenhouse (23/14 °C) at the end of June 2005. The number of flowers produced in each treatment were counted and statically analysed by ANOVA using MINITAB.

3.2.2 THE INFLUENCE OF LIGHT INTENSITY ON PLANT MORPHOGENESIS AND FLOWERING OF *WATSONIA* SPECIES

Attempts to separate daylength and light intensity effects were made in February 2005 by exposing *W. tabularis* and *W. borbonica* to different light intensities. Temperatures were maintained constant at 12/7 °C (day and night) at 16 h daylength. Initially, plants were grown in a 25 % shadehouse at natural summer temperatures. Plants were then exposed to light intensities in the growth chambers of either ±39.5 or 150 µmol $m^{-2}s^{-1}$, between one to second and third leaf stages using fluorescent tubes. Each treatment consisted of ten plants. When the treatment was completed, plants were returned to the greenhouse, with the light intensity of ±450 µmol $m^{-2}s^{-1}$.

Comparative studies were made with control plants maintained in the shadehouse where the light intensity was ±950 µmol $m^{-2}s^{-1}$. The photosynthetically active radiation (PAR) was measured using point and line quantum sensors (Apogee instrument, Inc., Logan, Utah) attached to a data logger (Campbell Scientific, Inc., Logan, Utah). The full sun (100 %) had an average of ±1403 µmol $m^{-2}s^{-1}$, measured at 12h00. At the end of the treatment, the quality of plants was assessed by measuring the width at the bottom of the youngest leaf. The quality was also visually inspected by assessing plant sturdiness, flower angle and physiological abnormalities. Numbers of leaves and flowers produced were counted at the end of the experiment. Data were analysed by ANOVA, using General Linear Models Procedure in MINITAB (12).

3.3 RESULTS AND DISCUSSION

3.3.1 PHOTOPERIODIC RESPONSES

Increasing daylength enhanced plant morphogenesis in *Watsonia* plants in general. LD regimes significantly increased the height and the number of leaves per plant ($P<0.001$) compared to SD regimes (Table 3.3.1). In the control plants, DN regimes, the height was significantly ($P<0.001$) reduced but the number of leaves was the highest. This was however, species-dependent. In general, *W. tabularis* produced a higher number of leaves compared to *W. borbonica* and *W. pillansii.* In addition, *W. borbonica* is generally a taller plant compared with the two other species. Thus, increasing daylength was effective at increasing plant height and leaf number. Similar results were reported in *Lilium longiflorum* and *Brodiaea* (HEINS *et al.*, 1982b; HAN *et al.*, 1994).

The onset of flower formation in *Watsonia* species was significantly promoted by LD compared to SD conditions, even though flowers were eventually initiated under certain SD conditions. This was, however, highly dependent on the temperature and species interaction (Table 3.3.1). The interaction was evident in *W. pillansii*, where a low temperature significantly ($P<0.001$) promoted flowering under both LD and SD regimes (Figure 3.3.1 A-C). At moderate and higher temperatures no flowering was observed in this species, irrespective of daylength. According to HEIDE (1977) the critical daylength is to a certain extend determined by phytochrome conversion rate, which is highly temperature dependent (HEIDE, 1977). This was speculated to be due to the finding that under low temperatures the amplitude of the rhythm is reduced or suppressed resulting in the escape from photoperiodic control of flowering at such temperatures (HEIDE, 1977).

Table 3.3.1. The effect of daylength regimes on flowering of *Watsonia* species. Plants were exposed to photoperiod treatments for 12 weeks. Data were analysed by ANOVA, MINITAB. Each species was analysed separately and the treatment consisted of 10 replicates per regime. Means with letters in common are not significantly different at 5 % ($P<0.05$), separated by Fisher's test.

Species	Temperature °C	Daylength	Leaf Length (cm)	Leaf Number	Flower Number (per plant)
W. borbonica	12/7	LD (16 h)	54.72 ± 1.16c	5.09 ± 0.13a	14.36 ± 0.74a
		SD (8 h)	38.90 ± 1.23d	4.20 ± 0.10b	0.00 ± 0.0c
	23/14	LD (16 h)	77.33 ± 1.53 a	5.20 ± 0.50a	10.00 ± 0.52b
		SD (8 h)	68.00 ± 1.71ab	5.10 ± 0.31a	0.00 ± 0.0c
	24/7	DN (12 h)	49.60 ± 1.05c	5.77 ± 0.17a	16.00 ± 0.32a
W. pillansii	12/7	LD (16 h)	41.00 ± 0.42b	4.50 ± 0.14b	12.17± 0.19a
		SD (8 h)	35.20 ±1.11bc	3.80 ± 1.75c	11.30 ± 0.35a
	23/14	LD (16 h)	45.30 ± 0.88a	5.50 ± 0.10a	0.00 ± 0.0b
		SD (8 h)	43.50 ± 1.42a	5.10 ± 0.17b	0.00 ± 0.0b
	24/7	DN (12 h)	29.27 ± 0.91c	6.00 ± 0.14a	0.00 ± 0.0b
W. tabularis	12/7	LD (16 h)	43.20 ± 0.77c	5.80 ± 0.15b	11.00 ± 0.48b
		SD (8 h)	41.40 ± 1.45c	4.50 ± 0.07c	0.00 ± 0.00c
	23/14	LD (16 h)	69.00 ± 1.91a	8.00 ± 0.10a	14.15 ± 0.48a
		SD (8 h)	61.00 ± 1.87ab	6.00 ± 0.13b	0.00 ± 0.0c
	24/7	DN (12 h)	52.20 ± 0.43bc	7.60 ± 0.11a	11.60 ± 0.13b

A minimum of five leaves was required for successful spike emergence. LD enhanced growth and flowering in *Watsonia* (Table 3.3.1). Similar results were reported for *Achimenes* (VLAHOS, 1990) and *Lilium longiflorum* (HEINS *et al.*, 1982b; MOE AND HEINS, 1990). In SD regimes, the number of leaves was compromised. As a result, poor or no flowering was observed under SD regimes (Table 3.3.1). Thus, lack of flowering observed in SD, could also indicate that this regime was apparently insufficient (too short) for shoots to reach a stage where they were able to perceive the floral stimulus and develop the inflorescence to anthesis (CHAILAKHYAN, 1936; THOMAS and VINCE-PRUE, 1997; LEVY and DEAN, 1998). This can be explained by the total number of leaves observed under SD regimes, which were below the critical number required for flowering (Table 3.3.1). Taken together the results indicate that *Watsonia* plants are facultative LD or DN plants as classified by ERWIN and WARNER (2002).

Even though flowering was promoted under LD regimes, a variation in flowering of certain species observed under DN and LD regimes seems to indicate that photoperiod plays a minor role in the flowering of Watsonias, when the temperature was not lowered. This can be clearly demonstrated by comparing flowering of all three species at LD and DN regimes at 23/14 °C and 24/7 °C, respectively. Flowering observed in *W. borbonica* and *W. pillansii* could be attributed to temperature rather than photoperiod. *W. tabularis* was an exception as a higher number of flowers occurred at 23/14 °C (Table 3.3.1). Similar observations were reported in *Osteospermum jucundum* (ADAMS *et al.*, 1998) and *Phlox paniculata* (RUNKLE *et al.*, 1998), where initial exposure to low temperature and subsequent transferral to LD and higher temperatures hastened and promoted flowering.

Flowering variation displayed by the three selected *Watsonia* species could also be attributed to ecological differences in the natural habitat of the species. ROSS and MURFET (1985) reported a similar variation for flowering responses of *Lathyrus odoratus* growing in winter (DNP), spring or summer species (FLDP). Furthermore, SERÇE and HANCOCK (2005) reported similar results in three *Fragaria* species: *chiloensis* (FLDP), *virginiana* (DN) and *ananassa* (LDP).

Interestingly, when the experiment was repeated in 2005, plants exposed to SD at the three leaf stage, flowered (Table 3.3.2). There was no significant difference (*P>0.05*) found in the height and the number of leaves between SD and LD regimes, in both species (Table 3.3.2). However, the total number of flowers produced was significantly (*P<0.01*) reduced under a SD regime. In addition to poor flowering, flowers were sparse in SD regimes (Figure 3.3.2). In the control plants maintained in DN conditions, flowering was poor due to flower blasting, caused by elevated temperatures. Comparing results obtained in both years (2004 and 2005) suggested that photoperiod effects were additive to those of temperature (Table 3.3.1 and 3.3.2). This was clearly demonstrated by the number of leaves and flowers obtained per plant in *W. tabularis* under either LD or SD at 23/14°C. These results suggested two things (i) flowering was not dependent on photoperiod and (ii) flower initiation was sensitive to photoperiod at certain developmental stages. These results were similar to those of SHILLO and HALEVY (1976c) for *Gladiolus* species.

The morphology and the angle of the flowers in SD regimes were different and slightly deflected downwards compared to those on LD and DN plants, which were upright (Figure 3.3.1 A-C and 3.3.2). Such deflection affects plant quality detrimentally, rendering it unattractive horticulturally. These results, thus demonstrated that photoperiod in *Watsonia* plants, plays a major role not only in plant morphogenesis, but also in flower morphology. In contrast, results on *Nephrolepis exaltata* (ERWIN *et al.*, 1993) and *Heliconia aurantiaca* (GEERTSEN, 1990) suggested that different photoperiodic regimes had no significant effect on either morphology or development rate or on the total number of shoots emerging on either of these plants.

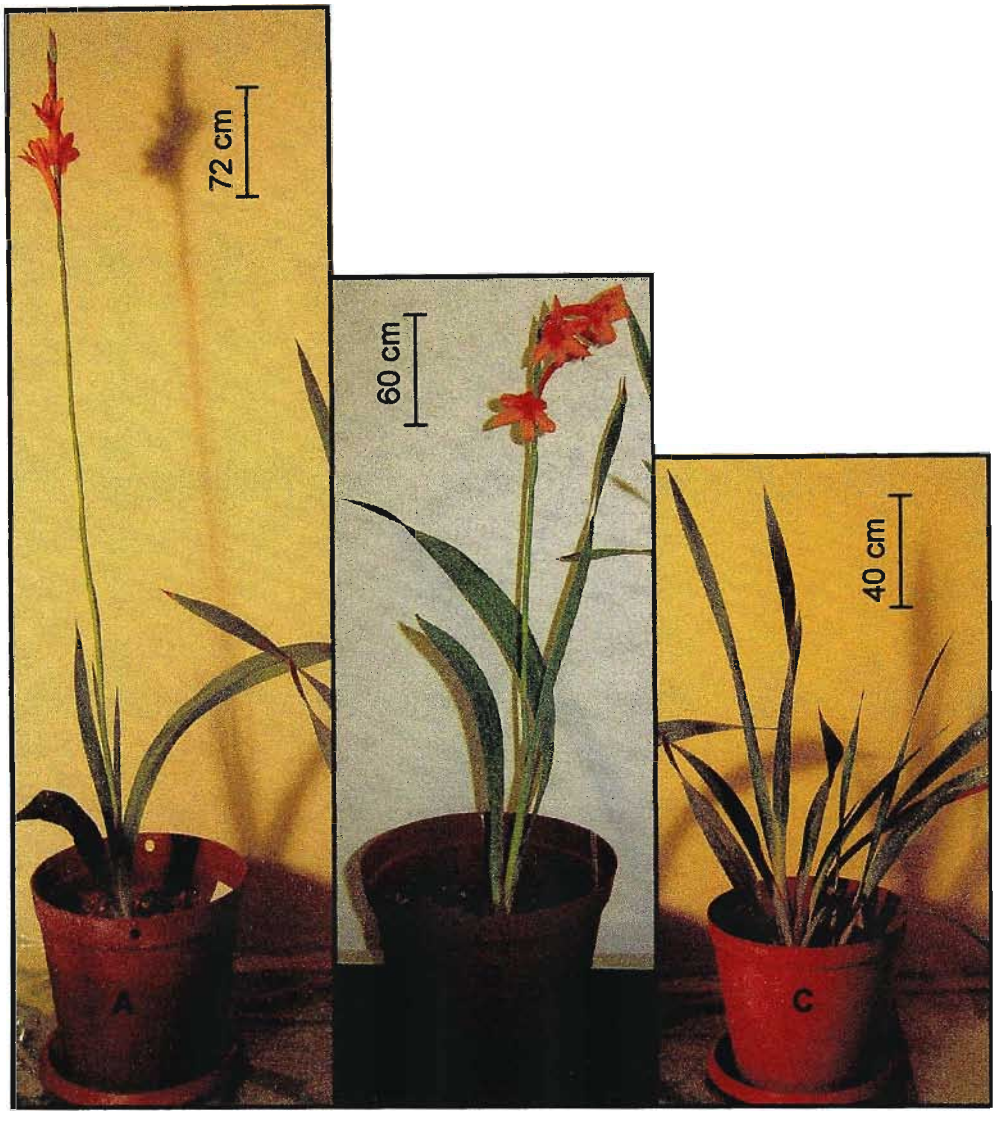

Figure 3.3.1 (A-C). The effect of daylength on flowering of *Watsonia pillansii*.
A: *W. pillansii* plant maintained at LD and low temperature regimes, flowered with five leaves. Note the appearance of the flowers.
B: *W. pillansii* plant maintained at SD and low temperature regimes, flowered with an average of five leaves. Note the deflection of the flowers.
C: *W. pillansii,* which served as a control plant, maintained in DN regime under natural temperature/s, produced more than 7 leaves but no flowers.

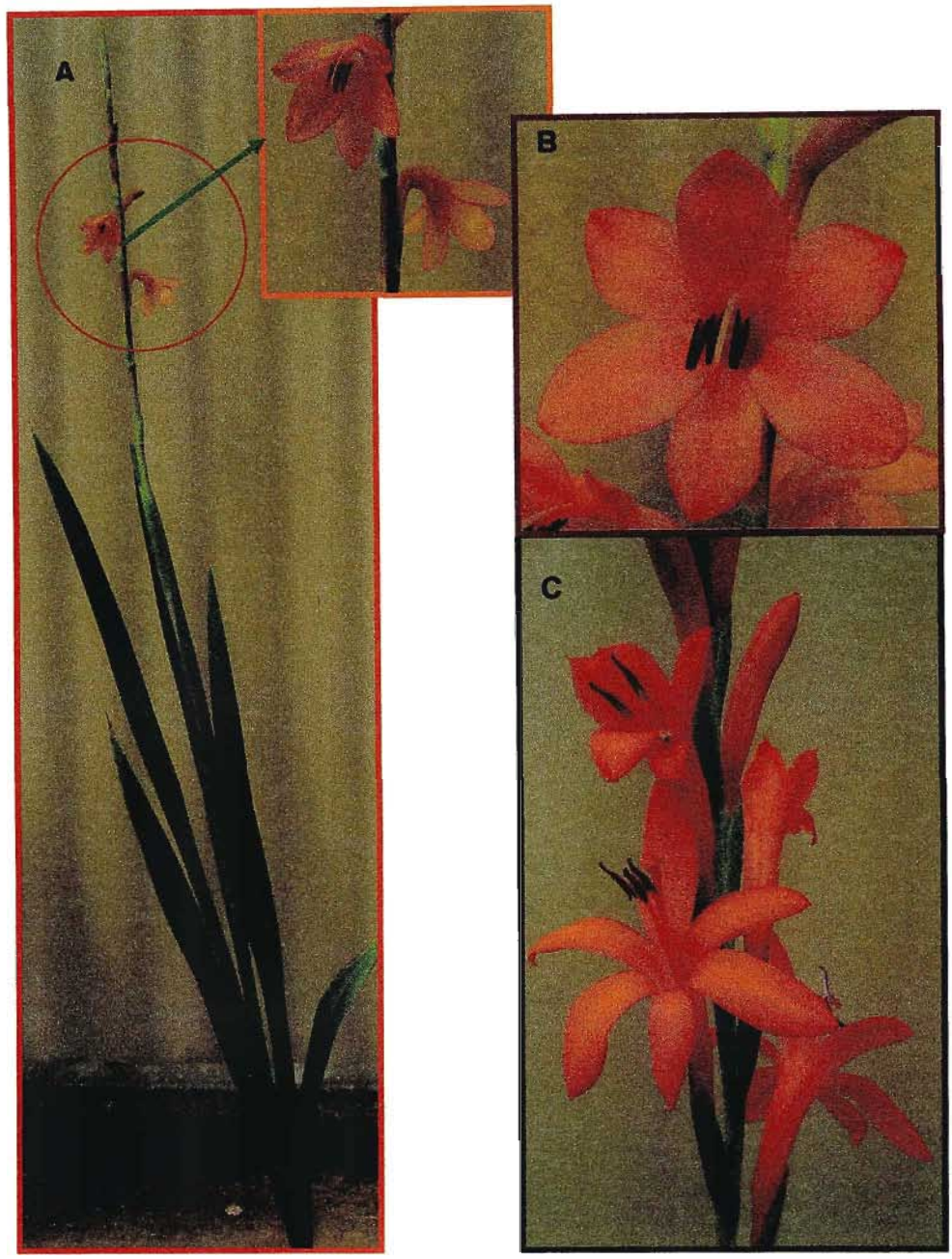

Figure 3.3.2 (A-B). The morphological effect of LD versus SD regimes on flowering of *Watsonia tabularis.*
A: A plant exposed to a SD regime slightly etiolated. The leaf number was reduced and flowers were sparse. Note the deflection (angle) of the flowers (either bent sideways or downwards).
B: A control plant exposed to a DN regime. Flowers had thick petals and flowers were positioned upright.
C: Plants exposed to a LD regime, with more attractive flowers. Note the angle and morphology of the flowers compared to those in Figure 3.3.2 A.

Table 3.3.2. A comparison of the influence of daylength at the third leaf stage. Experiments were conducted in 2005. The plants were maintained in the shadehouse until the formation of the third leaf. They were then subjected to either 16 or 8 h daylength for 7 weeks. Each species was analysed separately by ANOVA and the treatments each included 10 replicates. Means with letters in common are not significantly different at 5 % (*P<0.05*), separated by Fisher's test. Standard error values are presented.

Species	Daylength (h)	Temperature (°C)	Leaf Height (cm)	Leaf Number	Flower Number	Inflorescence Height (cm)
W. borbonica	16 h (LD)	12/7	74.33 ± 2.26a	6.00 ± 0.21a	19.00 ± 0.56a	92.80 ± 1.56a
	8 h (SD)		73.40 ± 1.59a	5.50 ± 0.15a	8.00 ± 0.40b	86.67 ± 2.84a
	12 h (DN)	24/7	63.00 ± 1.38b	6.40 ± 0.10a	6.89 ± 0.14b	85.29 ± 3.04a
W. tabularis	16 h (LD)	12/7	55.33 ± 1.58a	6.18 ± 0.35a	14.83 ± 0.22a	82.00 ± 3.17a
	8 h (SD)		46.00 ± 1.80a	5.83 ± 0.17a	8.33 ± 0.35b	79.00 ± 2.49a
	12 h (DN)	24/7	43.79 ± 1.80a	6.16 ± 0.16a	12.00 ± 0.24ab	81.33 ± 4.25a

3.3.2 THE EFFECT OF LIGHT INTENSITY ON PLANT MORPHOGENESIS AND FLOWERING

Plants exposed to a light intensity of either 39.5 or 150 µmol $m^{-2}s^{-1}$ displayed different degrees of sensitivity to reduced light during different developmental stages (Table 3.3.3). Exposure to 39.5 µmol $m^{-2}s^{-1}$, between the first to third leaf stage and above, resulted in more (*P<0.001*) etiolated plants compared to 150 µmol $m^{-2}s^{-1}$ and untreated plants at 950 µmol $m^{-2}s^{-1}$ (Table 3.3.3) (Figure 3.3.3 A). However, at the first to third leaf stage, plants were more sensitive to reduced light intensity; as a result, they died after being returned to the greenhouse. In contrast, plants exposed to low light beyond third leaf stage were less sensitive but unattractive (etiolated and thin leaves) (Figure 3.3.3 B-C). The leaves were longer and flaccid when compared to short and compact plants maintained at 150 µmol $m^{-2}s^{-1}$. Excessive elongation of lily stems due to insufficient light was also observed by BEATTIE and WHITE (1993). It was clear that light intensity is important in controlling height and plant quality, as observed by HEINS *et al.* (1982a) for lilies and DAVIES *et al.* (2002) for *Sandersonia* species.

Even though exposing plants to low light intensity after the third leaf stage greatly reduced leaf quality, the flowering ability was not impaired (Figure 3.3.3 B). However, the light intensity significantly (*P<0.001*) influenced the total number of flowers produced per plant. More flowers were obtained at 150 µmol m^{-2} s^{-1} compared to 39.5 µmol $m^{-2}s^{-1}$ (Table 3.3.3). This result indicated that increasing the light intensity had a strong effect on leaf number and number of flowers produced per plant. Light intensity thus affects both plant morphogenesis and flowering. Since the temperature was kept constant in 150 and 39.5 µmol $m^{-2}s^{-1}$, the differences are probably a true reflection of light intensity and not temperature. It is possible that under higher light intensities, the leaf temperature is increased to above air temperature. Higher temperatures accelerate development while high light intensity increases photosynthesis, which maintains plant quality as observed by HEINS *et al.* (1982a). In *Euphorbia pulcherrima,* low light (11 and 53 µmol $m^{-2}s^{-1}$) delayed flower initiation, but did not affect the rate of bud development following initiation (WANG *et al.*, 2003).

Table 3.3.3. The effect of varying light intensities on plant morphogenesis and flowering. The results for each species was analysed separately by ANOVA. Each treatment consisted of 10 replicates. The plants were exposed to light intensity treatments at either the first-third leaf stage or after the third leaf stage for 7 weeks. Means with letters in common are not significantly different at 5 % ($P<0.05$), separated by Fisher's test. Same plants were used (*).

Species	*Light Intensity* ($\mu mol\ m^{-2}s^{-1}$)	Developmental Stage	Temperature (°C)	Leaf Length (cm)	Leaf Number	Flower Number (per plant)
W. borbonica	39.5	1-3	12/7	51.90 ± 1.60a	2.10 ± 0.15c	0.00 ± 0.0c
	150	1-3	12/7	38.50 ± 1.29b	3.30 ± 0.20b	17.29 ± 0.59a
	950 (Control)	1-3	24/7	30.54 ± 0.87c	3.61 ±0.183a	10.00 ± 0.27b*
	39.5	>3	12/7	76.00 ± 0.32a	4.00 ± 0.10b	16.10 ± 0.80b
	150	>3	12/7	54.70 ± 0.76b	5.20 ± 0.12a	18.60 ± 0.30a
	950 (Control)	>3	24/7	50.00 ± 0.77b	5.50 ± 0.08a	10.00 ± 0.27c*
W. tabularis	39.5	1-3	12/7	42.30 ± 1.75a	2.70 ± 0.11c	0.00 ± 0.0c
	150	1-3	12/7	32.60 ± 1.08b	3.90 ±0.15b	13.00 ± 0.43a
	950 (Control)	1-3	24/7	34.55 ± 1.28b	4.30 ±0.66a	9.44± 0.28b*
	39.5	>3	12/7	63.50 ± 1.36a	4.70 ± 0.16b	8.60 ± 0.18c
	150	>3	12/7	42.30 ± 1.72b	5.70 ± 0.14a	11.80 ± 0.26a
	950 (Control)	>3	24/7	44.07 ± 0.49b	7.00 ±0.09a	9.44± 0.28b*

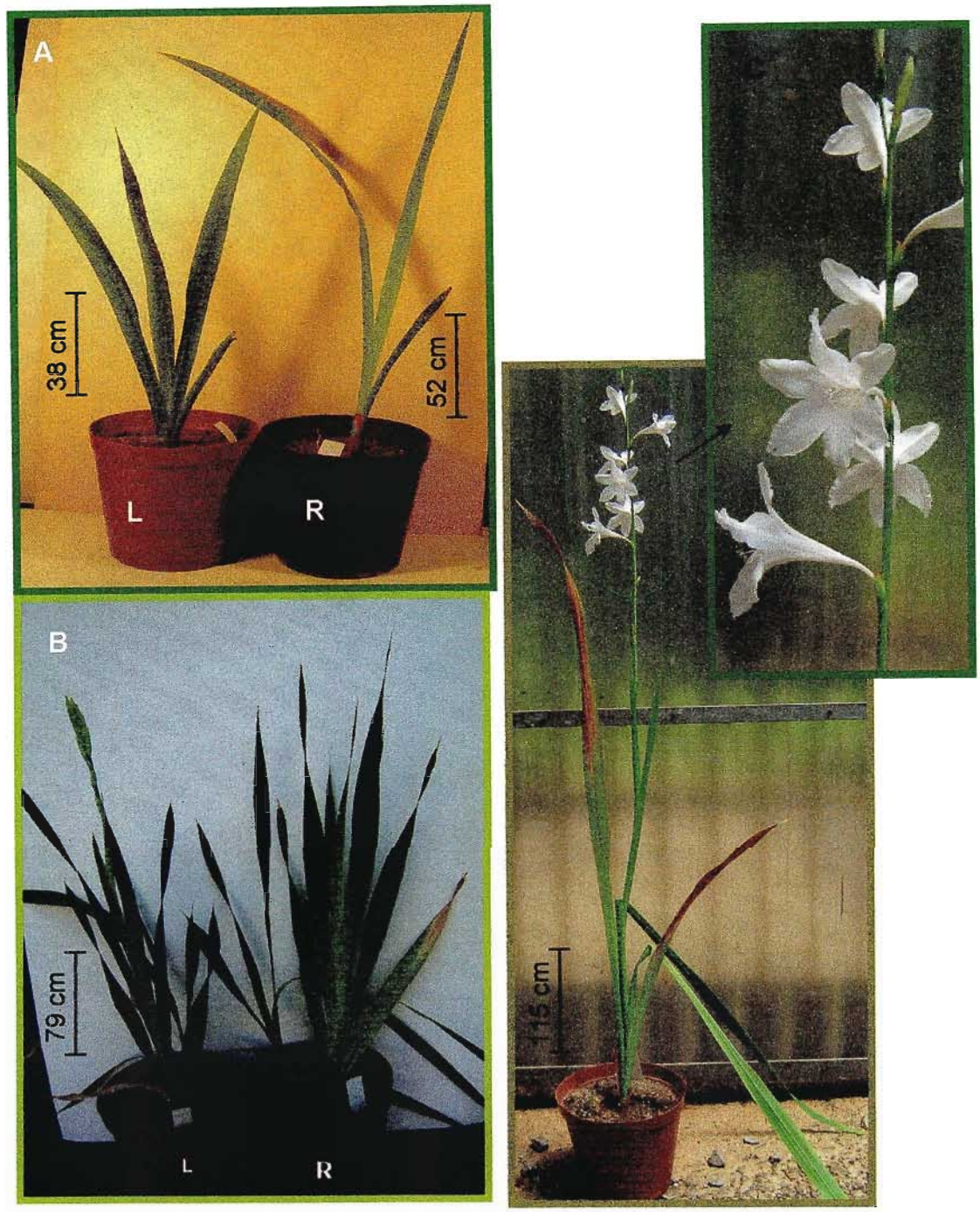

Figure 3.3.3 (A-C). Effect of light intensity on *Watsonia* species applied at different developmental stages.

A: *W. borbonica* plants (first-third leaf stage) exposed to 150 µmol $m^{-2}s^{-1}$ (L) versus those exposed to ±39.5 µmol $m^{-2}s^{-1}$ (R). Note the height, the width and the number of leaves.

B: *W. tabularis* plants exposed to light intensity after the third leaf stage. Note the appearance of the leaves. Flowering ability was not affected in appearance at either 150 µmol $m^{-2}s^{-1}$ (L) or 39.5 µmol $m^{-2}s^{-1}$ (R).

C: *W. borbonica* plant exposed to low light intensity (39.5 µmol $m^{-2}s^{-1}$) after the third leaf stage flowered. However, appearance was compromised. Note the increase in the leaf height.

The role of light intensity in plant morphogenesis was investigated by GRUEBER *et al.* (1984) and ISLAM *et al.* (2005) in *Eustoma*. Increasing light intensity reduced the time to flower transition and enhanced vegetative development (GRUEBER *et al.*, 1984; ISLAM *et al.*, 2005). Sensitivity to reduced light intensity also varied with the developmental stages of the plant. The results were in agreement with those of SHILLO and HALEVY (1976b) and IMANISHI and IMAE (1990), in *Gladiolus* species, where it was reported that low light intensity during fourth to fifth leaf stage, resulted in a decrease in the percentage of flowering and number of florets per spike.

The lack of flowering between the first to third leaf stages could be due to plants being placed under severe stress during this transitional stage. *Watsonia* plants need to maintain a high photosynthetic level due to high meristematic activity occurring at the SAM. It is possible that under insufficient light, all the assimilates were channelled to self-maintenance rather than to flower or corm initiation, which demand considerable resources. Furthermore, plants under low light intensity were etiolated, indicating an imbalance in the red and far-red light ratio, suggesting phytochrome action interference (BAGNALL, 1993; THOMAS, 1993). Recent studies in *Arabidopsis* have indicated that etiolation responses are mediated by phytochrome B, which is an important regulator of flowering in response to light quality and photoperiod (WHITELAM *et al.*, 1998).

Plant quality was assessed by height and leaf compactness (width). The width of the youngest leaf was significantly increased ($P<0.001$) in 150 compared to 39.5 µmol $m^{-2}s^{-1}$ treated plants (Figure 3.3.4 A and B). However, at 950 µmol $m^{-2}s^{-1}$ (control condition), leaf width was the greatest when compared to lower light intensities. The results demonstrated that a stronger plant and inflorescence were the result of light and temperature interaction. Studies in *Sandersonia aurantiaca*, showed that stem strength was responsive to irradiance, as under the lowest photosynthetical photon flux densities (PPF), the long stems were weak (CATLEY *et al.*, 2002a; DAVIES *et al.*, 2002). Furthermore, a study by HEINS *et al.* (1982a) in *Lilium longiflorum* clearly

showed that in order to maintain plant quality, light intensity must also increase as temperature increases.

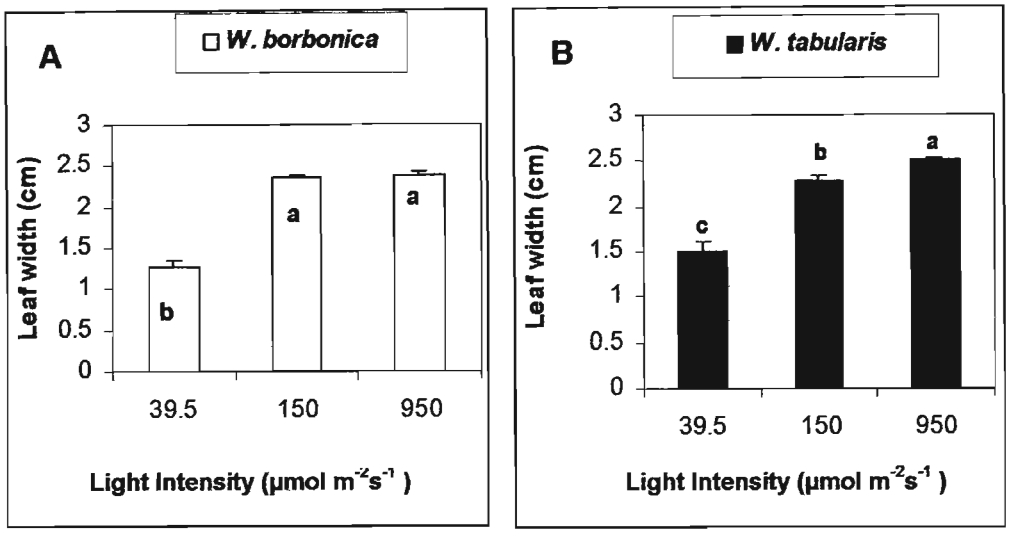

Figure 3.3.4 (A-B). A comparison of the leaf width in *Watsonia* species as a consequence of exposure to varying light intensity regimes after the third leaf stage. Each treatment consisted of 10 plants. Each species was analysed separately, by ANOVA. Means with letters in common are not significantly different at 5 % (*P<0.05*), separated by Fisher's test. The vertical bars represent standard error.

In the daylength response, there were additive effects of light intensity and/or temperature. The morphological appearance of flowers produced under SD and low light were similar. Under SD regimes, flowering was reduced and the flowers were bent sideways (Figure 3.3.1 A versus B and Figure 3.3.2 A versus C). Under low light intensities the flowering rate was reduced but the flowers were normal (Figure 3.3.3 B and C). The results were comparable with those of OYAERT *et al.* (2003) who investigated the flowering of *Spathiphyllum.* Flower initiation in *Spathiphyllum*, was hastened by SD but the effect of photoperiod was influenced by light intensity (OYAERT *et al.*, 2003). For instance, reducing light intensity (55 µmol $m^{-2}s^{-1}$) under LD or SD hastened or delayed flower development, respectively (in comparison with 110 µmol $m^{-2}s^{-1}$). This was thought to be mediated through the effect of light sum (= photoperiod x light intensity). Similar interrelated interactions of light intensity were

reported by SHILLO and HALEVY (1976b) for *Gladiolus*. BROSCHAT *et al.* (1984) reported that for *Heliconia psittacorum,* the main factors influencing flowering are irradiance and temperature even though increasing daylength resulted in increasing vegetative growth.

In summary, flowering was promoted by LD compared to SD regimes. However, there was a strong interaction between temperature and daylength. LD was inductive for flowering in *Watsonia tabularis* at 21/14 °C. However, in other species studied, photoperiod had a minor role if the temperature was not controlled. The results also indicated that there exists a potential to use LDs to hasten flowering when plants are grown at warmer temperatures after inflorescence initiation. The *Watsonia* plants studied were sensitive to both daylength and light intensity at younger (between 1-3 leaf stage) developmental stages. However, light intensity is more important than daylength, since it appears to play a role in both the vegetative and flowering stages. Although light intensity plays a central role in flower initiation, increasing the daylength can be beneficial for increasing the photosynthetic input even though it is not sufficient for flowering. In addition, low light intensity cannot be substituted for by low temperature, whereas, SD can be substituted for by low temperatures. Thus, in order to induce flowering and enhance plant quality, light intensity must be maintained as high as possible.

Chapter 4:

FLOWER INITIATION AND THE RELATIONSHIP BETWEEN CORM SIZE AND FLOWERING COMPETENCY

4.1 INTRODUCTION

The motivation behind the investigation of the relationship between corm size and competency to flower was prompted by several literature findings, where it has been reported that the size of a propagule determines its ability to flower (HALEVY, 1990; REES, 1992; DE HERTOGH and LE NARD, 1993). Failure to flower, lowered flowering percentage and a lack of uniformity and periodicity in flowering in many geophytes have been attributed to many factors, including propagule size (HAN, 2001; ROH, 2005). The minimum propagule size and weight required for flowering varies between genera and between species within a genus (DE HERTOGH and LE NARD, 1993; HAN, 2001). Although there has been speculation that the size of the apical growing point, more than propagule size, affect flowering (KOHL, 1962; DOSS and CHRISTIAN, 1979; HAN *et al.*, 1991), this was not investigated here.

Information on minimum corm size is therefore, critical prior to the introduction of a flowering species into commmercial production. In most cases propagules are sold according to weight. Consequently, large lily bulbs sell for a higher price because they have the potential to produce a plant with more flowers than those grown from small propagules (WANG and BREEN, 1984).

Environmental requirements for floral and corm development of *Watsonia* species are still not understood and need to be established in order to produce good quality pot plants. A basic understanding of the ontogeny of the inflorescence and the periodic development of the corm under various environmental factors (manipulated temperature and light regimes) is therefore necessary to understand and optimise flower production.

Flowering is a complex process, under both endogenous and environmental control. It is known that light and temperature play a crucial role in flower initiation and development (BERNIER *et al.*, 1988; HALEVY, 1990). However, flower initiation and subsequent flower development can respond differently to the same environmental condition (ADAMS *et al.*, 1998). Thus, it was crucial to establish environmental conditions that induce/inhibit flower commencement in *Watsonia* species. Anatomical observation was necessary to visualize the flowering transition in the shoot apex and inflorescence differentiation and maturation. In the early stages, the inflorescence is hidden amongst the leaves. Anatomical observation could therefore provide information on how flowering is progressing relative to the development of the whole plant under certain environmental conditions.

4.1.2 OBJECTIVES

-To characterise flower initiation in *Watsonia* species; and

-To determine the critical corm size required for flowering in selected *Watsonia* species.

4.2 MATERIALS AND METHODS

4.2.1 ANATOMICAL AND MORPHOLOGICAL STUDIES

Corms of *W. tabularis* were used for anatomical studies due to availability. However, corms of *W. borbonica* and *W. pillansii* were investigated during the course of the experiment to establish if the flowering process is similar between species. Corms of between 10-20 g were selected for the dissection. After shoot initiation, corms were dissected onto main shoot (± 1 mm thick), made at the planes parallel to the axis of the corms. This was conducted by means of serial hand sections. Plant sections were then observed under a dissecting microscope (without any chemical staining). Descriptions of inflorescence and apex anatomy were made with reference to the corresponding developmental stage of the plant. Anatomical observations were conducted to identify the flower initiation stages. Furthermore, stages in inflorescence development were examined and correlated with the vegetative development of *Watsonia* plants until the formation of the fourth leaf. In this study, individual verticals were not identified because no detailed study was made concerning flowering.

4.2.2 DETERMINING THE CRITICAL CORM SIZE REQUIRED FOR FLOWERING

Commmecially-sourced corms of *W. borbonica, W. tabularis* and *W. pillansii* were individually weighed. They were then planted in April 2004 as described in Chapter two, Section 2.2.4. The experiment was repeated in February 2005, when corms of *Watsonia angusta* were included with the above three species. After nine months, the corms were harvested and data on the flowering ability of individual corms was recorded. Data were pooled for all measured characteristics and where there was a significant year x treatment interaction, the comparisons were analysed separately for the 2004 and 2005 flowering seasons.

4.3 RESULTS AND DISCUSSION

4.3.1 MORPHOLOGY AND DEVELOPMENT OF THE SHOOT APEX

After the release of dormancy, observations made under the disserting microscope revealed no changes in the shoot apical mersitem (SAM) until the leaves were about 5 cm in length (after the first week) (Figure 4.3.1 A). During this initial stage (1-6 weeks), plants were characterised by vigourous vegetative growth. Flower initiation in the SAM was first observed at the second leaf stage, when both the leaves had exceeded 10 cm in length. Beyond this developmental stage, the SAM assumed a dome shape, indicating a transition from a vegetative to floral state (Figure 4.3.1 B). Bud initiation in *Iris* was similar to bud initiation in *Watsonia*, indicated by a depression in the SAM (Figure 4.3.1 C) (UHRING, 1973). Subsequently, the first lobes of the staminal primordia appeared on the upper sides of the dome (Figure 4.3.1 C). As time progressed, the presence of other structures became visible. Inflorescence differentiation could be seen starting from the second to third leaf stages (Figure 4.3.2 A-B).

At the second to third leaf stage, daughter corms were observed (Figure 4.3.2 C). In flowering *Watsonia* plants, the first two daughter corms were always lateral and positioned above the mother corm, irrespective of species (Figure 4.3.2 D). Consequently, the inflorescence extends from the shoot apex between the two daughter corms (Figure 4.3.2 E). In general, in non-flowering corms, one daughter corm was initiated first, this was later followed by multiple secondary cormels. After the formation of the 4th leaf, the leaf sheath took on a cylindrical appearance in order to accomodate the elongation of the internal spike (Figure 4.3.2F).

The flower formation process in *Watsonia* species resembled that found in *Gladiolus* described by SHILLO and HALEVY (1976a). The only difference being that *Gladiolus* is typically more foliose than *Watsonia* plants.

Flower initiation occurred simultaneous with daughter corm formation. In all three investigated species (viz. *W. angusta, borbonica* and *tabularis*) flowering was closely associated with corm formation (Figure 4.3.2 E).

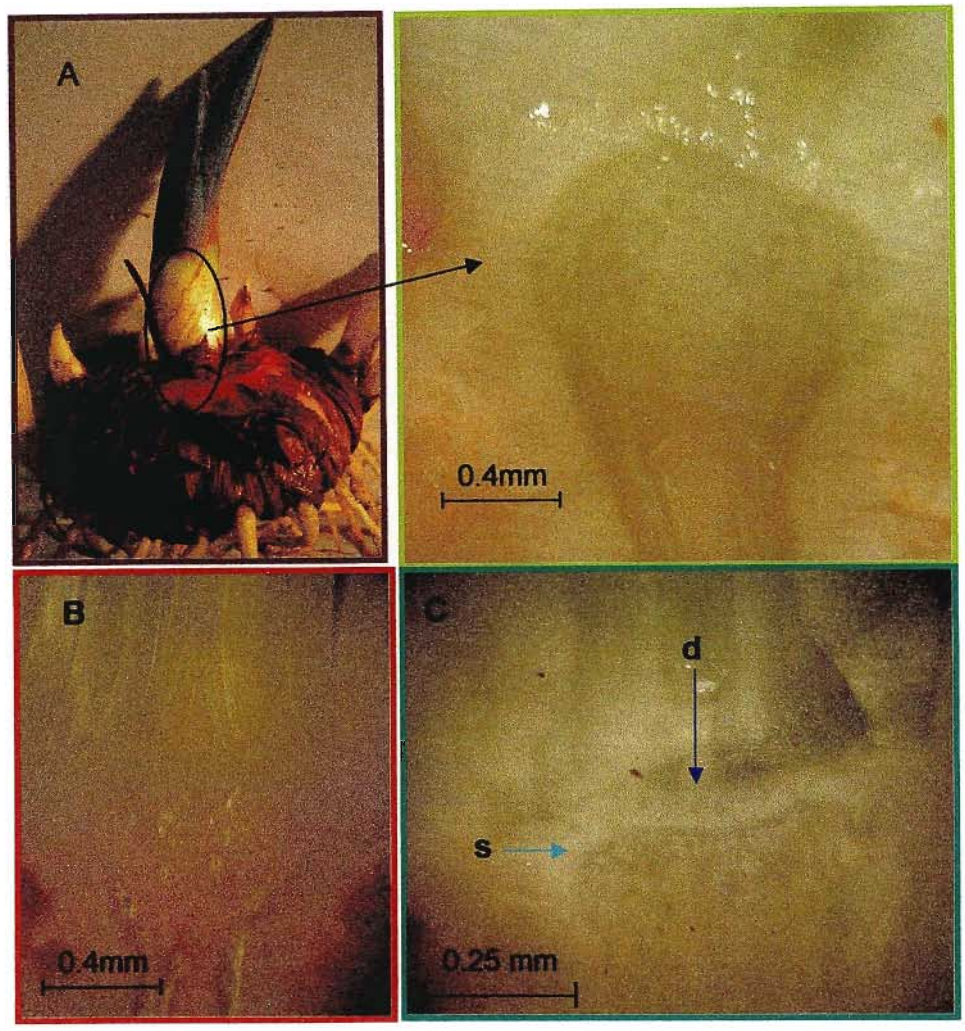

Figure 4.3.1 (A-C). Floral development in the shoot apex meristem (SAM) of *Watsonia.*
A: After the release of dormancy, leaves were initiated but no anatomical change was observed in the SAM, which remained flat. At this stage, the leaves were less that 10 cm in length.
B: Transitional stage of *Watsonia* bud growth. The apex was broadened and became convex at this stage.
C: A longitudinal section of *W. tabularis.* A depression at apex (d) is an early indication of flower bud initiation. Lobes (s) become staminal primordial.

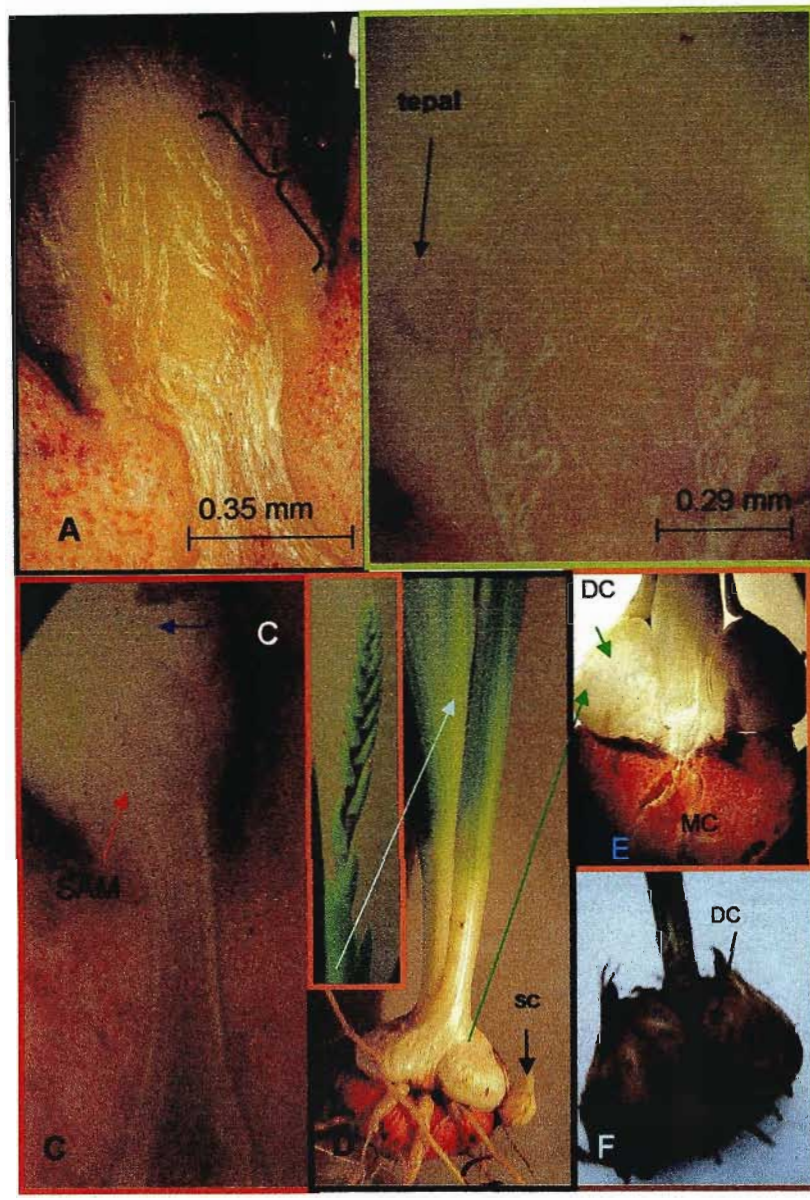

Figure 4.3.2 (A-F). Longitudinal sections of *W. borbonica* and *W. tabularis* corms, showing changes in the shoot apical meristem during the flower initiation process.
A: A domed-shape shoot apex (transition to flowering) after the formation of the second leaf. Inflorescence initiation can be seen (i).
B: After the formation of the second leaf, the inflorescence was clearly defined (i).
C: After second and third leaf formation, daughter corms (C) positioned above the mother corm could be clearly seen.
D: Inflorescence elongation and two daughter corms(C), enclosed with the leaf sheath. Note the formation of the secondary cormels on the right side (SC). Note spike appearance after external sheath extension.
E: A longitudinal section showing the inflorescence base and two daughter corms positioned laterally.
F: *W. borbonica* after anthesis. Note the enlarged dormant daughter corms (DC) still attached to the mother corm, which has been completely utilized and reduced to a hard woody disc.

Similar observations were reported in *Freesia,* where flower and daughter corm initiation occurred simultaneously (GILBERTSON-FERRISS *et al.*, 1981b; BERGHOEF *et al.*, 1986b). However, in *W. pillansii*, very few daughter corms were observed even in plants which had flowered. This could be due to the fact that *W. pillansii* is a summer-rainfall species. According to GOLDBLATT (1989), it grows in autumn (September-October) and it flowers later, at the end of summer (February-March) in contrast to other winter-rainfall species, which flower at the end of spring. This was reported as a difficult species to grow (GOLDBLATT, 1989). Spike elongation occurs after daughter corm formation, becoming externally visible only after extension of the fifth leaf (Figure 4.3.2 C-D). At this stage, individual florets were already developed but they remained closed for an additional three more weeks until the entire inflorescence had extended (Figure 4.3.2 D-E).

At the end of the flowering process, which takes approximately 9 months, the mother corm was completely utilised (Figure 4.3.2 F and 4.3.3). Only a hard woody disc remained. This can resist decay for many years (LE MAITRE and BROWN, 1992). The two daughter corms, located at the base of the spike accumulated weight (became filled) throughout the flowering process (Figure 4.3.3). These data suggested that the mother corm serves as a source of reserves for daughter corms, secondary cormels and flower development, which were the main sinks.

It is possibe that the assimilates are utilised on a competitive basis between the flower and developing cormels, since these processes occurred concurrently. Similar observations were reported by GILBERTSON-FERRISS *et al.* (1981b) for *Freesia.* The authors also suggested that temperatures (9-20 °C) inducive for flower initiation were also favourable for corm filling in *Freesia hybrida*, suggesting a competitive process. Watsonias are close relatives of Freesias, and the two genera likely display similarities in their flowering patterns.

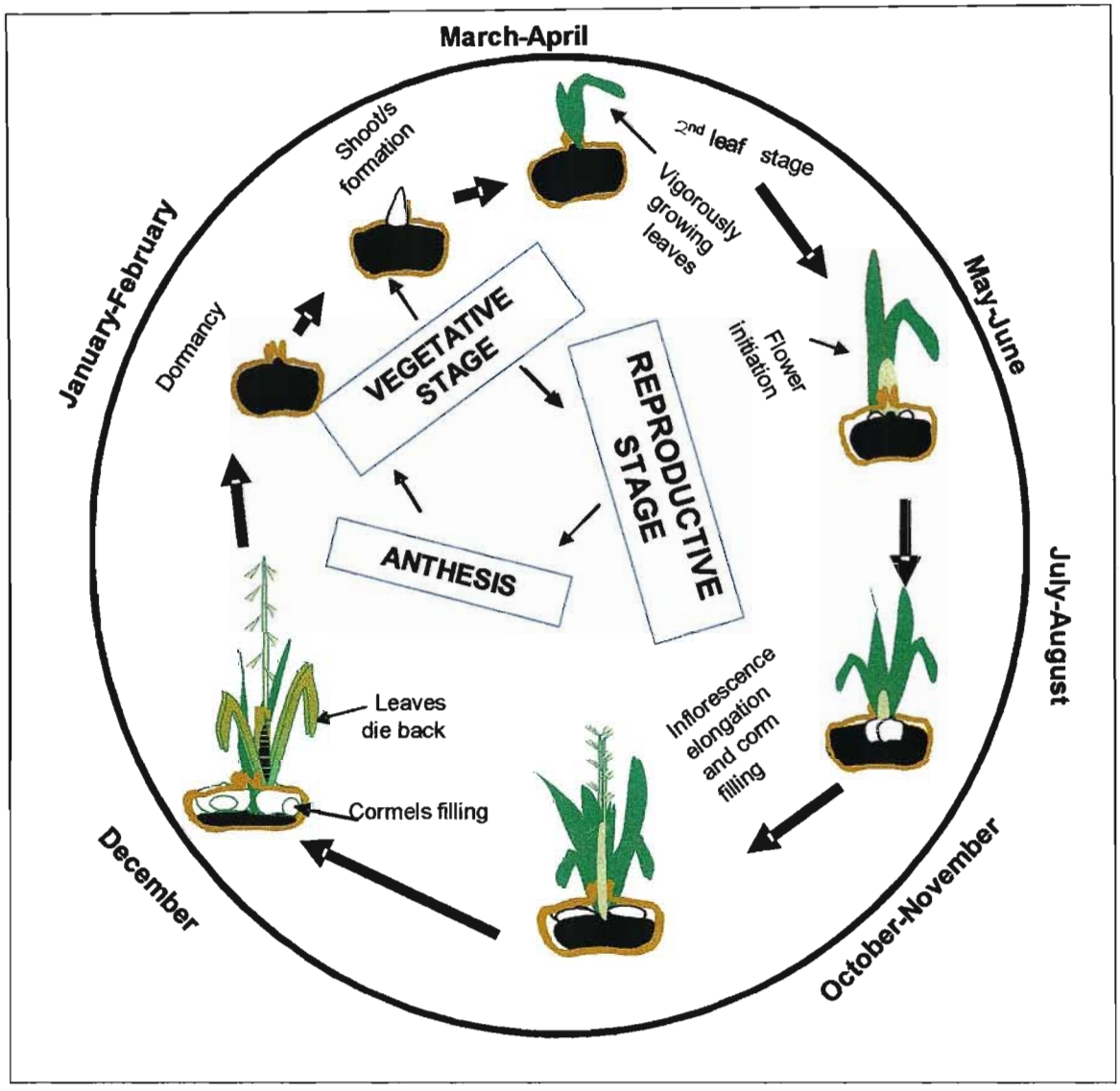

Figure 4.3.3. A schematic diagram depicting the flowering sequence of *Watsonia* corms. The diagram is based on data for *W. tabularis*. Similar events were observed in *W. borbonica* and *W. angusta*. The observations were conducted for 9 months, the average time taken to complete the flowering process. Corms were planted in the 2004 and 2005 flowering seasons. Initially, corms were planted between March and April 2004 and harvested in December 2004. The second batch was planted in February–December 2005.

At the end of anthesis, the daughter corms were dormant, as they did not show any further development (Figure 4.3.2F). In all three species, cormel initiation was related to flower formation. In general, a plant produced two daughter corms together and the rest of the cormels were initiated at later stages of growth, or not all (Figure

4.3.3). Corm and flower development was similar for *W. angusta, W. borbonica* and *W. tabularis,* except for the size of the corm and the number of leaves, which were species-specific.

4.3.2 THE RELATIONSHIP BETWEEN CORM SIZE AND FLOWERING

Larger *Watsonia* corms produced more robust and taller plants. Typically, small corms produced leaves but the plants did not flower even under inductive conditions (Figure 4.3.4 A-D). Furthermore, the number of leaves produced per plant in all three species, significantly increased with corm size ($P<0.01$) (Figure 4.3.4 A-C). The results are in agreement with those obtained for *Lilium longiflorum*, where the number of leaves increased with an increase in bulb size (DE HERTOGH *et al.*, 1969).

The critical corm size capable of flowering varied among *Watsonia* species (Figure 4.3.4). In *W. tabularis* and *W. borbonica,* the minimum corm size required for flowering was in excess 9 or 10 g, respectively. In *W. pillansii*, corms weighing over 5 g flowered. Corms of *W. angusta* were capable of flowering even at weights of 1-3 g (Figure 4.3.4 A-D). When the number of flowers produced per plant versus corm size was compared, no significant ($P>0.05$) difference was found (Figure 4.3.5 A-D). Results on temperature (Chapter 2), indicated that flowering was significantly influenced by temperature ($P<0.001$). All considered, the results clearly demonstrated that although bigger corms had a potential of producing bigger plants with a higher number of leaves and flowers, their flowering ability was hampered if the temperature was not controlled. This indicates that bulb or corm size alone does not determine the flowering potential of a geophyte as observed in *Iris hollandica* and saffron corms where the critical bulb size varies yearly depending on the field conditions in which the bulbs were produced (HAN, 2001). However, the results were in disagreement with the finding of HAN (2001) in three species of the *Brodiaea* complex, where it was shown that regardless of species, the flowering percentage of all plants increased as the size of the mother corm increased, despite the minimum corm size for 100 % flowering varying with species and the source of the corms.

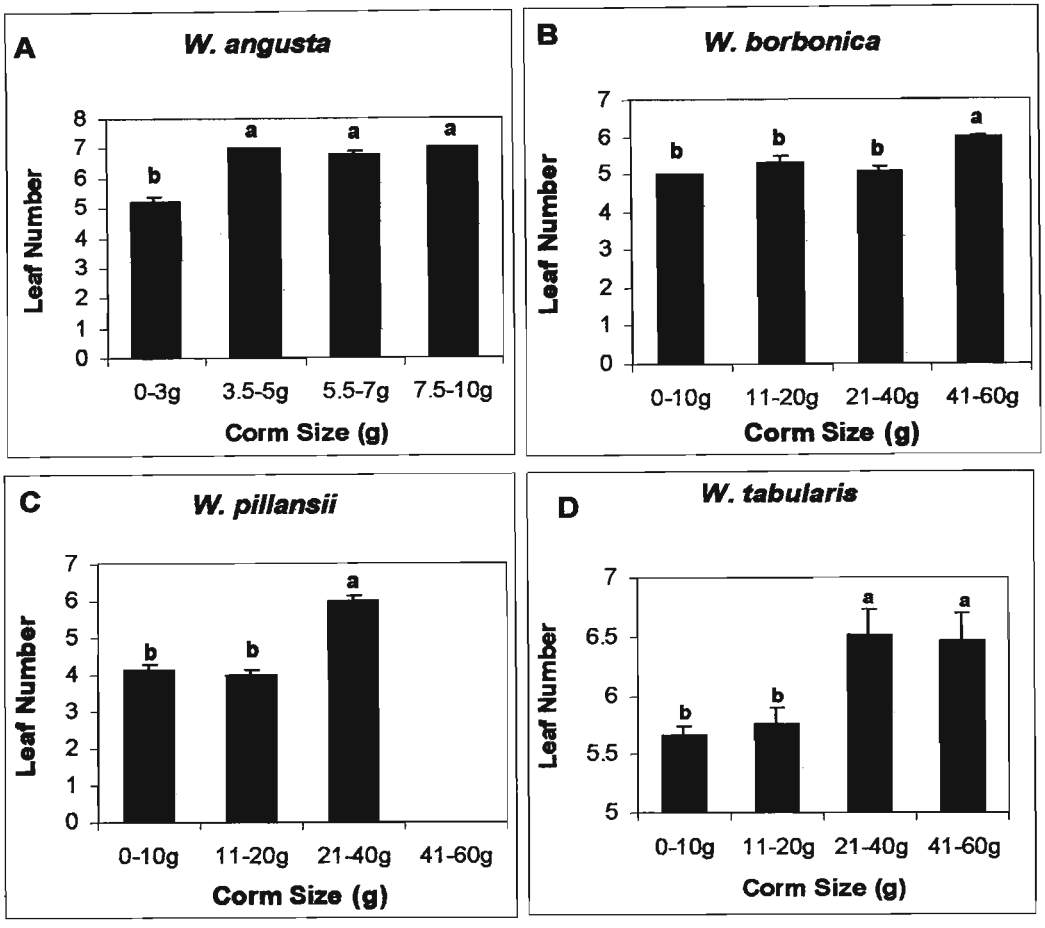

Figure 4.3.4 (A-D). A comparison of the number of leaves produced by different size corms in four *Watsonia* species. Similar letters indicate that there was no significant difference between the treatments. The means were separated by Fisher's test, where *(P<0.05)*. **A**: *W. angusta* (n=15). **B**: *W. borbonica* (n=64). **C**: *W. pillansii* (n=61). **D**: *W. tabularis* (n=60). The bars indicate standard error.

This study demonstrated that temperature is a major requirement for flowering in *Watsonia* plants. Studies by HAN (2001) on the *Brodiaea* complex indicated that environmental conditions during the time corms are produced play a significant role in their flowering potential. The author reported that corms of different species of *Triteleia* sourced from different environmental conditions responded differently in terms of their flowering ability. Consequently, *Triteleia ixiodes* corms from a natural habitat weighing 0.3 g flowered, whereas for those from a cultivated environment, a minimum flowering corm size of 3.6 g was required. Data for *T. ixiodes* thus illustrate that environmental conditions during which the corms are produced may play a significant role in the eventual flowering potential of the species.

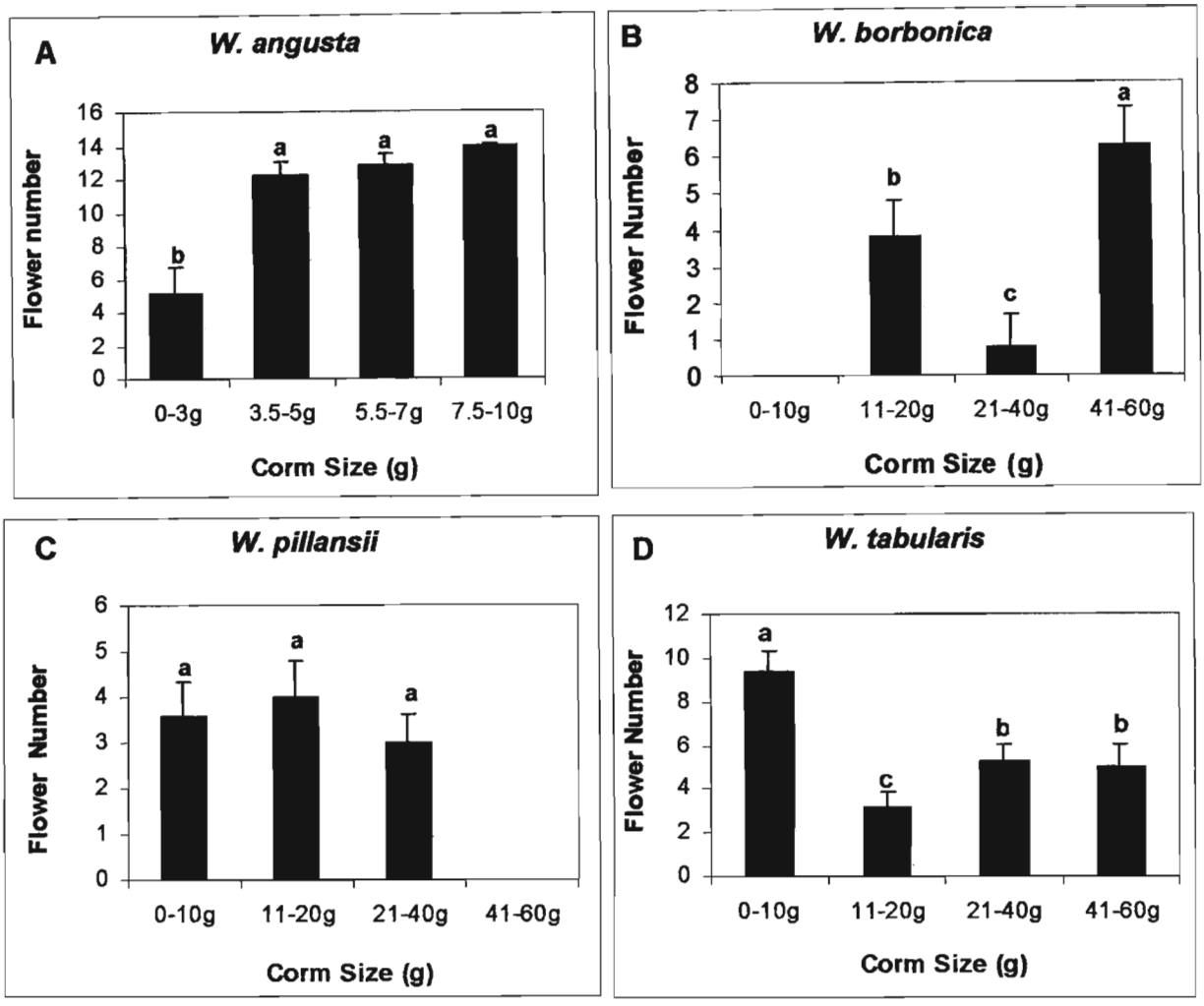

Figure 4.3.5 (A-D). A comparison of flower number produced by different size corms of four *Watsonia* species. Similar letters indicate that there was no significant difference between the treatments. The means were separated by Fisher's test, where *P<0.05.* **A**: *W. angusta* (n=15). **B**: *W. borbonica* (n=64). **C**: *W. pillansii* (n=61). **D**: *W. tabularis* (n=60). The bars indicate standard error.

In all species studied, there exists a critical corm size above which plants typically produced a maximum number of flowers (Figures 4.3.5 A-D). For instance, *W. angusta* corms between 3.5-7 g produced the same number of flowers per plant as those between 5.5-7 g (Figure 4.3.5 A). A similar trend was observed for *W. pillansii* and *W. tabularis* (Figure 4.3.5 C and D). The results are in agreement with those of

HAN *et al.* (1991), where flowering percentage was not affected by a 10-fold increase in corm size above a critical weight.

The variation in terms of critical corm size required for flowering *Watsonia* species, clearly indicated that corm maturity or potential to flower was not related to corm size in Watsonias. The suggestion that flowering potential depends on the size of the apical meristem rather than overall corm size could be feasible, as observed in lilies, Iris and *Brodiaea* (KOHL, 1967; DOSS and CHRISTIAN, 1979; HAN *et al.*, 1991). Even though in this study the apex size was not measured, it is possible that the apices inside the small corms were similar in size to those inside large corms, as observed in *T. laxa* (HAN *et al.*, 1991). However, this suggestion remains to be verified in *Watsonia*. More recent studies by CLARK and BURGE (2002) demonstrated that flowering performance in *Sandersonia* tubers is influenced by tuber weight rather than by the size of the apical meristem.

In summary, propagule size alone may not be a reliable indicator of the flowering potential of *Watsonia* corms. Flowering potential of each species varies significantly and thus, it is necessary to investigate each species independently. Furthermore, the environment in which the propagules are produced should be considered when determining the flowering potential of each species.

Chapter 5:

5. GENERAL DISCUSSION AND CONCLUSIONS

THE INTEGRATED EFFECT OF TEMPERATURE, DAYLENGTH AND LIGHT ON FLOWERING OF WATSONIAS

Investigation of the cues that induce flowering at the physiological level in *Watsonia* species are valuable as these plants can make effective horticultural subjects. Attempts were made to establish: (i) the critical corm size capable of flowering; (ii) to clarify the influence of temperature on growth and flowering; (iii) and lastly, to investigate the influence of light on flowering.

Flowering ability in selected *Watsonia* species was not influenced by corm size. The critical corm size required for flowering varied significantly from species to species. Newly formed corms lack the capacity to flower. Thus, corm maturity is not dependent on corm size (WAITHAKA, 1986) but may be influenced by apex size (KOHL, 1967; DOSS and CHRISTIAN, 1979; HALEVY, 1990). The ability to flower is highly influenced by environmental conditions, especially temperature (HAN, 2001; LEE *et al.*, 2003). Consequently flowering each year is different since environmental conditions surrounding corm production is not tightly controlled.

Temperature plays a pivotal role in the growth and flowering cycle of *Watsonia* species. It was observed that in the studied *Watsonia* species, dormancy of the corm was induced at higher temperatures, usually after flowering. Furthermore, in summer-rainfall species, low temperatures of either 4°C or 10 °C could break this dormancy. This suggests that *Watsonia* corms (winter-rainfall) are dormant in summer as observed in many geophytes found in Mediterranean regions, e.g. *Gladiolus* and *Freesia* (HALEVY, 1990).

The results demonstrate that temperature is the main cue controlling flowering in Watsonias. Furthermore, results from reciprocal experiments showed that the 'overall

appearance' of winter-rainfall species could be enhanced by exposing the plants to 'warm-cold-warm' cycles, since they displayed a quantitative response to vernalisation. This strategy allows the plants to produce a maximum number of leaves, and then a subsequent low temperature treatment induces a uniform flowering response. However, plants need to be exposed to low temperatures for 9-12 weeks for the low temperature to be effective. Elevated temperatures (± 21-25 °C) increased the total number of flowers produced per plant. However, spike emergence was sensitive to higher temperatures, as flower blasting and wilting was observed above 26 °C. Thus, the temperature needs to be lowered (12-18 °C), just before the opening of the first flower, to promote overall plant and flower-keeping qualities.

In contrast to winter-rainfall species summer-rainfall species displayed a qualitative response to vernalisation, as no flowering was observed in non-vernalised plants. According to this study, they need a 'cold–warm-cold' temperature sequence, which is also in line with their natural flowering cycle (GOLDBLATT, 1989). These plants appear most sensitive to cold temperature (7-12 °C) at the first to second leaf stage. Exposure to low temperatures at this stage ensured flowering even though the plant had a minimum of five leaves (Figure 2.3.5). Once vernalisation treatment was satisfied, the effect of a SD or LD regime was masked. In many species, flowering response to photoperiod is modulated by low temperature (CHOUARD, 1960; LANG 1965), which interferes with the circadian rhythm, thus making the critical photoperiod required by the plant shorter (HEIDE, 1977). Studies in *Arabidopsis* revealed that the response to vernalisation eliminates or diminishes both light quality and daylength responsiveness (BAGNALL, 1993), which may change the sensitivity of the apex to stimuli or inhibitors (LANG, 1965; BAGNALL, 1993). Furthermore, studies conducted in pear showed that the vernalisation–photoperiod interaction is similar to the vernalisation–light quality interaction (MURFET, 1985).

Anatomical and morphological observation revealed that flower initiation commences at formation of the second leaf. During the transitional stage, *Watsonia* plants need to maintain high photosynthetic levels due to high meristemactic activity and demands

that occurs at the SAM. It is obvious that considerable resources are diverted to flower and cormel production, thus, nutritionally stressful events must be avoided. Studies on light intensity clearly showed that reduced light intensity hampered plant development and flower initiation. Leaves grew etiolated and the stem became weak and unable to support the leaves. This resulted in lodging and ultimately plant death.

The injurious effect of low light intensity was quantitative and accumulative in *Watsonia*, as was observed in *Gladiolus* and *Chrysanthemum* (SHILLO and HALEVY, 1976b; KARLSSON *et al.,* 1989; IMANISHI and IMAE, 1990). It was further demonstrated that the sensitivity of plants to environmental factors changed according to the different developmental stages. *Watsonia* species in this study appeared most sensitive to temperature and light at the first up to the third leaf stage.

Parallel studies conducted on daylength revealed that flowering was promoted by LD as no flowering was observed under the SD. The lack of flowering in SD could be due to the fact that SD regimes slowed down growth compared to LD regimes. Flowering observed under DN and SD regimes at the third leaf stage, suggested that daylength plays an important role in morphogenesis but not in flowering. However, daylength regimes interacted with temperature and light for an overall effect in flowering of *Watsonia* plants. It may be possible that under SD and low light intensity regimes, the photosynthetic capacity (photoperiod x light intensity) was reduced (SHILLO and HALEVY, 1976b and c; OYAERT *et al.*, 2003; MATTSON and ERWIN, 2005), thus compromising photosynthesis and phytochrome action, which both play important roles in flowering (MOE and HEINS, 1990; BAGNALL, 1993; THOMAS, 1993).

With respect to the theory of florigen, floral induction requires the transport of signal molecules between different parts of the plant, in particular from leaves to the shoot apex (CHAILAKHYAN, 1936; BERNIER *et al.*, 1993). Flowering is thus a dependent event and interacts with other changeable environmental factors and the endogenous circadian clock (to sense temperature and daylength) (COUPLAND, 1997; CREMER *et al.*, 1998; DEVLIN and KAY, 2000). Photoperiod affects the partitioning of

photosynthates between reproductive and vegetative tissues/organs (DEVLIN and KAY, 2000), whilst the photoperiod x temperature interaction controls the direction of photosynthate flow and the tendency to flower (DEVLIN and KAY, 2000).

Observations at the SAM provided evidence that flower and corm initiation occur simultaneously. Whether differentiation in the two processes is enhanced by the same temperature is still questionable. In the reciprocal studies, MTR induced flowers and cormels, but continuous elevated temperature exposure resulted in flower abortion. However, it appears that cormel growth was enhanced under this stressful condition. Corms with aborted flower spikes were observed to have more cormels, which suggests that when the florets were aborted the excess assimilates were channelled to daughter corm initiation. During bulb production in lily, flowering could reduce bulb size through competition for available assimilates (WANG and BREEN, 1984). In *Freesia*, it was observed that higher temperatures inhibited floral initiation because under such conditions the corm becomes the more competitive sink (GILBERTSON-FERRIS *et al.*, 1981). Overall, these observations are in agreement with the nutrient diversion hypothesis suggested by SACHS and HACKETT (1983).

Flower and daughter corm initiation in selected *Watsonia* species closely resembled those in *Gladiolus* and *Freesia* (SHILLO and HALEVY, 1976a; BERGHOEF *et al.*, 1986b). However, flowering in *Watsonia* was not under autonomous control like *Gladiolus*, where flowering always followed the development of a definite number of leaves (HARTESEMA, 1937; SHILLO and HALEVY, 1976a). In this regard *Watsonia* rather resembles *Freesia* in which flowering is highly-controlled by temperature (GILBERTSON-FERRIS *et al.*, 1981). Low temperature exposure in *Watsonia* species reduced total leaf number but also reduced time to flowering. Warm temperatures led to progressively higher final leaf number, but delayed flower initiation.

The appreciable variation in time to flower in selected *Watsonia* species was not surprising considering that they originate from different climates. There was a

significant difference between days to flower in winter- and summer-rainfall species. *W. angusta, W. borbonica* and *W. tabularis* took 189, 171 and 180 days to flower, respectively. *Watsonia pillansii,* a summer-rainfall species, took 270 days to flower.

Although this study addressed light and temperature separately, overall flowering was influenced by the interaction of these two environmental factors. However, it must be stressed that to produce a horticulturally desirable plant these factors need to be understood and controlled. To optimize flowering and flower quality, a plant may need to be exposed to different temperatures and/or certain light regimes at different developmental stages. Daily and seasonal fluctuations in temperature and light (irradiance) are not the only variations that occur in a growing environment. Obviously, in an experiment involving biological material, it is difficult to remove all variables that may result in differences. These differences could have attributed to varying sprouting times and times of flowering in *Watsonia* corms (pre-histories).

CONCLUSIONS:

This study has shown that *Watsonia* species have the potential to be commercialised as a cut or pot flower. For the purpose of this study the main objectives were accomplished and a few recommendations can be made to improve the quality and quantity of commercial *Watsonia* species:

- *Temperature is the main cue inducing flowering in Watsonia. Temperature also controls other aspects of development such as corm dormancy. Dormancy can be released effectively by low temperatures of either 4 or 10 °C, which ensures synchronized growth.*
- *Summer-rainfall species follow an obligate vernalisation response, whereas winter-rainfall species displayed a facultative-response.*
- *Elevated temperatures and long days promoted high total leaf and flower numbers. In terms of a light requirement, daylength plays a minor role in the flowering of Watsonias but light intensity is indispensable, as low light intensity inhibited flowering. Furthermore, photoperiod can be replaced by low temperatures whereas low light can not.*

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