

FACTORS AFFECTING FLOWER INITIATION AND DEVELOPMENT
IN DUTCH IRIS

by

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ABSTRACT

Flower initiation and development to anthesis in Dutch iris were investigated using three cultivars, 'Wedgwood', 'Ideal' and 'Professor Blaauw'. In particular, environmental effects on flower initiation and on flower abortion were studied.

With the aid of a thermal-time model, the rate of initiation (the reciprocal of time to initiation) was shown to be linearly related to the temperature used to store dry bulbs, provided that this was constant. When bulbs were transferred from one temperature to another, however, the rates of initiation differed from those expected. It was concluded that the sequence of temperatures was important and a possible mechanism for flower induction was discussed. Following the start of flower initiation per se, a different optimum temperature for flower-organ differentiation was observed.

The effect of both pre-planting and post-planting temperatures on the growth and development of the flower were investigated. The proportion of bulbs with successful flower development to anthesis increased with mean flower and stem dry weight. The heaviest flower and stems were produced by those bulbs with the greatest leaf weight. This was attributed to the increased availability of current photosynthates in plants with the largest foliage frames.

Flower development was affected by an interaction between time of planting, bulb size and glasshouse temperature for a given light integral. With later bulb plantings, between January and March, higher light integrals were required for the same flower development at moderately high temperatures (16-18°C). This higher light requirement was detected with the largest bulbs first and not until the last planting date with smaller bulbs. At higher temperatures (20°C) flower development was poor regardless of the light integral. At lower temperatures (14°C) the same flower development achieved regardless of the light integral within the range tested. Further investigation in controlled

environments enabled the detection of a photoperiodic effect at the moderately high temperatures. Daughter-bulb growth was promoted by long photoperiods and high temperatures increasing sink strength there for assimilates with a corresponding decrease in flower development.

The partitioning of assimilates under high and low light was examined by determining the distribution of ¹⁴C-labelled assimilates during growth in the glasshouse. Daughter bulbs under low light had a higher relative specific activity than those under high light, at the time when the flower was most prone to abortion. Application of cytokinin to the flower bud resulted in a reduced daughter bulb weight, but heavier flower buds. It was concluded that flower development was affected by the total current assimilate available and the partitioning of these assimilates between daughter bulbs and flower bud.

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1. INTRODUCTION

1. INTRODUCTION

The Dutch iris is a complex hybrid derived from I. xiphium Linn., (Spanish iris) I. tingitana Boiss. and Reut, and possibly also I. latifolia Miller, (English iris) (Mathew, 1981). Dutch irises are bulbous; the bulb is a storage organ formed from modified swollen leaves on a compressed stem. In 1984 an estimated 41,500 bulbs were forced in the UK (Anon, 1985); i.e. grown out of season for the production of cut flowers. Cut flowers can be produced all-year-round and are frequently promoted because they are the only blue flower available throughout the year. The most popular cultivars grown for commercial production are 'Ideal', (a sport of the older 'Wedgwood'), and 'Professor Blaauw'. Their flowering and growth can be controlled by storage temperatures to produce flowers out of the natural season, which, in the UK, is April-June.

A study of flower initiation and development in the Dutch iris was considered necessary for the following reasons:-

- 1) Bulbs subjected to the usual commercial temperature treatments occasionally fail to initiate a flower. This occurs particularly with the smaller grades of bulbs which are often used in the UK. Since each bulb planted is a relatively costly item, no great loss of flowers can be tolerated by the growers (Walla and Kristoffersen, 1969).
- 2) The temperature treatments applied to the bulbs are based on the original work of Blaauw (see review by Hartsema, 1961). These recommendations have continually been refined and improved with additional temperature combinations. The physiological reasons for these improvements have been poorly understood, and there is little scientific evidence of improvement in flowering resulting from these additional temperature treatments.

- 3) A developmental disorder, 'blasting', in which the flower fails to develop to a harvestable stage, i.e. abortion, is common in iris bulbs forced during low irradiance conditions in winter. It is during this time, however, that there is a great demand for high quality iris flowers.

- 4) The physiological reasons for flower-bud blasting are again poorly understood. The influence of pre-planting storage temperatures and conditions during forcing on flower and whole-plant development requires investigation in order to establish the reasons for such treatments causing reduced or increased flower failure.

Definitions of the various stages of flower formation are frequently confused. In this thesis the following terms will be used:-

Induction: those events which are responsible for the evocation of the apex to initiate flowers.

Evocation: the events which occur at the apex between the arrival of a floral stimulus (or removal of an inhibitor) and the first macroscopic signs of flowering.

Initiation: First appearance of flower initials.

Here, the term flower development is concerned more with the growth of a flower to a harvestable stage from the complete embryo bud. A flower is considered to have reached a harvestable stage once it has developed to anthesis, following stem extension, bud growth and bud colouring.

When flower-buds blast they become papery, dry and brown, they fail to increase in length or weight (general atrophy), and stem extension stops. Plate 1.1 shows a bud developing normally on the right, while that on the left is at a slightly later stage of development but has aborted. The bud abortion is accompanied

by the drying and abortion of the two spathe leaves, (Plate 1.2), but rarely any of the other leaves. Neither abscission of the buds nor of the spathe leaves usually follows abortion.

In tulip and tomato, in which similar flower abortion can occur, it is generally the anthers which abort first (Rees, 1982; Newell, personal communication). With iris, however, it appears that the gynoecium fails first. Plate 1.3 shows a bud which has developed to a reasonable size but the gynoecium shows the first signs of loss of colour and withering. The anthers can be seen clearly to show no signs of drying at this stage. The second bud has completely blasted. In this respect blasting in iris is similar to that in Lilium (Durieux, Kamerbeek and van Meeteren, 1983) and Rosa (Lindstrom, 1956).

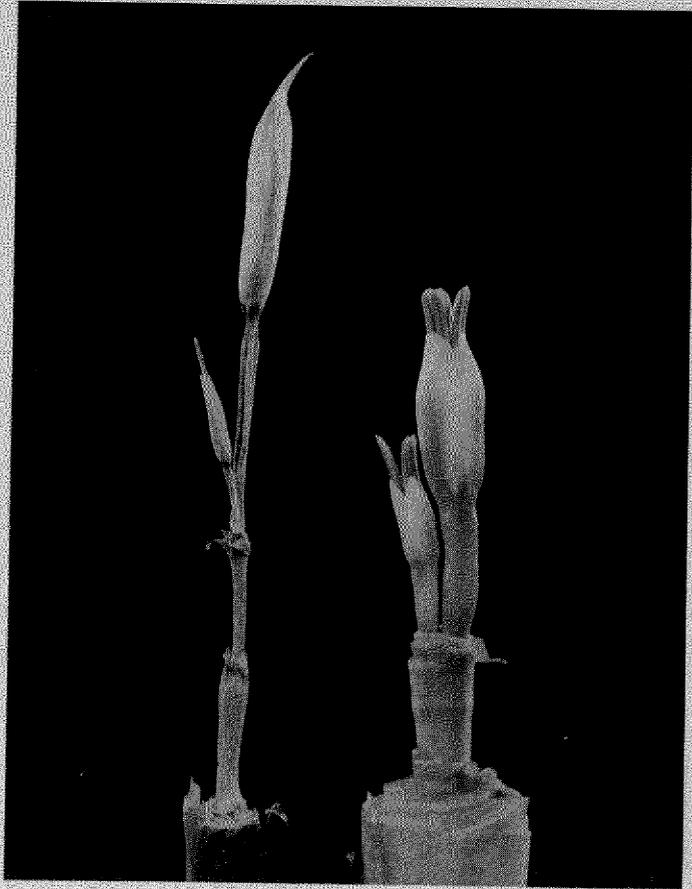


Plate 1.1 Leaves removed from two Iris plants to show the young buds. The buds of the plant on the right are developing normally, but those of the plant on the left have both aborted.



Plate 1.2 Folliar leaves removed apart from radical leaf, showing the dried and aborted spathe leaves enclosing the aborted flower-buds.



Plate 1.3 Early signs of abortion, the gynoecium has started to wither and dry but the anthers still appear normal.

2. LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Bulb structure and morphology

Dissection of a large iris bulb centripetally reveals the following morphological structures. First a number of brown fibrous tunics enclose the bulb, (Rees, 1972) which are the remains of the previous year's leaf bases. The number of tunics is dependent on the development of the mother-bulb from which the bulb is derived. If this parent bulb did not flower, then a 'round' bulb is produced with 2 to 4 tunics (Schipper, 1980). Round bulbs are particularly resistant to the handling required for forcing bulbs throughout the year. If the mother-bulb flowered, then the flower stem causes the innermost daughter-bulb to have a distinct flat side. These 'flat' bulbs have only a single tunic, are more easily damaged and show greater water loss during storage than round bulbs. Aoba (1967) described these tunics as protective leaves.

On removing the tunics, the storage leaves are revealed. These are modified leaves, swollen into large white scales which completely encircle the bulb, the edges just meeting. (Rees, 1972). There are normally four of these scales in large bulbs, the largest being the outermost one (Blaauw, 1935). The most central scale is usually referred to as a half scale, it is thinner and smaller than the others, only completely encircling the bulb at the base.

Within these storage leaves are the sheath, or sprout, leaves. There are usually two or three in number and they emerge above soil level with green tips (Rees, 1972). They are reasonably fleshy, suggesting a structure intermediate between that of the scales and the foliage leaves which they enclose. The foliage leaves vary in number from three up to about ten. In flowering bulbs the first of these leaves is radical or a basal leaf, while the others are attached to the flower stem. In non-flowering bulbs all the leaves are basal. Daughter bulbs are



Plate 2.1 Dutch iris bulbs stored dry, in plastic trays as used commercially for forcing temperature treatments

formed from the axillary meristems in the axils of the scales, sheath leaves and basal foliage leaves. In non-flowering bulbs the apex also forms a daughter bulb. The innermost daughter bulbs are smallest early in the season, but their growth rate is higher than that of the outer ones, so they soon become larger (Rees, 1972).

In flowering bulbs 6-8 foliage leaves are usually produced. The two primordia below the flower develop into spathe leaves, which are more papery but broader than foliar leaves. In the vegetative bulbs only three or sometimes four foliar leaves are formed. These grow longer and are narrower than the leaves of flowering plants.

The flower is actinomorphic (Rees, 1972) and its development from the vegetative apex has been well documented. In Hartsema's review (1961), the various stages of flower formation were numbered (Table 2.1). In more recent work these stages have been given the first letter of the various organs formed (Cremer, Beyer and De Munk, 1974).

Table 2.1 The stages of flower development from the vegetative apex as described by Hartsema (1961) and Cremer et al. (1974).

Stage of flower formation		Description of state of apex
I		Vegetative flat apex
II		Apex raised into a dome shape
III		Three primordia differentiate
	A	Stamens differentiate from the upper part of these primordia
IV	P1	First set of tepals (first perianth whorl) differentiate from the base of the three primordia
V	P2	Second perianth whorl forms, alternating with the first
VI	G	Three carpels develop opposite the stamens

The flower, therefore, consists of three stamens which are petaloid, two perianth whorls of three (the 'standards' and the 'falls') and 3 carpels. A second bud is usually initiated in the axial of the last spathe leaf; this, in turn, is enclosed in a further spathe leaf.

2.2 Development and physiology of commercially used temperature treatments for forcing Dutch iris

Commercial flower production, year round, involves temperature storage treatments which are applied to unplanted or 'dry' bulbs, (Plate 2.1). These treatments have been developed to give a rapid flower initiation and subsequent development for early markets or to retard growth and development to produce flowers later than the natural season. Following retarding treatments, initiation and growth must be rapid so that the glasshouse periods remain short.

2.2.1 The initial warm storage

Bulbs for flower production are lifted from the bulb fields in the autumn when they are vegetative with three or four foliage leaves. They are cleaned and graded before placing in an initial warm treatment or heat 'curing'. The temperature treatments given are based primarily on the work of Blaauw and co-workers, whose work has been reviewed by Hartsema (1961). This work established that an initial high temperature treatment of 1 week at 31°C, given before a treatment in which flower initiation could occur, delayed flowering but increased the number of bulbs in which a flower was initiated. Kamerbeek (1965) has shown that the initial high temperature treatment, with temperatures greater than 20 but less than 41°C, enables the initiation of further foliage leaves. At the time of lifting there are approximately three foliage leaves already present at the bulb apex. Kamerbeek observed that when bulbs were given a cold treatment, below 17°C, at this stage

in their development, further primordia developed into bulb scales of a new daughter bulb. This would produce the typical vegetative plant, the 'three leaf' or alternatively in Dutch 'drieblader' plant. These have three or occasionally four foliar leaves and then a daughter bulb at the apex, thus preventing the formation of a flower. The initial warm treatment has often been referred to as the pre-induction or pre-florigene phase (Blaauw, 1941a). It is not, itself, thought to be responsible for flower induction, but necessary to prevent the leaf primordia developing into daughter bulb scales. This permits the mother-bulb apex to continue to a state of development when it can respond to an inductive treatment.

The heat curing treatment also appears to be responsible for the breaking of dormancy, or earlier sprouting of the bulb, (Tsukamoto and Ando, 1973(a); Sano, 1973; Le Nard, 1980) once they are placed in lower temperatures. The earlier breaking of dormancy could be responsible for the shortened chronological time to flower harvesting reported by many workers when heat-cured bulbs are compared to unheated ones (Blaauw, 1941a; Griffiths, 1936; Hartsema and Luyten, 1955; Doss, 1981). Tsukamoto and Ando (1973b) extracted inhibitors from bulbs stored at 10°C or 20°C. They discovered two inhibitors, one in the acid fraction and another in the neutral fraction which were identified as abscisic acid (ABA) and a fatty acid, capric acid, respectively. Both these inhibitors decreased in activity in bulbs stored at 20°C, while there was no change in bulbs stored at 10°C. The decline in inhibitors at the high temperature was found to coincide with the elongation of the first leaf when bulbs were dissected. Le Nard (1980) suggested that heat curing was necessary for the mobilization of bulb reserves, high temperatures enabling the carbohydrates to be converted to easily transported sugars.

In addition to the accelerated rate to flower harvest, Le Nard (1980) and Doss (1981) have observed a decrease in leaf number at flower initiation with increased time at curing temperatures when followed by a cool treatment. This leads to a dilemma, in that this reduced leaf number suggests that heat curing may be responsible for flower induction, which contradicts

conclusions drawn by previous workers as stated above. It seems more likely that the reduced leaf number is due to a slower rate of leaf initiation at high temperatures. This is supported by observations of a gradual reduction in mean leaf number from 6.93 with no heat curing to 5.84 with 5 weeks at 32°C (Doss, 1981). If high temperatures cause induction it might be expected that the leaf number would fall sharply when the threshold for induction was achieved, with no further major reduction once this critical period has been passed.

An initial high temperature treatment is not only capable of accelerating subsequent flower development and increasing the proportion of bulbs with flowers initiated, it can also be used to retard bulbs for forcing later in the season. This was first attempted by Beijer (1952) who investigated both the possibility of high, (25.5°C) or low temperatures (-0.5°C) for retarding bulbs. High temperature was found to be most successful for retarding growth and increasing subsequent flowering performance, presumably because of the prevention of the formation of a daughter bulb at the apex. Similarly, the growth of leaves was strongly retarded by a constant 25.5°C storage. Despite the fact that high temperatures cause a reduction in the growth inhibitor activity, the reduced respiration rate may be a cause rather than effect of retarded growth and development (Halevy, 1962; Kamerbeek, 1962; Rodrigues Pereira, 1962).

When bulbs are retarded for long periods, the number of leaves formed before flower initiation increases (e.g. Hartsema and Luyten, 1962). Flowers cannot be initiated at the high temperature but leaf primordia are initiated slowly (Beijer, 1952; Kamerbeek, 1965), therefore these plants are at a later stage of development when placed in a lower temperature suitable for flower initiation.

Bulbs must be above a critical size in order to initiate a flower (Blaauw, 1934). Blaauw (1941,b) stressed, however, that the critical size in iris was unrelated to bulb age. In iris, as in tulip, the mother bulb exists only for one season, leaving daughter bulbs in the autumn. Therefore, all bulbs are approximately the same age whether large flowering bulbs or small

non-flowering bulbs. For 'Wedgwood' and sports the critical bulb size is 7-8cm in circumference (Kamerbeek, 1965). Heat curing encourages intermediate size bulbs (7-8, 8-9 cm in circumference) to flower.

Recently, it has been possible to replace most of the heat curing process with a smoke or ethylene treatment to the dry bulbs (Kamerbeek, Durieux and Schipper, 1980; Schipper, 1981; Imanishi and Fortanier, 1982; Schipper and Weijden, 1982). Ethylene gas has also been shown to accelerate flower initiation (Stuart, Asen and Could, 1966; Uhring, 1973), as well as bringing about flower initiation in bulbs that would otherwise form three leaf plants. (Stuart et al, 1966; Durieux and Kamerbeek, 1974; Imanishi and Fortanier 1982). The process involved in the response of the bulb to such a treatment is not understood. Ma^{ri}assen and van der Plas (1986) have identified an alternative respiration pathway which was found in bulbs capable of flowering when given a heat treatment but did not occur in bulbs which were non-flowering. Ethylene and smoke which contains ethylene were found to stimulate this alternative pathway quicker than heat treatment of bulbs.

Kamerbeek, et al (1980) described how ethylene or ethephon induce the apex to switch from vegetative to reproductive development. Kamerbeek and Verlind (1972), also observed a rise in respiration at the apex when ethylene was applied. This rise in respiration was also observed to occur naturally at the apex when turning from vegetative to reproductive growth (Kamerbeek, 1962; Rodrigues Pereira, 1962). Ethanol has also been shown to be capable of breaking dormancy in Gladiolus, Freesia and Lilium longiflorum (Hosoki, 1983).

The heat treatment which has been developed for commercial early forcing (i.e. for cut flowers from December to March) is that first proposed by Kamberbeek and Beijer (1964); the bulbs are first subjected to 35°C for 2 weeks and then 40°C for 3 days. It was generally found that the higher the temperatures the more successful the promotion of flowering. The two weeks at 35°C, however, were necessary to accustom the bulbs to high temperatures before bulbs were placed in 40°C. Temperatures higher than 40°C were considered lethal. Longer periods than 3 days should be avoided because of excessive water loss from the bulbs.

The recommended treatment for retarding bulbs (25.5°C) was based for many years on Beijer's results. Bulbs could be stored for at least a year at this temperature and then given treatments which would enable them to flower. Recently 30°C has been considered a better retarding temperature, the first-leaf length and number of leaf primordia were both further reduced more at 30 than at 25.5°C (Schipper, 1983).

For bulbs which are grown in warm areas of Japan or Israel, there is no necessity for an initial warm treatment for early forcing (Sano, 1974a,b; Halevy, Shoub and Rakati-Aayalon, 1964).

2.2.2 The cool treatment

Following the high temperature treatment, a cool treatment is given. The metabolic block of the high temperature is removed, enabling accelerated leaf initiation, flower induction and initiation (Hartsema, 1961). This was referred to as the 'florigene' phase by Blaauw (1941a) or the 'generative' phase by Kamerbeek (1965).

Flower induction and initiation can occur at any temperature below 25.5°C but at temperatures above 20°C the flower soon aborts (Rees, 1972). Kamerbeek (1965) recommended temperatures of 17°C or below. The optimum temperature for the shortest chronological time to initiation is 9-13°C (Hartsema and Luyten, 1955b). In terms of development of the plant, the optimum temperature is unknown. Since iris is a determinate plant the number of foliage leaves formed before flower initiation gives the time of flower initiation in terms of the stage of development of the plant. With decreasing temperature from 25°C to 2°C, Blaauw (1941), Hartsema and Luyten (1955b), and Sano (1974a) observed the flower to be initiated at a lower leaf number. For example at 17°C Blaauw found that an average of 6.9 further leaves from the start of the cold treatment were initiated, before a flower, but at 2°C on average of only 2.6 further leaves were initiated.

There are three possible hypotheses for flower induction in Dutch iris:

1) Induction is autonomous. This was defined by Evans (1969) as occurring in a plant which requires the passage of time more than a specific environmental condition (such as photoperiod or temperature) for flowering to be evoked. Examples of plants which exhibit autonomous induction are Rosa which produce flowers continuously if conditions are suitable for growth. However, Citrus and Malus sp. also induce autonomously but show distinct periodicity, flowering at specific seasons (Malevy, 1984). There is an inherent (or genetically fixed) pattern of behaviour at the apex, which will pursue its appointed course, independently of the differentiated portions of the plant.

2. Induction occurs in response to environmental stimuli. The behaviour of the apex ^{can be} is affected by influences arising from the mature parts of the plant, as in photoperiodic induction, although in iris this could be due to cold treatment influencing the mature plant parts, which in turn affect the apex. Vernalization is an inductive effect of low temperature, flower initiation usually then occurs at following higher temperatures. In iris, however, induction may occur at relatively warm temperatures which are also suitable for initiation. The biochemistry of vernalisation is largely unknown but Salisbury (1963) suggested it was a balance between an inhibitor and promotor of flowering. If the Q_{10} for the production of the inhibitor was greater than that of the promotor, then it is possible that at high temperatures the rate of production of the inhibitor will be greater than that of the promotor, while the reverse situation may exist at lower temperatures.

3. Induction is dependent upon the gradual accumulation of certain metabolites at the apex, which must reach a threshold concentration before flowering will occur. The nutrient diversion hypothesis (Sachs, 1977) is a version of this last hypothesis. This states that nutrients are mobilized to specific areas

(central zone) of the apex and that this is responsible for flower initiation. Induction is therefore the factor which is responsible for mobilization of nutrients to these specific areas and initiation can be influenced by factors which affect competing sinks for assimilates at appropriate times.

Any of these hypotheses individually could explain induction in iris, or it is possible that a combination of two or all three may be involved.

Halevy (1984) classifies iris as a plant with autonomous induction. Several workers, however, have reported an acceleration in iris flower initiation with low temperatures (Blaauw, 1941; Hartsema and Luyten 1955b; Sano 1974a,b and c), although low temperature was not essential for initiation (Sano, 1974c). Dutch iris could, therefore, be said to have a quantitative low temperature/vernalization response, where a low temperature promotes but is not essential for flowering.

Plants with a vernalization response can often be devernalized with a period of high temperature following the low temperature for induction. Sano (1974a and c) showed a high temperature treatment before the low temperature induction was complete delayed flower initiation, but the low temperature induction was relatively stable to following high temperatures..

The scales of the bulb have been shown to play a part in the floral induction. Sano (1974b) removed the scales prior to a cold period and a flower was not initiated. If removal followed the cold period then flower buds were formed. A carbohydrate requirement for thermoinduction has been reported in many cold requiring plants, e.g. winter cereals, radish, carrot and cabbage (see review, Lang, 1965). The removal of leaves, tubers or roots has reduced or abolished the effectiveness of thermoinduction to varying extents in different plants. Since iris bulbs usually induce and initiate flowers in the dark, before the emergence of the foliar leaves from the bulbs, the scales are the only major source of carbohydrate. Rodrigues Pereira (1962) concluded that the transition from the vegetative to reproductive state involved

a considerable increase in carbohydrates at the apex. This increase occurs during evocation of the apex. A similar increase was observed in cauliflower, with an increase in the amount of starch that was stained after an inductive cold treatment (Sadik and Ozbun, 1967). The accumulation of carbohydrates in the apex of iris was not considered to be the trigger for flower initiation (Rodrigues Pereira, 1962). Raising the soluble carbohydrate level of an excised apex by adding sucrose to the medium did not cause flower initiation even once the level was equivalent to that of an apex of an intact bulb which had initiated a flower.

Rodrigues Pereira (1962, 1964, 1965) investigated flower induction and initiation with isolated explants consisting of the stem-disc with a growing point with two or three young leaf primordia. The presence of young foliar leaves and/or scales promoted flower initiation. A factor in the scales could diffuse across an agar bridge to the apex. Incubating excised scales on agar at 13°C caused them to release three growth substances. If the scales were then removed and excised apices placed on the agar, flower initiation was promoted in comparison with those on media which had not previously had the scale incubation period. The three growth substances, identified from the scales, reached peak concentration in the apex or bud at specific times in the floral development. The first, A, was at a maximum concentration just before the apex showed morphological signs of initiation, while the second and third, B and C, peaked at specific stages of flower organ initiation (Rodrigues Pereira, 1964). On the strength of bio-assays (Rodrigues Pereira, 1964) and exogenous gibberellin application (Halevy and Shoub, 1964), it was concluded that A had no gibberellin-like properties, but fractions B and C were gibberellin-like (Rodrigues Pereira, 1970). Exogenous gibberellins applied soon after flower initiation accelerated flower development.

The three growth substances identified were present in both the shoot tip and scales before the low temperature induction treatment. The low temperature, therefore, was thought not to be responsible for the formation of these substances but it might activate their transport to the apex. Iris would, therefore,

differ from other cold responsive plants where the shoot tip is the site of preception eg celery (Curtis and Chang, 1930) and chrysanthemum (Schwabe, 1954). Alternatively, the low temperature treatment may render the apex fit to respond to the growth substances. The first factor, A, might cause the diversion of the carbohydrates to the central zone of the apex, thereby satisfying Sachs's hypothesis (Sachs, 1977).

2.2.3. Standard temperature treatments

The requirement of a high temperature treatment to obtain bulbs of adequate maturity, followed by a cool treatment for flower induction and initiation, coupled with the discovery of high temperature retarding treatments have lead to the development of standard treatments for year round forcing. Certain refinements and improvements have been incorporated into the basic requirements. These have not been concerned with flower initiation per se but mainly with reducing the time required for growth in the glasshouse; improving the chances of development to anthesis or to enable the production of a "balanced" plant i.e. encouraging the flower to emerge well above the leaves. It was discovered that an intermediate treatment at 17°C between the heat curing and cooling improved the changes of flower production, mainly due to a reduction in the occurrence of blasting (Kamerbeek, 1965). By reversing the treatment so that the 17°C was applied after the cool treatment, the time to harvest was reduced and the occurrence of blasting further diminished (Kamerbeek and Beijer, 1964).

Several variations of standard treatments now exist, but basically they can be represented by the following examples:-

Very early flowering (Dec-Jan): 35°C (2 weeks); 40°C (3 days);
17°C (2 weeks) and 9°C (6 weeks)

or : 35°C (2 weeks); 40°C (3 days);
9°C (6 weeks) and 17°C (2 weeks)

Early flowering (Feb-April): 30°C (4 weeks); 40°C (3 days);
17°C (2 weeks) and 9°C (6 weeks)

or : with the reversal of the 17 and
9°C treatments

Normal, late and (May-Dec): 30°C followed by 17°C (2 weeks)
very late flowering and 9°C (6 weeks)

2.3 Thermal time

Thermal time (or accumulated temperature) offers a technique for relating the rate of plant development to temperature. It can be defined as the equivalent time required for development to a specific state if the temperature was a single degree above the base temperature. Its use can be two-fold; first, as a predictive method to determine the time of a specific developmental stage in fluctuating temperatures (eg seed germination, emergence, leaf initiation or flower initiation). Secondly, thermal time can be used to study the influence of different temperature sequences to determine whether or not plants respond differently to that expected from constant temperature responses, i.e. is the rate of development under fluctuating temperatures different from that expected from the rate obtained under a range of constant temperatures?

Briggs (1973) and Millar (1976) used a thermal time method to predict when narcissus bulbs had developed to a state of 'readiness to house'. This was traditionally assessed by feeling for the bud in the neck of the bulb before bulbs were moved from a standing ground to a glasshouse for forcing. Both Briggs and Millar logged temperatures below 15.5°C as a basis for calculating 'accumulated day-degrees' (ADD), however there were differences between standing ground bulbs and constant temperature stored bulbs, even when both had accumulated the same day-degrees. Bulbs from standing ground had longer shoots and shorter forcing times.

Rees and Hanks (1984), calculated ADD for narcissus by accumulating day-degrees below 30°C and found a good correlation between ADD and required glasshouse periods, but all temperature treatments were given as constant temperatures. Rees (1972) considered there to be two components to the cold treatment for narcissus. First, root and shoot growth is favoured by a higher temperature, 13°C, and secondly a lower optimum temperature, 4.4°C, is required for the latter stages of flower development. Generally, 9°C is used, being a compromise for both processes. Rees and Briggs (1975), also identified two components in the cold treatment for tulip forcing. The low temperature requirement, in this case, is for ^{the induction of} stem extension and the higher temperature requirement for stem growth. If the cold requirement is not satisfied, then growth may be fast but the stem will not extend to its full length. If the temperature is too low, then the potential for long stems is present but growth is slow.

Rees (unpublished), developed a day-degree system for narcissus which considered both of these components. For the satisfaction of the cold requirement for flower development, temperatures below 15.5°C were accumulated, day-degrees cold (DDC). Then, as a measure of temperatures allowing shoot growth, temperatures above 4.4°C were accumulated, day degrees for growth, DDG. These were combined to give ACCTEMP:-

$$\text{ACCTEMP} = \text{DDC} + (\text{DDG}/2).$$

ACCTEMP was found to work reasonably well with 'Golden Harvest' but less well with 'Fortune' using data from both Kirton, (Lincolnshire) and Rosewarne, (Cornwall).

There are no reports of the use of thermal time to study the cold requirement for iris bulbs. Kimura and Stuart (1972) and Kimura (1981), reported methods for ensuring that bulbs had received sufficient heat curing treatment necessary for subsequent successful flower initiation. Kimura and Stuart used an equivalent unit method, one EU being defined as 1 day at 32°C (the usual temperature for heat curing in controlled temperature rooms in the USA), or an equivalent heat exposure at different time

temperature combinations, calculated from a logarithmic relationship between temperature and heat curing satisfaction. Kimura (1981) reported that a total of 18 EU were required from 1 June, to ensure flowering under W.Washington USA field conditions.

Garcia-Huidobro, Monteith and Squire (1982 a,b) developed a method for calculating the thermal time requirements for the germination of pearl millet (Pennisetum typhoides). This method will be adopted here to investigate the temperature effect on flower initiation in iris. There are two conditions which must be satisfied for thermal time to be successful as a predictive method. The relationship between temperature and the rate of development must be linear. Secondly, the rate of development at an instant must depend only on the current temperature and not be influenced by previous temperature sequences.

Garcia-Huidobro et al (1982) stressed the importance of establishing three cardinal temperatures. These are:-

- 1) The base temperature, T_b , the temperature at which and below which the rate of development is zero.
- 2) The optimum temperature, T_o , is the temperature at which the rate of development is at its maximum.
- 3) The maximum temperature, T_m , is the temperature at which and above which the rate of development is zero.

When the rate of development is plotted against temperature the relationship should be linear between T_b and T_o , the rate increasing by the same increment for each degree rise in temperature. Similarly there should be a decreasing linear response for increasing temperatures from T_o to T_m . By extrapolation of these linear lines back to $1/t = 0$, T_b and T_m are defined respectively. The two straight lines intercept at the optimum temperature, T_o .

If a linear relationship does exist then the equation for the straight line between T_b and T_o is:-

$$1/t = \text{constant} \times (T - T_b) \quad (\text{eq 2.1})$$

The constant is the slope of the line and the intercept is zero if $(T - T_b)$ is the x component.

Similarly for temperatures above T_o :-

$$1/t = \text{constant} \times (T_m - T) \quad (\text{eq 2.2})$$

The thermal time, θ , is the multiple of time and effective temperature, so for temperatures below T_o thermal time, θ_1 , is defined by:-

$$\theta_1 = t \times (T - T_b) \quad (\text{eq 2.3})$$

Similarly θ_2 for temperatures above T_o is defined by:-

$$\theta_2 = t \times (T_m - T) \quad (\text{eq 2.4})$$

Hence from equations 2.1 and 2.2 it can be seen that the reciprocal of the slope of the straight lines are θ_1 and θ_2 respectively.

Since there is no simple function to describe the accumulative way in which a population of plants germinates or initiates a flower with time, all five parameters, T_b , T_o , T_m , θ_1 and θ_2 should be calculated for various arbitrary subsets of a sample, eg for each successive 10 per cent of the sample. Differences in the rate of development of these subsets, due to between plant variation, could be either due to differences in the cardinal temperatures but equivalent rates or similar cardinal temperatures but different rates, Fig 2.1 (from Garcia-Huidobro et al 1982a).

The method of calculation of thermal time suggested by Garcia-Huidobro et al. (1982a), has the following advantages over previous calculation methods:-

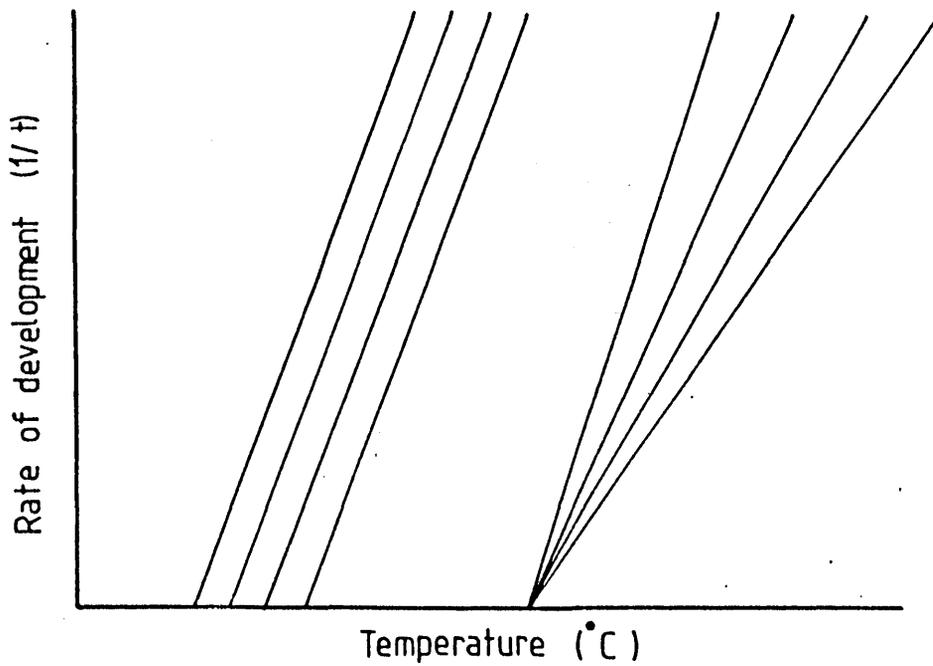


Fig 2.1

Hypothetical relationships between rate of development and temperature for different proportions of the population, on the left similar rates with different base temperatures, and on the right, different rates but similar base temperatures. (from Garcia-Huidobro et al 1982a).

- 1) The base temperature is selected by experimental methods, rather than by an arbitrary procedure.
- 2) The response of rate of development to temperature is confirmed as linear, rather than assuming that each °C rise produces an equal response.
- 3) The procedure can be applied to supra-optimal temperatures, as well as sub-optimal. In addition the two can be combined to give an overall response when temperatures experienced are both above and below the optimum, (Garcia-Huidobro et al 1982b), and any detrimental effect of temperatures beyond the optimum can be taken into account.

2.4 Post-planting flower development and blasting

Once the flower is fully formed in stored bulbs, i.e. all flower organs are differentiated, further development does not usually occur until after the bulbs are planted. Once planted the leaves emerge first, followed by flower stem extension. The flower organs grow and the tepals then develop colour. The flower then emerges from the two spathe leaves, at which point they are harvested commercially, the outer petals 'falls' then open and the anthers release their pollen, i.e. anthesis occurs.

Flower blasting is the term used for flower-bud abortion particularly in bulbous plants. It is the premature cessation of growth and development of the flower and flower stem (Kamerbeek, 1966). The symptoms vary with the stage at which the physiological disorder occurs. If blasting occurs early in the flower's development then plants are produced with a similar appearance to those in which a flower was not initiated. It is only on dissection of the bulb that a dried flower is observed. The most common stage for blasting, however, is at the time of maximum stem elongation, i.e. during the 14 days before anthesis (Fortanier and Zevenbergen, 1973). Mae and Vonk, (1974) claimed the critical period, when the flower was most prone to blasting,

to be 14 to 7 days before anthesis. Symptoms at later stages of development include the failure of the flowers to open correctly; the 'falls' open but the rest of the flower remains closed ('strangled' flowers) or, in a mild form, pale flowers may be produced (De Hertogh, 1985).

Flower blasting occurs when bulb are forced at high temperatures and low irradiance, or if water stressed (Hartsema and Luyten, 1955a; Kamerbeek, 1969; Fortanier and Zevenbergen, 1973). Pre-planting treatments can, however, enhance or reduce the chances of flower blasting. The cool treatment which accelerates flower initiation, also accelerates and improves subsequent flower development (Sano, 1974). Similarly, insufficient high temperature treatment has been shown to lead to an increase in blasted flowers (Le Nard, 1980), possibly due to insufficient mobilization of reserves.

Flower-bud abortion is often attributed to a shortage of carbohydrates at the growing and developing flower. A low light effect has also been shown to lead to flower abortion in other plants, eg Lilium (Kamerbeek and Durieux, 1971); Rosa (Moe, 1971); Gladiolus (Shillo and Halevy, 1981); Bougainvillea (Hackett and Sachs, 1966) and tomato (Cooper, 1964). This light effect has been partly, or entirely, attributed to the level of photosynthesis and, thus, to the availability of assimilates for growth and development.

Iris bulb reserves were thought to be exhausted under low light conditions, before flowers developed to a harvestable stage. Current assimilates were, therefore, required for the production of a flower to anthesis (Hartsema and Luyten, 1961; Rees, 1972). High temperatures cause quicker growth and therefore less time to either mobilize reserves or supply carbohydrates by photosynthesis, while their utilization was greater because of an increased respiration rate. Wassink and Wassink-van Lummel (1952), lowered the night temperature during forcing in the glasshouse. This was expected to reduce blasting at a given light energy, since less assimilates were expected to be utilized by respiration during the night, thus allowing greater availability for the extending stem and growing flower. However, no

appreciable difference in the number of plants blasting was observed between those grown at normal and those at low night temperatures.

During forcing in the glasshouse, high temperatures at night (15-18°C) while maintaining normal day temperatures (13-15°C) results in similar percentages of flowers developing to anthesis as a constant day and night temperature (13-15°C). The high night temperature, however, reduces the required glasshouse days for development to a harvestable stage (Kamerbeek, 1966; Fortanier and Zevenbergen, 1973). The respiration rate, and utilization of assimilates at night has, therefore, been shown not to affect the flower development. If the temperature remains high (15-18°C) during daylight hours at any time, however, flower development is affected, the most sensitive stages causing blasting appear to be dawn and dusk when irradiance is low (Kamerbeek, 1966). The fact that flower development is unaffected by the night temperature suggests that high temperatures are antagonistic to flower development only in the light, possibly due to influence on processes which occur only in the light eg photorespiration or transpiration. Fortanier and Zevenbergen (1973), suggested that the differential night/day effects of temperature could be related to differences in plant water status. Low temperatures during the night and high temperatures during the day result in soil temperatures lagging behind increasing air temperatures at dawn, which can lead to water stress.

Mae and Vonk (1974) investigated flower development under normal (56 W m^{-2}) and low (2.6 W m^{-2}) light and normal (0.03%) and low (0.003%) CO_2 concentration during the critical light sensitive period (14-7 days before anthesis). Normal irradiance, even when the CO_2 concentration was a tenth of its ambient level, gave 100 per cent flower development. Low irradiance and normal CO_2 concentration, however, resulted in reduced number of flowers developing. They concluded that the light does not act on flower development primarily by synthesizing large enough quantities of photosynthates. Labelling plants with ^{14}C and ^{32}P and observing the distribution following a dark or normal light treatment showed the light effect to be connected with the distribution pattern of

carbohydrates. In particular, the transport of ^{14}C photosynthetic products from the leaves in the direction of the developing bud was negatively influenced by a dark treatment. Mor and Halevy (1980a) demonstrated the same response to light and low light at the shoot tip on the distribution of ^{14}C -assimilates to the developing rose bud. In reduced light this decline in ^{14}C distribution to the bud preceded its abortion.

Many workers have considered the establishment of sinks to be mediated through hormonal control, in particular gibberellins (Harris, Jeffcoat, and Garrod, 1969; Jeffcoat and Harris, 1972) and cytokinins (Mae and Vonk, 1974; Tse, Ramina, Hackett and Sachs, 1974). Cytokinins applied to the dark treated iris flower buds both increased the number of flowers developing (Mae and Vonk, 1974) and the movement of ^{14}C to the flower bud (Vonk and Ribot, 1982). Cytokinins (BA) followed by gibberellin (GA_3) application to tomato inflorescences stimulated their development (Kinet, 1977). Growth substance treatment does not increase the photosynthetic rate or the proportion of assimilates exported from the leaf, but supply to the treated inflorescence increases, while that of the vegetative apical shoot decreases (Leonard, Kinet, Bodson and Bernier, 1983). Benzylaminopurine was more active in overcoming bud blast in Dutch iris than was gibberellic acid (Mae and Vonk, 1974).

Leonard and Kinet (1982) investigated endogenous cytokinin and gibberellin levels in ^{tomato} inflorescences from high and low light treatments. Those from the low light were found to have reduced cytokinin activity, but gibberellin activity remained high. Vonk and Ribot (1982) found an increase in ABA content in ^{iris} buds given a dark treatment. They concluded that this ABA reduced the sink strength of the bud, possibly by preventing GA biosynthesis. Alpi, Ceccarelli, Tognoni and Gregorini (1976) reported an increase in GA activity in the floral parts of iris at anthesis. Cytokinin application was thought to lead to a build up of bound ABA and a reduction in the free ABA (Vonk and Ribot, 1982) while in light-cultured buds applied ABA was shown to be rapidly converted to ABA-glucose.

With the background information outlined here, factors affecting flower initiation and development in the Dutch iris were investigated. Flower induction and initiation were investigated to determine the action of cold treatment, whether this was vernalization or a direct response to lower temperatures permitting growth and development to a stage where flowers were formed autonomously. Flower development was investigated by first identifying the main sinks in competition with the flower and how light and temperature affected these sinks.

3. GENERAL MATERIALS AND METHODS

3. GENERAL MATERIALS AND METHODS

The experiments described in this thesis were carried out using the storage facilities, laboratories and glasshouses at the Glasshouse Crops Research Institute, Littlehampton. Saxcil growth cabinets at the University of Nottingham, School of Agriculture, Sutton Bonington were also used. The experiments described were completed during the period October 1982 to September 1985.

In this chapter those materials and methods either for specific techniques or common to most experiments will be described. Details of materials and methods which apply only to certain experiments will be described in the relevant chapters.

3.1 Plant material and general care and growing procedures

Dutch iris of three cultivars were used during the course of these investigations; 'Wedgwood' which has been used as a standard cultivar for much of the previous work; 'Ideal' a modern sport of 'Wedgwood' and 'Professor Blaauw' which is a later, slower maturing cultivar. The bulbs were grown at GCRI on the brickearth soils (Hamble series). The original stock bulbs were obtained from Rosewarne EHS (Cornwall).

On lifting in August-September, bulbs were cleaned and separated from the remaining old mother-bulb and then graded using a round grading riddle. All bulbs were then dipped for control against aphids and Penicillium corymbiferum in a solution containing 2.5 per cent pirimicarb, carbamate insecticide; 0.2 per cent benomyl, systemic fungicide and 1% captafol, fungicide. The bulbs were then left overnight on a drying bench.

Bulbs to be given flowering treatments were then placed either in the retarding temperature store at 25.5°C for later use or at 35°C for early forcing.

The temperature treatments given to the bulbs will be described in the relevant chapters. The pre-planting treatments were all applied in the dark in controlled temperature rooms

(Prestcold Southern Ltd, Worthing) or in small incubators constructed at GCRI. In both cases temperatures could be controlled to within $\pm 1^{\circ}\text{C}$.

Bulbs were planted in 210 mm diameter full sized pots in GCRI potting compost without additional nutrients as iris roots are particularly sensitive to high salt concentrations. Five bulbs were planted in each pot with the nose of the bulb just below the surface of the compost. This was calculated to give a planting density equivalent to that used commercially when pots were pushed closely together.

Plants were watered frequently with tap water, no feed was given during the forcing period.

3.2 Plant apical examination and growth analysis

Bulbs were dissected for apical examination to determine leaf number and the time of flower initiation as shown in Fig 3.1. The top and old hard base plate were removed, the scales were cut away to form a cube. Leaves could then be peeled away and counted. The final leaf removal, counting of the leaf primordia and apical examination were carried out under a binocular microscope ($\times 50$). The youngest leaf primordia was defined as by Hussey (1963) as the smallest initial with its adaxial surface showing a positive angle.

The start of flower initiation was taken from the time the apex formed a dome shape and the centre of the apex was above the last leaf primordium to be initiated.

When plants were harvested, frequently at the time of anthesis, they were usually divided into mother-bulb (scales, sheath leaves and base plate), roots, foliar leaves, daughter bulbs, flower buds and flower stem. Dry weights were determined by placing these into individual aluminium cans and drying in an oven at 80°C until constant weight was achieved. This usually occurred after 72 hours drying. The stem length was taken as the length from the base plate to just below the gynoecium on the flower. Similarly, the length of the first leaf was measured as

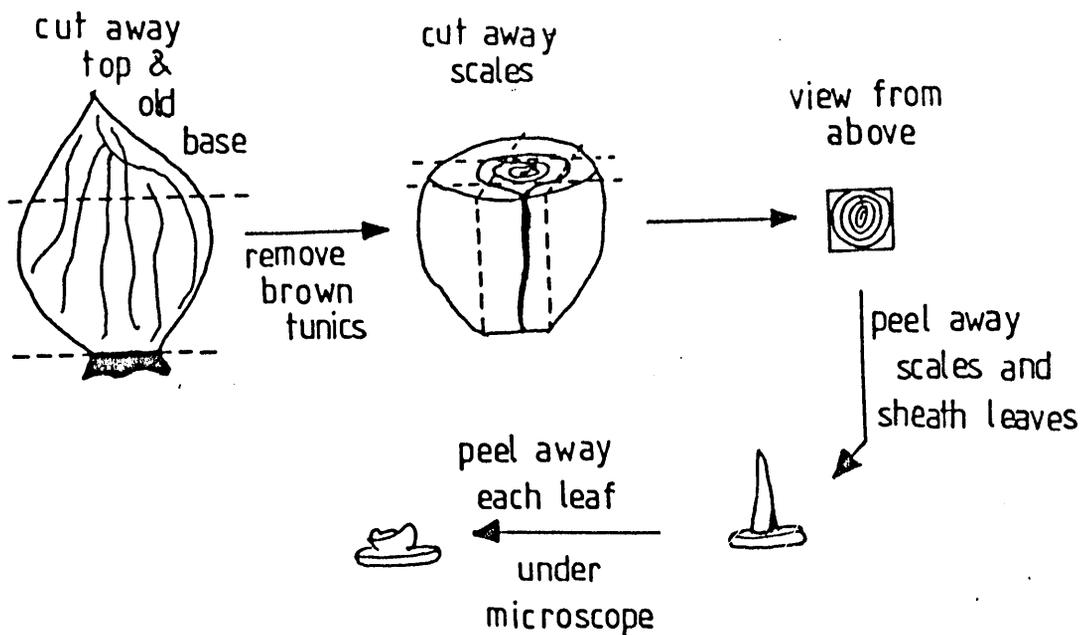


Fig 3.1

Diagrams illustrating method of dissecting iris bulbs to determine leaf number and time of flower initiation

the length from the base plate to the tip of the leaf. Days to anthesis was taken as the time from planting to the time of anther dehiscence. A flower was said to have aborted if it did not open or failed to develop to anthesis. The second bud was said to have aborted if it appeared papery and dry when the plant was harvested.

3.3 Carbohydrate analysis

Fresh flower bud material was cut into small pieces, approximately 2 mm cubed, and dropped immediately into boiling absolute ethanol, with a little calcium carbonate added, for 30 minutes. This prevented endogenous enzyme action and provided the first extract of alcohol soluble sugars. The bud material was filtered from the ethanol and placed in a cellulose thimble (33 mm x 100 mm, Whatman). The thimble was in turn placed inside the soxhlet equipment and the soluble sugars extracted for a number of hours with 80 per cent (V/V) aqueous ethanol. The number of hours necessary for extraction was established by replacing the aqueous ethanol every two hours. When reducing sugars were no longer detectable in the ethanol, the extraction was considered complete.

When all the soluble sugars were considered extracted, the extracts were combined and reduced in volume to about 50ml under pressure with a rotary evaporator. The aliquot and washings were then placed in a beaker on a steam bath to evaporate off all the alcohol - until the odour of alcohol had disappeared. After cooling to room temperature the extracts were then clarified by adding a saturated solution of neutral lead acetate (AnalaR), the excess of lead was then removed with sodium oxalate (AnalaR) as described in the "Official Methods of Analysis of the Association of Official Analytical Chemists" (Anon, 1975).

The clarified extracts were then made up to 100ml with MPP solution (2g methyl 4 hydroxybenzoate and 0.2g n propyl p-hydroxybenzoate per litre of glass distilled water). This solution prevents fungal and bacterial growth in the sugar extract. After the necessary dilution of this 100 ml extract

(e.g. 1 ml of this extract diluted 50 times), the total reducing sugars were determined on a Technicon autoanalyser using a copper-neocuproine method (Bittner and Manning, 1967).

A further 50 ml of clarified extract had 5 ml of concentrated HCl added giving a solution of 1NHCl. A test for the required time for complete hydrolysis revealed that the reaction was not complete until the reaction mixture was left for about 24 hours at room temperature. After this time the samples were neutralized with sodium carbonate (AnalaR) solution and the final volume adjusted to 100 ml with MPP. This was diluted (1 ml to 50 ml with MPP) before the total reducing sugars were again determined. The quantity of sucrose in the samples could then be calculated from the difference in reducing sugars before and after hydrolysis.

All tests included standards, made up in MPP, of glucose and sucrose; blanks containing no sugars but which received the same treatment as the extracts; sucrose standards which were not hydrolysed and glucose standards which were given the hydrolysis treatment. The recovery rates of sucrose standards were in the range of 102-108 per cent, while those for reducing sugars (fructose and glucose mixed) were 95-103 per cent.

The starch content of the buds was estimated by determining the reducing sugars after enzymic hydrolysis. The alcohol insoluble material remaining in the thimble after soxhlet extraction was washed with absolute alcohol and then with diethyl ether. The solid material was then dried in an oven at 80°C for 48 hours and weighed. The bud tissue was then finely ground in an electric-mill (Culatti) and 50mg of this powdered material weighed out for each enzyme assay. For the enzyme reaction 5ml of 0.2M acetate buffer, pH 4.6 (25.5 ml 0.2M acetic acid and 24.5 ml 0.2M sodium acetate made up to 100 ml with MPP) was added to this 50mg in a test tube. These were heated to 105°C in an electric block heater to gelatinize the starch. The contents of the tube were stirred vigorously with a vortex mixer at regular intervals. Two gelatinizing times were tested, 30 and 60 minutes, but no different effects of the two times could be demonstrated, therefore 30 minutes was considered adequate. An additional 4ml of buffer were added to wash down the sides of the tube, which were then allowed to cool before adding the enzyme.

'Agidex' (Glaxo Ltd) which contains α -1-4 amyloglucosidase, was compared with two commercial pure enzymes (Boehringer and Sigma) with either the alcohol insoluble flower material, pure potato starch (AnalaR) or cellulose powder (Whatman). No detectable differences were seen between the Agidex and the two pure enzymes with the flower or potato starch substrates. The cellulose test was included to test the specificity of the Agidex; none of the enzyme preparations gave any detectable reducing sugars with the cellulose substrate.

The concentrated Agidex, as supplied, contains a considerable quantity of glucose which was removed by dialysis. Visking tubing (The scientific Instrument Centre Ltd.) was sealed at the bottom end and 10ml of Agidex added. The top end was then tied around a glass rod which was placed across the top of a 500 ml beaker, so that the tube was suspended in the beaker. Tap water was run into the beaker for 48 hours. Finally the tubing was placed in distilled water for 30 minutes. The glucose-free Agidex was then diluted to 500ml with MPP and 1ml of this added to the substrate in acetate buffer.

A sample blank, containing an extra ml of buffer but no enzyme and an enzyme blank containing the enzyme but no substrate were included in all the tests.

All samples were incubated in a water bath at 50°C for 48 hours for the hydrolysis of starch to glucose. The enzyme was de-activated by heating, the solution filtered and diluted to 100ml with MPP. The reducing sugar content of the solution was then determined by autoanalysis. The amount of standard potato starch recovered by this method was 99-101%.

3.4 ¹⁴C radio-isotope experiments

Radio-isotope, ¹⁴C, was used to trace the distribution of photosynthetic assimilates and stored carbohydrates from the mother bulb.

3.4.1. Generation of $^{14}\text{CO}_2$ for labelling plants

A cylinder containing $^{14}\text{CO}_2$ was prepared by the method described by Ludwig and Canvin (1971). In this method a small cylinder, such as those commonly used by sub-aqua divers, was evacuated. A sealed flask was attached to the evacuated cylinder, this flask contained a vial with excess 2N sulphuric acid and 5000 μCi barium [^{14}C] carbonate (Amersham International plc, England). The vial was tipped to release the $^{14}\text{CO}_2$. The valve of the small cylinder was opened and the $^{14}\text{CO}_2$ drawn in under pressure.

The flask containing the acid reaction mixture was then gently warmed to obtain the maximum release of $^{14}\text{CO}_2$. Air was then introduced into the system from a pressurized cylinder via a pressure reducing valve. This allowed the $^{14}\text{CO}_2$ to be flushed out of the flask into the small cylinder. The flask was then disconnected and the air cylinder attached directly to the small cylinder. The small cylinder could then be pressurised to give a total equivalent volume of about 120l at atmospheric pressure. From the activity remaining in the release flask, the activity of each litre in the cylinder was calculated to be approximately 41.4 μCi .

3.4.2 Labelling plant material

Two sets of plant material were fed $^{14}\text{CO}_2$. Firstly, 50 'Wedgwood' (6-7 cm) bulbs were planted, 3 bulbs per 210 mm pot on 23 March 1984. These plants were grown outdoors, surrounded by a 1 m high plastic wind shelter. They were fed during August 1984. It was intended that with this first batch of plants, the daughter bulbs were to be labelled. At harvest these would then be given temperature treatments for forcing the following year, enabling the re-distribution of mother bulb reserves to be traced.

The second set of plants fed were to investigate the current assimilate partitioning during forcing. 'Wedgwood' 8-9 cm grade bulbs were used, 5 bulbs per 210 mm pot giving planting densities similar to those used commercially.

Plants were fed in a fume cupboard, under a bank of warm white fluorescent tubes giving an average light intensity at pot level of $21.1 \pm 0.9 \text{ W m}^{-2}$. When labelling the first batch of plants, to examine the re-distribution of reserve materials, all the pot was enclosed in a large polythene bag. The open end of the bag was sealed around the lip of the pot with strong adhesive plastic tape. The bag had a single direction valve sealed into the top end so that gas from the cylinder could be released into the enclosed area containing the plants. Plants were first left for about 30 minutes, enclosed in the bag under the lights. A known volume of gas from the cylinder (usually 4l) was then released, through a flow meter, into the bag. The plants were then left for 3 hours to assimilate the $^{14}\text{CO}_2$. During the initial feedings, samples of air (5 ml) were taken from the bag and the activity determined. Fig 3.2 shows the decline in activity with time as $^{14}\text{CO}_2$ was assimilated by the plants.

This process of feeding was repeated twice with each pot of plants, giving, on average, each plant an activity of just over 100 μci .

With the second batch of plants, to examine assimilate distribution during forcing, a polyethylene tube was placed over the second foliar leaf, above the radicle leaf. A plastic tube from the cylinder was fixed into the open end of this tube, which was then carefully sealed around the base of the leaf. The plants were then fed in the same manner as above, using smaller volumes of gas. On average the plants were fed to give a total activity of about 3-4 μci .

3.4.3 Partitioning and drying of plant materials

At harvest the plants were carefully washed and then divided into roots, scales and sheath leaves of the motherbulb; base plate, fed (source) leaf (for second batch of plants only); other foliar leaves (by removing from the stem at the node) and the flower bud plus flower stem. The various plant parts were rapidly frozen by dipping in liquid nitrogen and placing in labelled

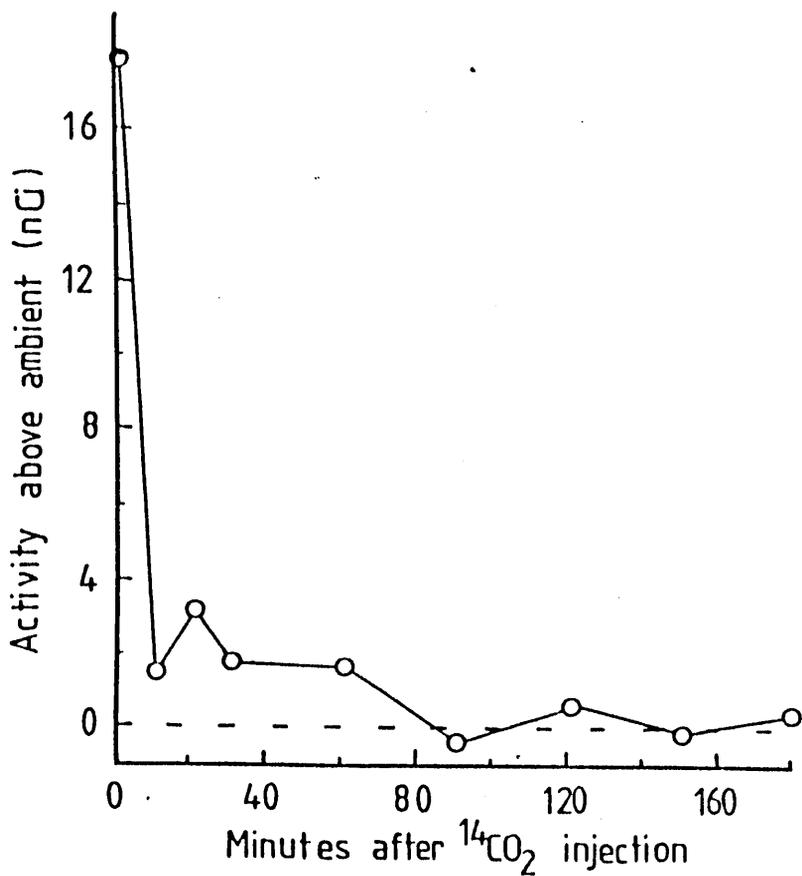


Fig 3.2

The decline of activity of a 5ml air sample, taken from the bag enclosing the plants to be labelled, at various times after injecting ¹⁴CO₂ gas.

plastic bags for freeze drying in an Edwards "Modulyo" unit with a custom-built sublimation chamber housed in a domestic freezer and under a vacuum of about -400 Pa.

3.4.4. Oxidation and scintillation counting

After drying, the contents of each envelope were weighed to determine the total dry matter of the various plant parts. The contents were then finely ground using a 'Glen-creston' (M280) sample mixer mill. Between 10-20 mg of this material was then weighed into a small aluminium boat. The samples were then oxidised (burnt) in a stream of pure oxygen at 900°C in a Carbon-hydrogen Analyser (Coleman 33). The water produced by the oxidation was trapped by a chemical absorption column. The carbon dioxide containing ^{14}C was absorbed in methoxyethylamine using the method of Adkin and Ho (1981). The outlet for the exhaust gas, containing the $^{14}\text{CO}_2$, was adapted so that a glass vial containing 1.5ml of methoxyethylamine was attached so that the gas bubbled through the CO_2 absorbant.

For scintillation counting the organic amine was mixed with the toluene based scintillation fluid Tpp4 (4g PPO, 100 mg POPOP, 40mg naphthalene, 1000ml toluene). Activity (distintegrations per minute) was counted on a pre-programmed "Beckman LS700" counter. Each sample was counted for 1 minute and an average of three counts were used.

3.5. Experimental design and statistical analysis

In most experiments a randomised complete block design was used. Since five plants were grown in each pot, the average for each pot was usually considered as a replicate, three or more pots being used per treatment.

Data were analysed using Genstat programmes, to give analysis of variance (ANOVA). Regression analyses were also used using either Genstat or Minitab. Tests of significance refer to change of probability at the five per cent level. Results were expressed in the form of tables or graphs.

4. TIME, TEMPERATURE AND FLOWER INITIATION

4. TIME, TEMPERATURE AND FLOWER INITIATION

Introduction

Flower initiation in iris occurs in direct response to low temperature (Hartsema, 1961). It is not known whether this is a vernalization response, which is an inductive phenomenon and does not itself evoke flowering (Vince-Prue, 1975), or whether low temperatures permit autonomous induction. The optimum temperature for flower initiation is claimed to be 13°C (Blaauw, 1941; Hartsema, 1961), but this could be a compromise temperature between two or more processes for example flower induction and flower initiation.

The cold period of storage before planting occupies about 8 weeks commercially. If the induction and development of the flower had different optimum temperatures and these could be identified then the cold storage period could be shortened. The reliability of the cold storage in causing complete flower initiation could also be improved.

Thermal time provides a suitable procedure for investigating such a problem. The procedure described by Garcia-Huidobro et al. (1982), for the prediction of germination in millet (see literature review) was adopted to study leaf, flower and flower organ initiation. This method requires the rate of development to be linearly related to the temperature. A second requirement is that the rate of development at an instant, is dependent only on the current temperature and not the thermal history, (thermal history being the previous temperature treatments or thermal sequence). Garcia-Huidobro et al. (1982b) suggested that the hypothesis that a physiological process was unaffected by thermal history could be tested by comparing measured and calculated values for the rate of development from a thermal-time equation. This equation is determined by studying development at constant temperatures.

Leaf initiation in response to temperature was first studied as the production of more than three foliage leaves is a prerequisite to flower initiation. Information can also be obtained about the rate of progress to flower initiation.

The aim of the experiments was to determine whether a relationship existed between the thermal time and leaf, flower and flower organ initiation at constant storage temperatures. The hypothesis that these processes were unaffected by thermal history could then be tested.

4.1 Material and Methods

Bulbs of 'Ideal', 9-10 cm grade, were imported from Holland (M. Verdegaal & Sons) and had been stored at growth retarding temperatures from lifting (30°C). On arrival they were stored for a further 8 days at 25°C before being placed in the 16 different temperature treatments on 20 November 1984:

- 1) Constant 2°C
- 2) Constant 5°C
- 3) Constant 9°C
- 4) Constant 13°C
- 5) Constant 17°C
- 6) Constant 20°C
- 7) Constant 25°C
- 8) 5°C 2 weeks 13°C thereafter
- 9) 5°C 4 weeks 9°C thereafter
- 10) 5°C 4 weeks 13°C thereafter
- 11) 5°C 4 weeks 17°C thereafter
- 12) 13°C 2 weeks, 20°C 6 weeks, 13°C thereafter
- 13) 13°C 2 weeks, 5°C 2 weeks, 13°C thereafter
- 14) 13°C 2 weeks, 5°C 2 weeks, 1°C 4 weeks, 13°C thereafter
- 15) 17°C 2 weeks, 9°C thereafter
- 16) 17°C 2 weeks, 13°C thereafter

Bulbs were stored at constant temperatures throughout in order to establish the thermal time model by obtaining rates of development at these fixed temperatures. Transfer temperatures included different lengths of time at 5°C to determine whether a vernalization response was discernible with temperatures below 13°C. A range of temperatures following this 5°C treatment were applied to determine if the optimum temperature was altered following a low temperature treatment. The treatments applied also provided bulbs at a given time which had received the same temperature treatments but in different orders e.g. 13°C for 2 weeks followed by 5°C for 2 weeks, treatment 13, or the reverse sequence, treatment 8. A standard cold treatment was included for comparison with the other treatments.

Twenty bulbs from each treatment were periodically sampled, their leaf number counted and the state of the apex determined under a stereo-binocular microscope.

4.2 Results

4.2.1. Determination of cardinal temperatures for leaf initiation

The cardinal temperatures (the base temperature, T_b ; optimum temperature, T_o ; and maximum temperature, T_m , for leaf initiation) were determined using data from bulbs stored at constant temperatures. The rate of leaf initiation was calculated by subtracting the mean leaf number once a flower had been initiated from the mean leaf number at the start of the treatment. These means were based on an average of 40 plants, except at 25°C when only 28 plants initiated a flower. The leaf number included the two spathe leaves around the first flower bud. From the curves of the proportion of bulbs with a flower initiated against time (Fig. 4.1) the weighted mean time to initiation was calculated, adopting the method described by Heydecker (1966) for seed germination, i.e. $\Sigma(L't')/L_m$ where L' is the number of leaves initiated on day t' and L_m is the maximum number of leaves initiated. Hence, the average rate of leaf initiation was taken as the increase in leaf

Fig 4.1

The proportion of bulbs with a flower initiated (R) against time in days.

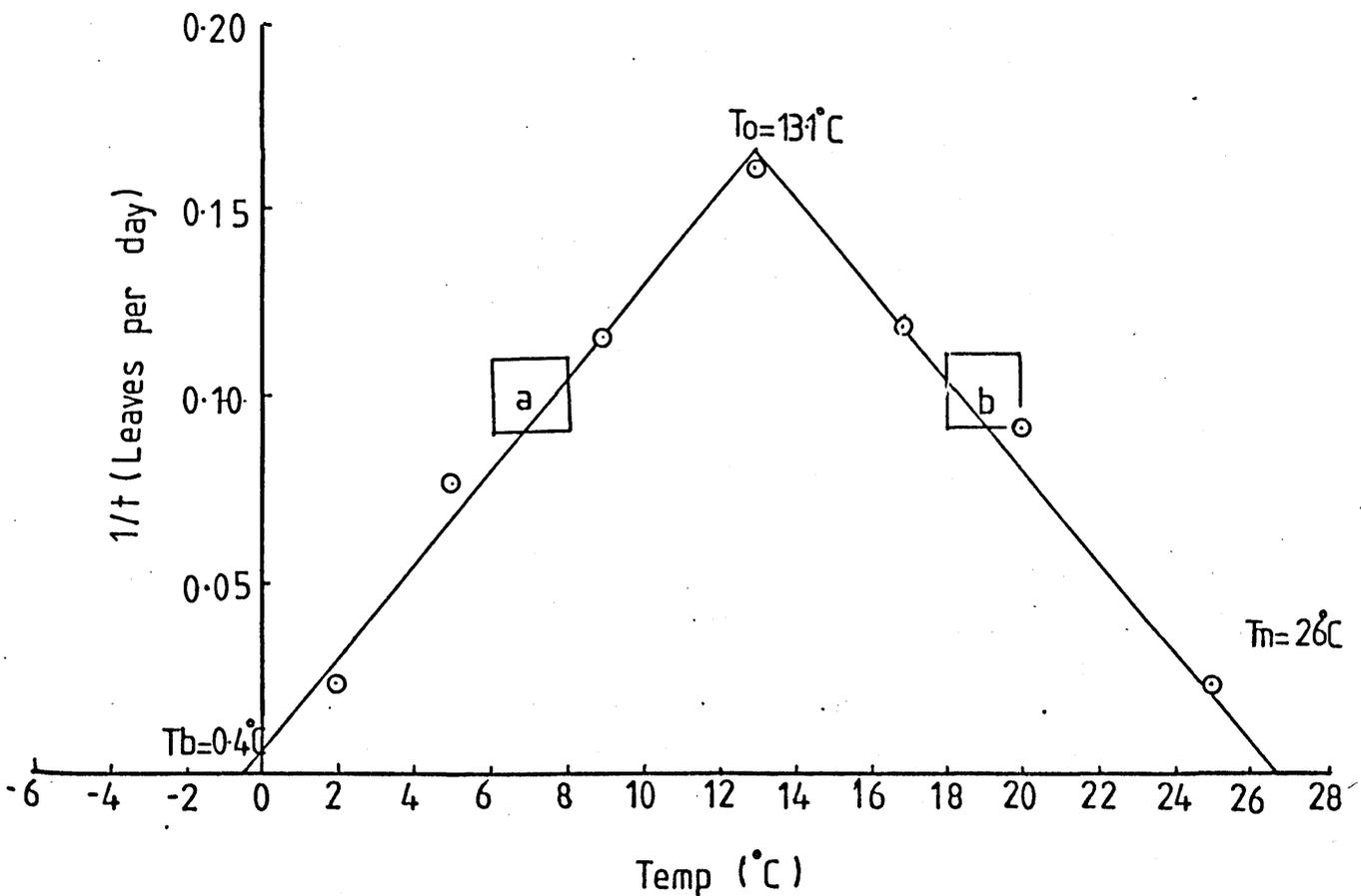
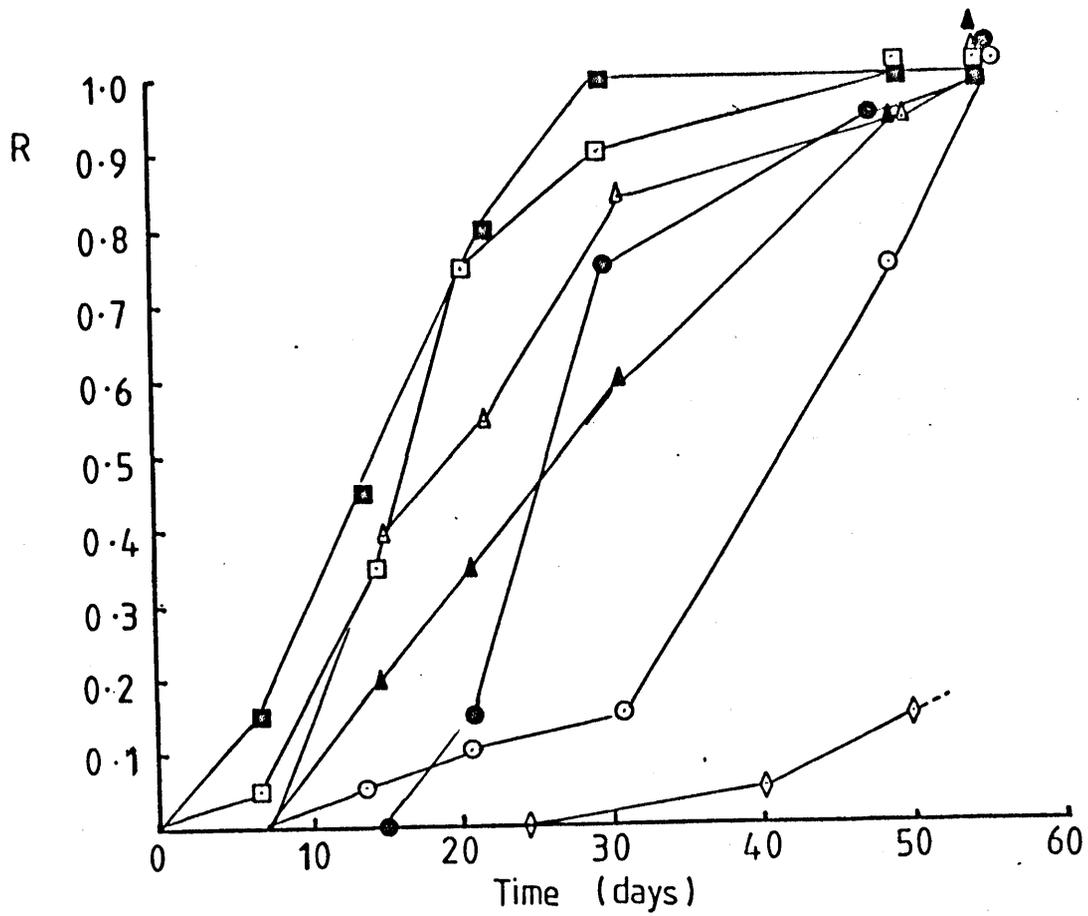
(○) = 2°C (●) = 5°C (□) = 9°C (⊠) = 13°C (△) = 17°C
(▲) = 20°C (◇) = 25°C

Fig 4.2

Rate of leaf initiation as a function of constant storage temperature.

$$\text{a) } \frac{1}{t} = 0.0049 + 0.0123T \quad r^2 = 0.99 \text{ (p < 0.01)}$$

$$\text{b) } \frac{1}{t} = 0.326 - 0.122 T \quad r^2 = 0.98 \text{ (p < 0.01)}$$



number from the beginning of the cool storage period up until flower initiation during the weighted mean time for flower initiation.

A linear relationship was shown to exist when the average leaf initiation rate was plotted against temperature, between 2 and 13°C ($r^2 = 0.99$, $p < 0.01$), as shown in Fig. 4.2. Similarly, a negative linear relationship existed between 13 and 25°C ($r^2 = 0.98$ $p < 0.01$). By extrapolating the two fitted lines, T_b and T_m were defined as -0.4°C and 26.7°C respectively. The optimum temperature, T_o , was estimated from the intersect of these two lines as 13.1°C .

4.2.2 Thermal time for leaf initiation

Using the cardinal temperatures obtained above, the thermal time for leaf initiation was calculated. The relationship between the thermal time and the number of leaves initiated is shown in Fig. 4.3. The thermal time required for the initiation of each subsequent leaf gradually increased. It is possible that there is a physiological maximum leaf number, at which a flower is initiated regardless of the temperature within the range tested here. The maximum leaf number in 'Ideal' was nine on average. A single line fitted the data adequately, for both temperatures above and below the optimum. This single fit is supported by the similarity of the slopes of the two lines in Fig. 4.2.

4.2.3 Predicted and measured leaf initiation rates

Since the thermal time required for the initiation of different leaves is not constant, it is not possible to predict the rate of leaf initiation by the method described in the literature review. Plotting the average leaf number of a sample against the known thermal time provided considerable scatter. This could be due to differences in the time the daughter bulbs were formed and their position in the mother bulb, which are known to affect their rate of early development (Rees, 1972).

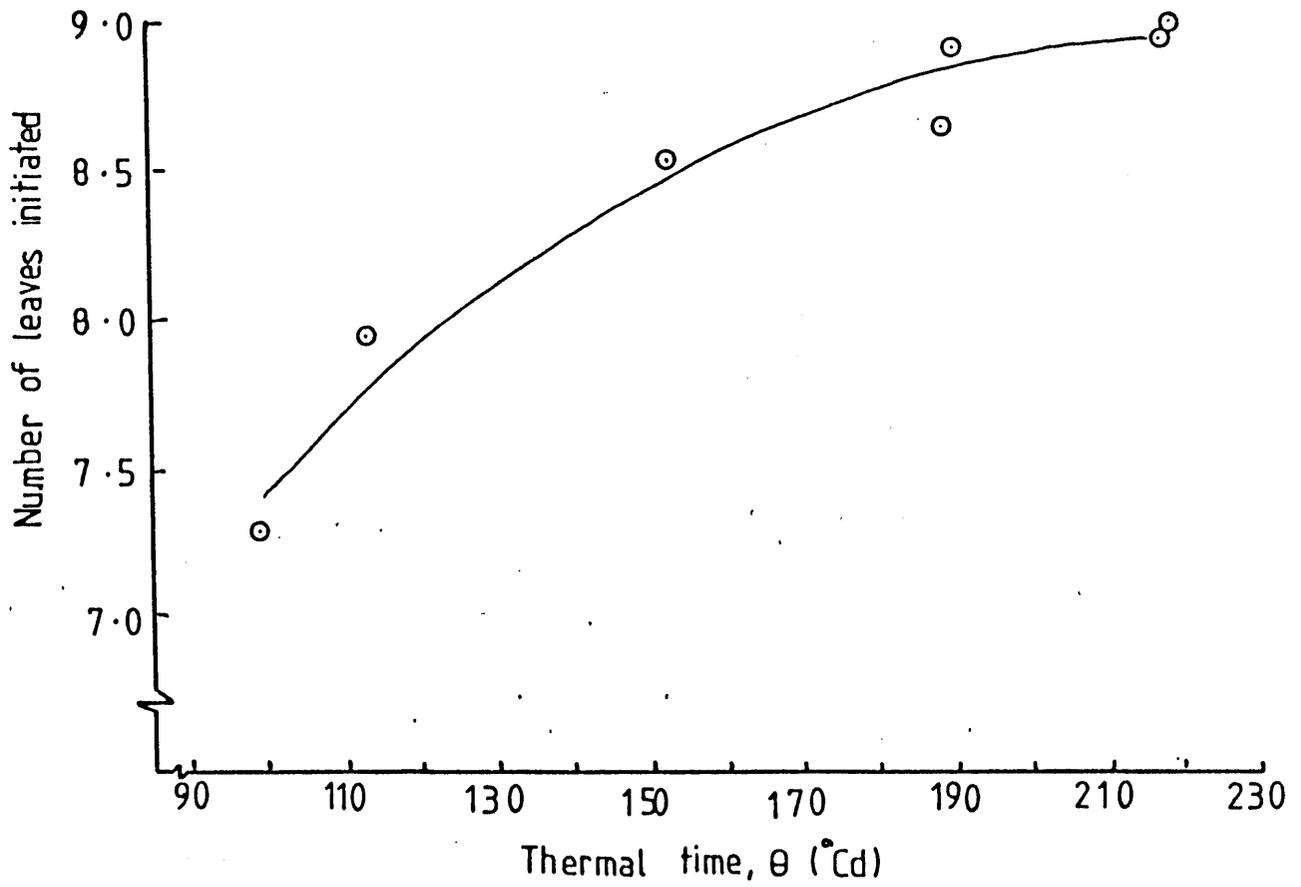


Fig 4.3

Number of leaves initiated against thermal time

$$\text{No. of leaves} = 3.77 + 0.0483 \theta - 0.00133 \theta^2$$

$$r^2 = 0.93 \text{ (} p < 0.01 \text{)}$$

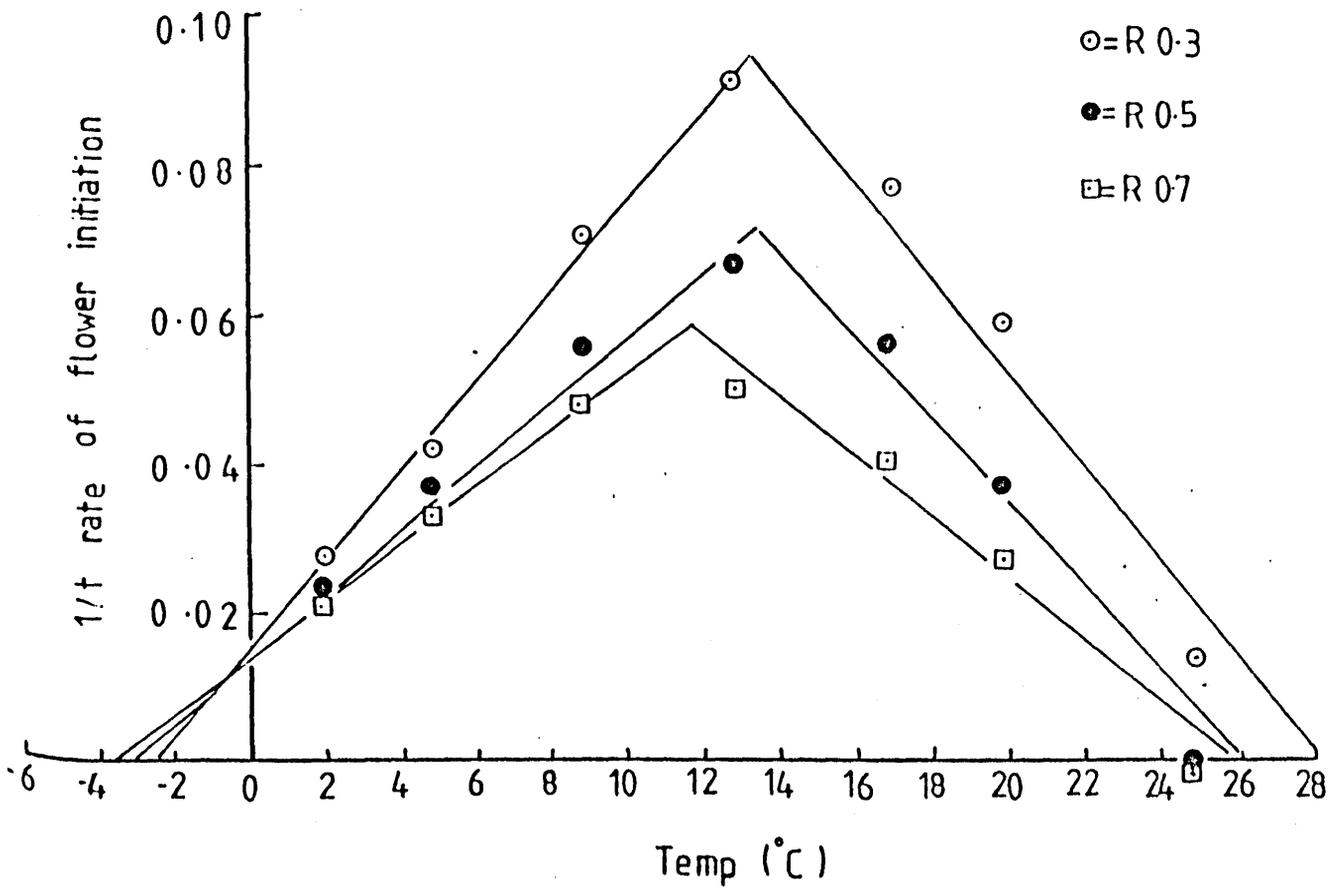


Fig 4.4

Examples of the rate of flower initiation ($1/t$) against temperature, for different proportions of a sample to have initiated, R (see Table 4.1 for parameters of linear regression).

4.2.4 Determination of cardinal temperatures for flower initiation

Fig 4.1 shows the proportion of bulbs with a flower initiated against time for constant temperatures between 2 and 25°C. From these curves the time taken for a given proportion of bulbs to initiate a flower (R) was estimated. The rate of initiation against temperature for different values of R was then plotted (Fig. 4.4). Linear relationships existed, both above and below the optimum temperature, for values of R between 0.1 and 0.8 (Table 4.1). By extrapolating and calculating the intercept of the two lines T_b , T_m and T_o were defined and θ_1 and θ_2 calculated from the reciprocal of the slopes of the two lines.

Table 4.1 Parameters of linear regressions for flower initiation rate, $1/t$, against temperature, $T(^{\circ}\text{C})$ for various proportions of bulbs to have initiated a flower, R.

a) Where $T < T_o$				
R	T_b	θ_1	r^2	(probability)
0.1	-0.1	69	0.94	($p < 0.05$)
0.2	-0.8	111	0.99	($p < 0.01$)
0.3	-2.6	169	0.99	($p < 0.01$)
0.4	-3.9	214	0.99	($p < 0.01$)
0.5	-3.2	233	0.98	($p < 0.05$)
0.6	-3.0	237	1.00	($p < 0.05$)
0.7	-3.5	260	1.00	($p < 0.05$)
0.8	-4.3	317	1.00	($p < 0.05$)
b) Where $T > T_o$				
R	T_m	θ_2	r^2 (probability)	T_o (from intercept of a + b)
0.1	27.2	88	0.97 ($p < 0.05$)	12.0
0.2	27.4	113	0.99 ($p < 0.01$)	13.0
0.3	28.1	154	0.96 ($p < 0.05$)	13.5
0.4	26.7	134	0.97 ($p < 0.05$)	14.9
0.5	25.9	176	0.96 ($p < 0.05$)	13.6
0.6	25.7	216	0.97 ($p < 0.05$)	11.8
0.7	25.6	237	0.97 ($p < 0.05$)	11.7
0.8	25.6	266	0.98 ($p < 0.05$)	12.1

From table 4.1, T_b , T_o and T_m are seen to be reasonably constant over the range of initiated proportions although the possibility of a drift to a lower base temperature with larger proportions exists. The means for these three temperatures are:- $T_b = -2.7 \pm 0.5^\circ\text{C}$; $T_o = 12.7 \pm 0.4^\circ\text{C}$ and $T_m = 26.5 \pm 0.3^\circ\text{C}$ but these are only significant if the drift in the cardinal temperatures is due to random variation. The relationship between rate and temperature when $R = 0.9$ and 1.0 was not linear. This was because estimates of $1/t$ were inaccurate because of the difficulty in estimating the time at which all bulbs were first initiated.

4.2.4.1 Initiation of the second flower-bud

The cardinal temperatures and the regression parameters for the initiation of the second flower bud in the terminal inflorescence are shown in Table 4.2.

Table 4.2 Parameters of linear regressions for the rate of initiation of the second bud in the terminal inflorescence, $1/t$ against temperature, $T(^{\circ}\text{C})$ for various proportions of bulbs to have initiated a second bud, R_2 .

R_2	T_b	θ_1	r^2 (prob.)	T_o	T_m	θ_2	r^2 (prob)
0.1	-0.22	214	0.89 (p < 0.01)	16.9	25.1	103	0.99 (p < 0.05)
0.2	1.63	199	0.99 (p < 0.01)	13.4	26.7	220	0.81 (p > 0.05)
0.3	0.85	214	0.91 (p < 0.05)	13.1	26.0	226	0.92 (p < 0.05)
0.4	0.67	258	0.89 (p < 0.05)	12.9	26.0	275	0.90 (p < 0.05)
0.5	0.24	308	0.81 (p < 0.05)	12.8	26.2	329	0.90 (p < 0.05)
0.6	0.40	347	0.84 (p < 0.05)	12.9	25.9	365	0.93 (p < 0.05)
0.7	0.35	394	0.84 (p < 0.05)	14.5	26.0	412	0.91 (p < 0.05)

A t test showed there to be no significant difference between the means for T_o and T_m but T_b for the second bud was higher than T_b for flower initiation per se. The mean values were:- $T_b = 0.6 \pm 0.2^\circ\text{C}$, $T_o = 13.8 \pm 0.6^\circ\text{C}$ and $T_m = 26.0 \pm 0.2^\circ\text{C}$.

4.2.5 Thermal time for flower initiation

Regression analysis showed a very highly significant fit ($p < 0.001$) between the proportion of a bulb sample which had initiated a flower and the thermal time, Fig. 4.5, for constant temperatures above and below T_0 , i.e. θ_1 and θ_2 of Table 4.1. From these regressions all bulbs in the sample were estimated to have initiated a flower after a thermal time of 340°Cd when the average temperature, T , was below the optimum, 12.8°C , or 261°Cd when T was above the optimum.

4.2.5.1 Thermal time for the second flower-bud to initiate

Again, the proportion of bulbs with a second bud initiated and the thermal time were linearly related (Fig. 4.7). When T was below T_0 , the thermal time required for all bulbs to initiate a second bud was estimated as 514°Cd and 575°C when T was above T_0 . From Fig. 4.6 it appears that the response to thermal time could be the same for temperature both above and below T_0 . This was tested by fitting a common line for both sets of data, two parallel lines were also fitted and two separate lines with the least squares. The sum of squares of these different lines were then compared and both the parallel lines and the common line were found to be significantly different from the two separate fitted lines, as shown.

4.2.6 Comparison between predicted and measured initiation rates for flower initiation

The time taken to $R = 0.5$ was estimated from Fig. 4.1; and $1/t$ gives the measured rate of initiation. By using the parameters in Table 4.1 for $R = 0.5$, the rate of initiation was predicted for constant temperatures using either:-

$$1/t (R = 0.5) = (T - T_b)/\theta_1 (R = 0.5) \quad \text{eq (4.1)}$$

Fig 4.5

The relationship between the proportion of bulbs with a flower initiated (R) and thermal time (θ) at temperatures (a) below (○) and (b) above (●) T_o

$$\begin{aligned} \text{a) } R &= -0.142 + 0.00294 \theta_1 \\ r^2 &= 0.95 \quad (p < 0.001) \end{aligned}$$

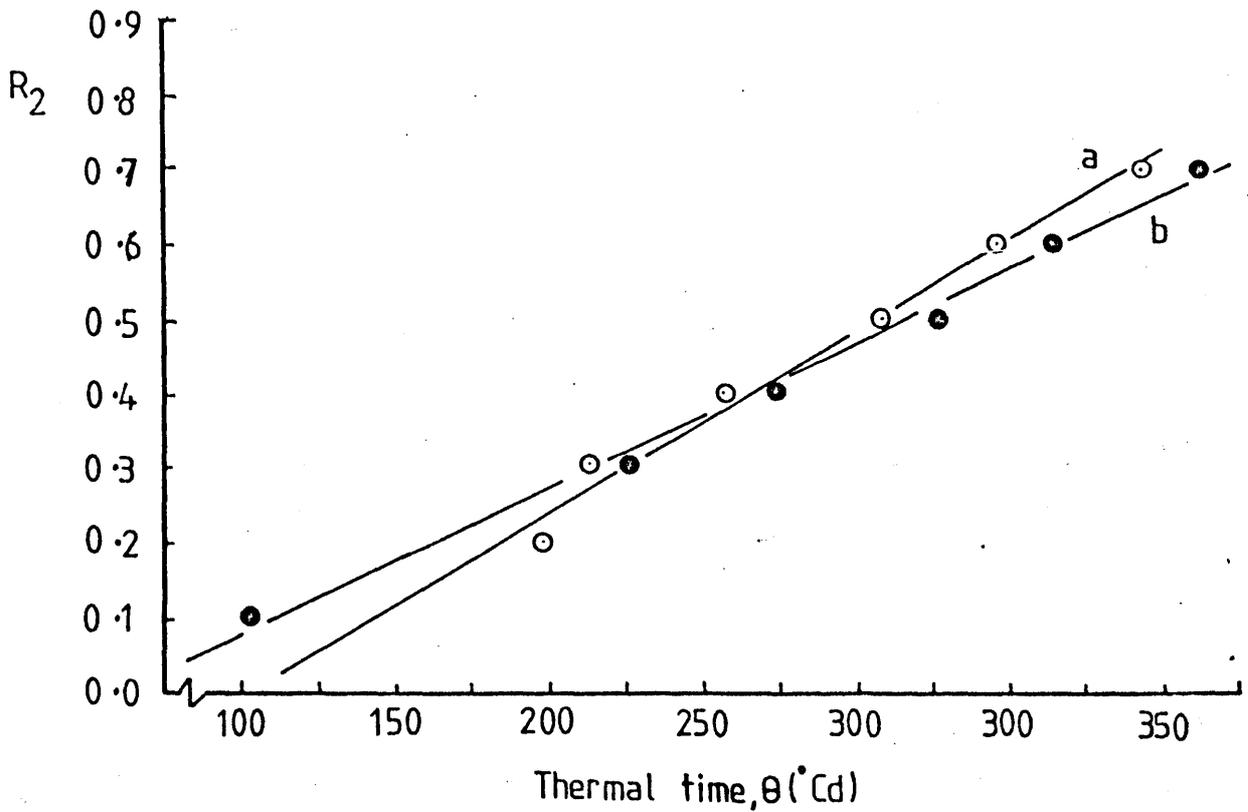
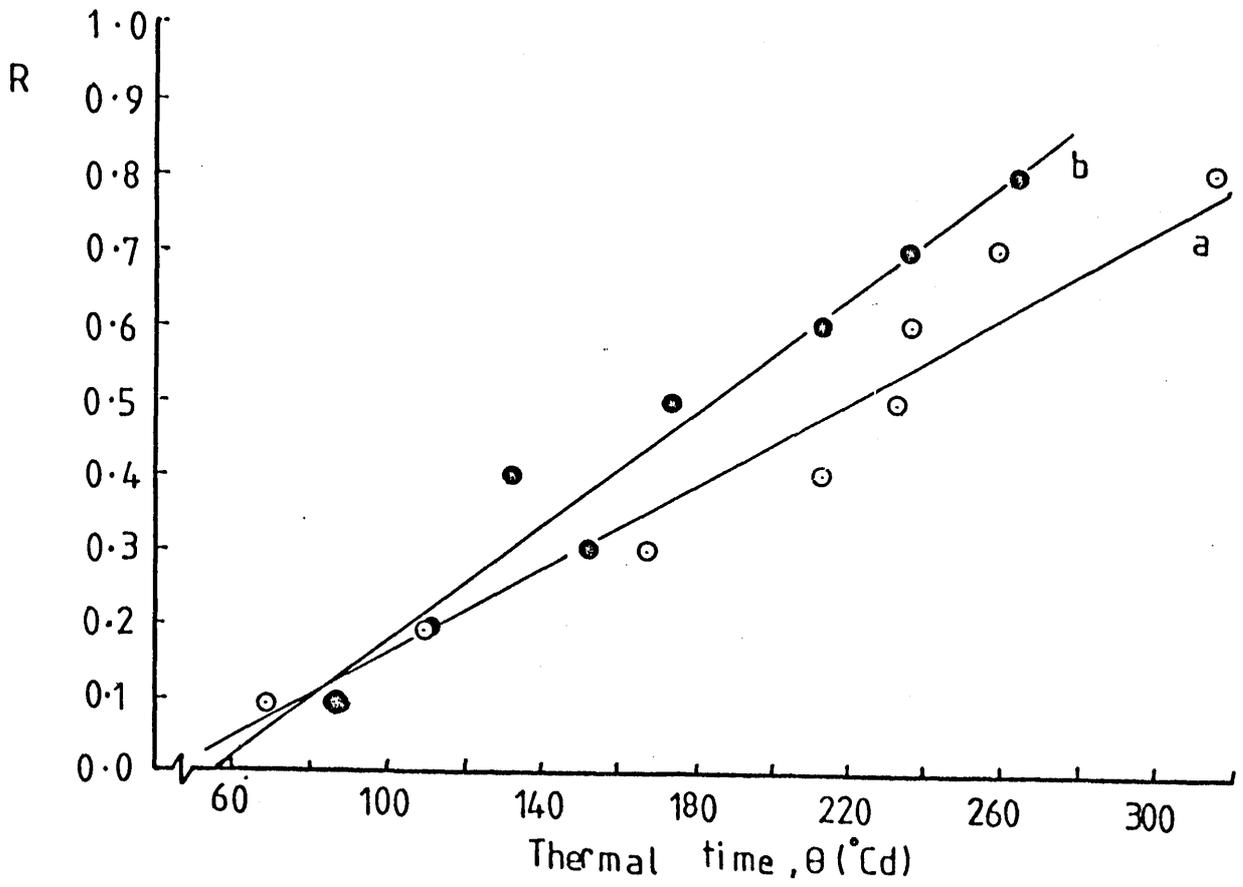
$$\begin{aligned} \text{b) } R &= -0.213 + 0.00383 \theta_2 \\ r^2 &= 0.96 \quad (p < 0.001) \end{aligned}$$

Fig 4.6

The proportion of bulbs with a second bud initiated (R_2) against thermal time for constant temperatures (a) below (○) and (b) above (●) T_o

$$\begin{aligned} \text{a) } R_2 &= -0.244 + 0.00242 \theta_1 \\ r^2 &= 0.98 \quad (p < 0.001) \end{aligned}$$

$$\begin{aligned} \text{b) } R_2 &= -0.121 + 0.00195 \theta_2 \\ r^2 &= 0.99 \quad (p < 0.001) \end{aligned}$$



for temperatures below the optimum but above the base temperature
or

$$1/t (R = 0.5) = (T_m - T)/\theta_2 (R = 0.5) \quad \text{eq (4.2)}$$

for temperatures above the optimum but below the maximum
temperature.

For transfer temperatures, measured rates were obtained from
curves similar to Fig. 4.1. The predicted rates were calculated
as above or if the temperatures were both above and below the
optimum:-

$$\theta_1 = t_1 (T_1 - T_b) + t_2 (T_m - T_2) (\theta_1/\theta_2) \quad \text{eq (4.3)}$$

θ_1 , θ_2 , T_b and T_m are all known from Table 4.2. Initiation to $R = 0.5$ had occurred when $\theta_1 (R = 0.5)$ was reached, t_1 was the time spent below T_0 and T_2 the time above T_0 . The rate of initiation is then $1/(t_1 + t_2)$. Occasionally initiation to 0.5 had occurred before bulbs were transferred to different temperatures. In those cases $\theta_1 (R = 0.5)$ was less than either $t_1 (T_1 - T_b)$ or $t_2 (T_m - T_2) \theta_1/\theta_2$, depending on whether the initial temperature treatment was above or below the optimum temperature.

Fig. 4.7 shows a plot of measured against predicted rates for both constant and transfer temperature treatments. If the thermal-time model was successful a straight line passing through the origin with a slope of 1 should be obtained. For constant temperatures a regression analysis passing through the origin accounted for 97.8% of the variation and the slope of the line was not significantly different from 1. However, for transferred temperature treatments, when a regression was fitted through the origin only 58.1% of the variance was accounted for and the slope was significantly different from 1 ($p < 0.001$). The predicted rate of initiation appears to be higher than the measured rate with transferred temperature treatments.

Fig 4.7

Measured flower initiation rates to $R = 0.5$ against predicted rates for constant (O) and transfer (●) temperature treatments. The line is the regression for constant temperatures passing through the origin

$$y = 1.0217x$$

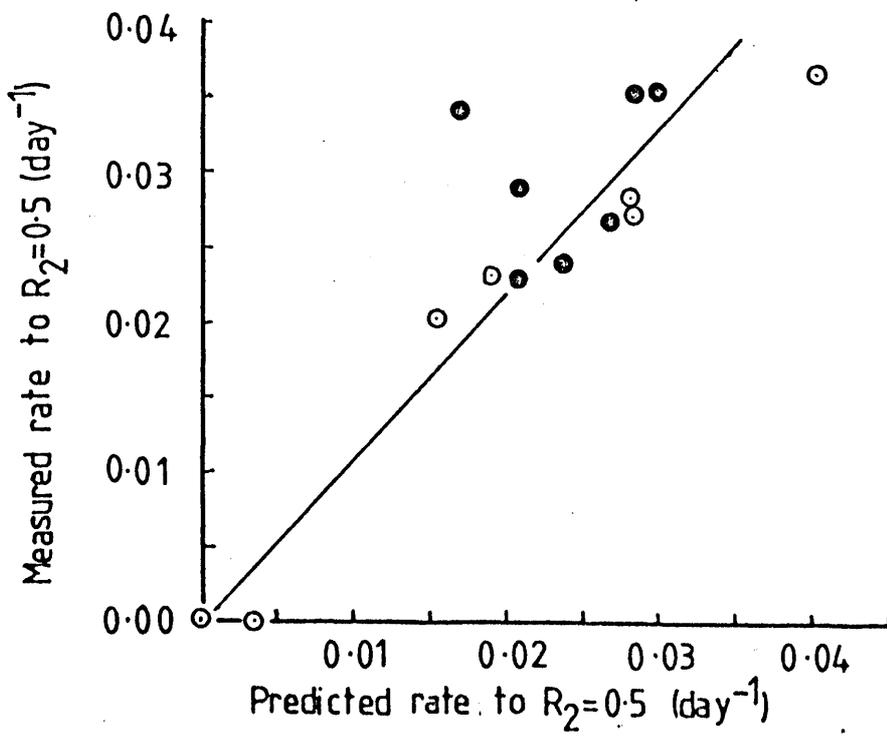
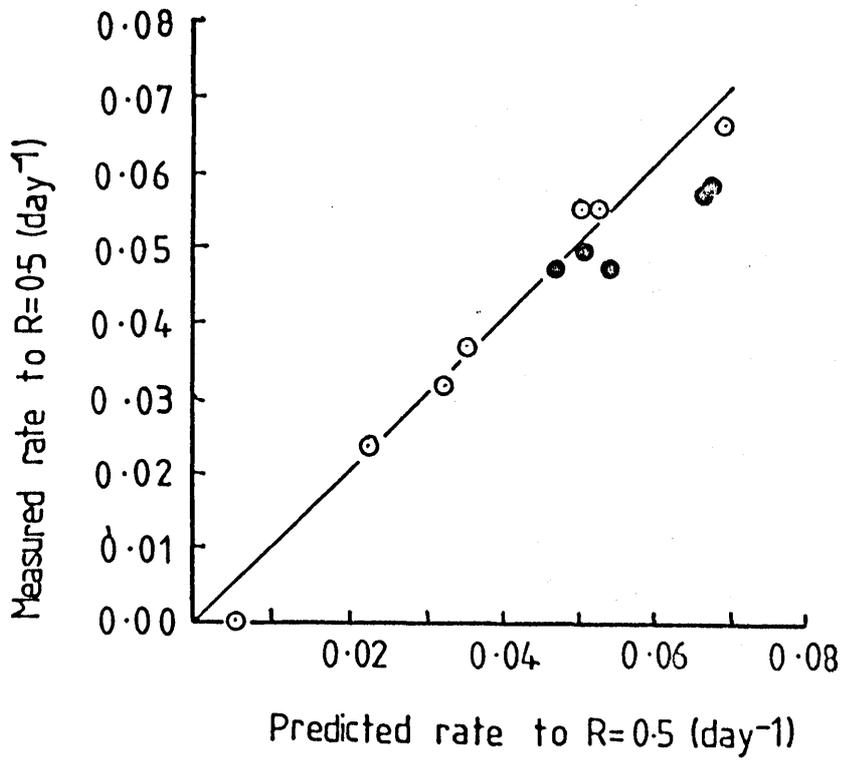
$$r^2 = 0.98 \text{ (} p < 0.001 \text{)}$$

Fig 4.8

Measured against predicted rates of second flower-bud initiation to $R_2 = 0.5$ for constant (O) and transfer (●) temperature treatments. The fitted line is the regression for constant temperatures passing through the origin

$$y = 1.13x$$

$$r^2 = 0.93 \text{ (} p < 0.001 \text{)}$$



4.2.6.1 Comparison of predicted and measured rates for the initiation of the second bud.

Fig. 4.8 shows the comparison for constant and transferred temperature treatments. For constant temperatures a regression line constrained to pass through the origin accounted for 92.6% of the variance and the slope was not significantly different from unity. For the transferred temperatures the residual variance exceeded that of the variance of the measured rates. The model was, therefore, unsuitable for plants transferred between temperatures. A regression analysis without constraints showed that there was not a significant linear relationship between measured and predicted values when transferred between temperatures. All the measured rates were greater than those predicted by thermal time when bulbs were transferred between temperatures. Some values differed by as much as 52% from the value predicted.

4.2.7 Flower organ initiation

The cardinal temperatures were determined as previously stated. Table 4.3 shows the regression parameters for those lines which were shown to be linear for anther initiation and table 4.4 for the first perianth whorl.

Table 4.3 Parameters for anther initiation. A = the proportion of bulbs in a sample of 20 with anthers initiated.

A	θ_1	r^2	(probability)	Tb	Tm	To	θ_2	r^2	(probability)
0.05	214	0.94	(p < 0.01)	-2.1	-	-	-	-	-
0.10	243	0.89	(p < 0.05)	-2.5	24.8	16.1	113	0.99	(p < 0.05)
0.20	216	0.88	(p < 0.05)	-0.6	26.1	12.7	218	0.91	(p < 0.05)
0.25	223	0.91	(p < 0.05)	0.8	26.0	13.3	228	0.94	(p < 0.05)
0.30	233	0.93	(p < 0.05)	0.9	25.8	13.1	242	0.95	(p < 0.05)
0.35	239	0.94	(p < 0.05)	1.0	25.6	13.0	251	0.97	(p < 0.05)
0.40	253	0.95	(p < 0.05)	1.0	25.6	13.0	268	0.97	(p < 0.01)
0.45	272	0.94	(p < 0.05)	1.0	25.6	13.0	287	0.97	(p < 0.05)
0.50	298	0.94	(p < 0.05)	1.0	25.7	13.0	315	0.96	(p < 0.05)
0.60	-	-	-	-	25.9	-	365	0.93	(p < 0.05)
0.70	-	-	-	-	26.1	-	417	0.92	(p < 0.05)

Table 4.4 Parameters of linear regression for the initiation of the first perianth whorl.

P_1 = the proportion of bulbs, from a sample of 20, with the first perianth whorl initiated.

P_1	θ_1	r^2	(probability)	Tb	Tm	To	θ_2	r^2	(probability)
0.1	233	0.95	(p < 0.05)	1.2	26.7	13.2	252	0.88	(p < 0.05)
0.2	301	0.92	(p < 0.05)	0.9	26.7	13.3	325	0.79	(p > 0.05)
0.3	348	0.91	(p < 0.05)	0.8	26.8	13.3	376	0.76	(p > 0.05)
0.4	389	0.91	(p < 0.05)	0.8	26.9	13.4	418	0.76	(p > 0.05)
0.5	361	0.87	(p < 0.05)	3.0	27.4	13.6	469	0.63	(p > 0.05)
0.6	391	0.87	(p < 0.05)	3.0	26.7	13.4	495	0.81	(p > 0.05)

A linear relationship was significant only when the proportion of bulbs at A was below 0.5 for temperatures below the optimum. This is possibly because too few samples were examined between A = 0.5 and 1.0. The mean cardinal temperatures were: Tb = $0.06 \pm 0.29^\circ\text{C}$; To = $13.4 \pm 0.39^\circ\text{C}$ and Tm = $25.7 \pm 0.14^\circ\text{C}$. The base temperature is a little higher (p < 0.001) and the maximum temperature a little lower (p < 0.05) than for flower initiation. This was because at 25°C and 2°C few, if any, flowers initiated

anthers within the period in which samples were taken. The last samples were dissected 65-70 days after the start of the treatments. The optimum temperature for flower initiation was shown not to differ from that for anther initiation. A prerequisite for anther initiation is flower initiation, therefore the cardinal temperatures would be expected to be similar to those for flower initiation. To remove the effect of flower initiation on the rate of subsequent development of flower organs, the weighted mean time for anther initiation was calculated. By subtracting this value from the weighted mean time for flower initiation the rate of anther initiation once flower initiation had occurred could be calculated. Fig. 4.9 shows the two straight lines fitted to these rates, the base temperature, T_b , was then 1.36; $T_o = 16.8^\circ\text{C}$ and $T_m = 25.0^\circ\text{C}$. This illustrates further the narrowing of the band of temperatures over which anther initiation will occur within 65-70 days. In addition, it shows that once flower initiation has occurred the optimum temperature for anther initiation is nearer 17°C than 13°C .

From the regression analysis of proportion of bulbs with anthers initiated and the thermal time, Fig. 4.10, the thermal time required from the beginning of the cool treatment for all bulbs to initiate anthers was estimated as 487°Cd for average temperatures below T_o and 557°Cd for temperatures above T_o . This linear regression did not hold for the first bulbs in the population to initiate anthers. Anthers were initiated in these bulbs at much later thermal times than would have been estimated from the linear regression extrapolated back from larger proportions of the population. This was probably because flower initiation had only just occurred at this time in these first bulbs and a certain time is required before anthers are then capable of being formed. When measured and predicted rates to $A = 0.5$ were plotted for anther initiation, then for constant temperatures the measured rates were greater than the predicted rates. A regression passing through the origin accounted for 98.6% of the variance but the slope was significantly steeper than unity. When a similar regression analysis was attempted for alternating temperatures the residual variance exceeded the variance of the measured rates of anther initiation.

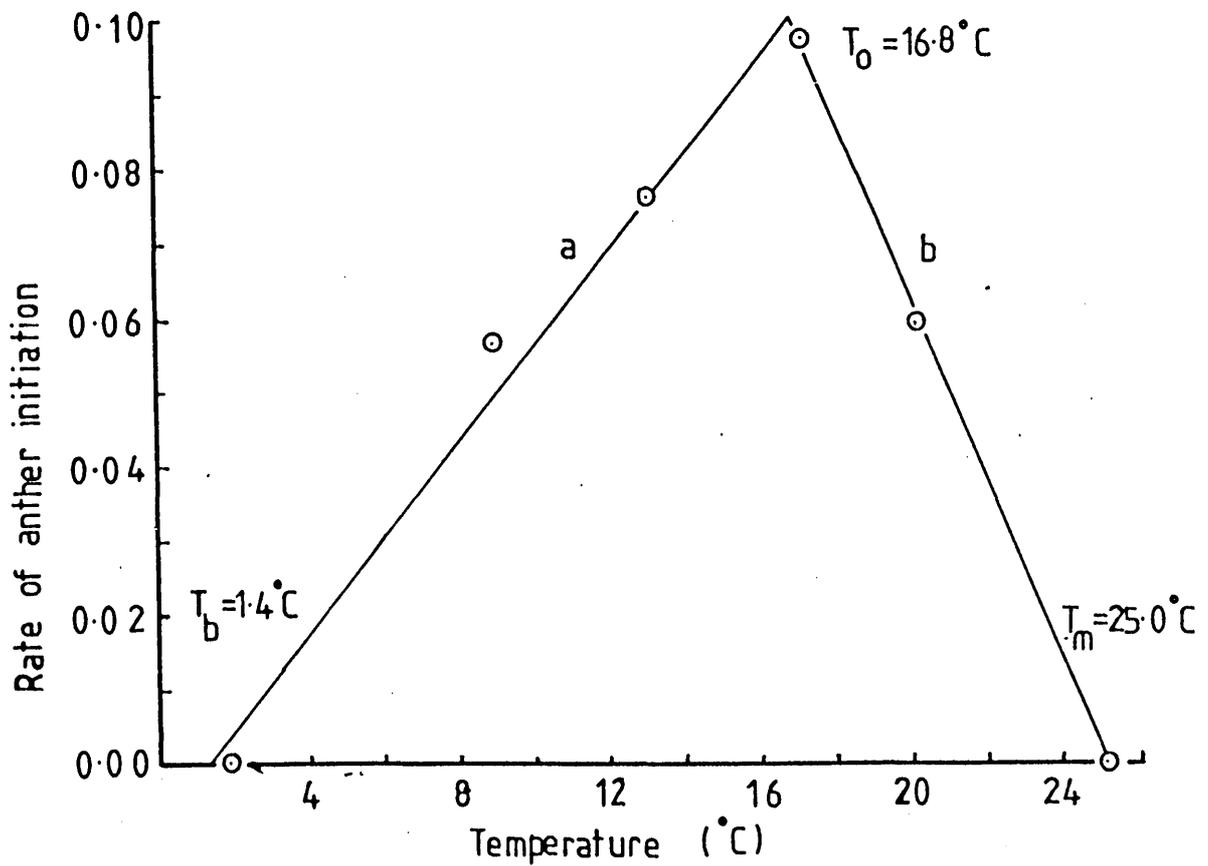


Fig 4.9

The rate of anther initiation following flower initiation as a function of constant storage temperature

a) $\frac{1}{t} = -0.00881 + 0.00647T$
 $r^2 = 0.98$ ($p < 0.01$)

b) $\frac{1}{t} = 0.303 - 0.121T$
 $r^2 = 1.0$ ($p < 0.05$)

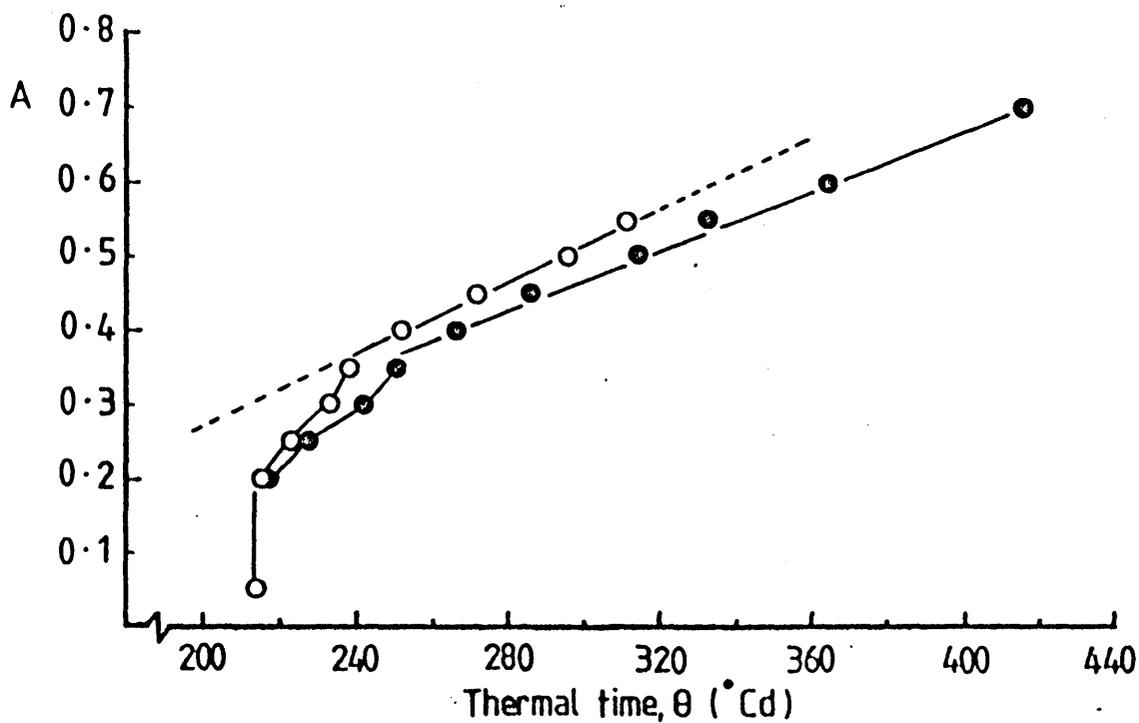


Fig. 4.10 The proportion of bulbs with anthers initiated on the first bud (A) against thermal time (a) below (o) and (b) above (●) T_o .

Linear part of relationship

$$(a) \quad A = -0.262 + 0.00259 \theta_1$$

$$r^2 = 0.99 \quad (p < 0.001)$$

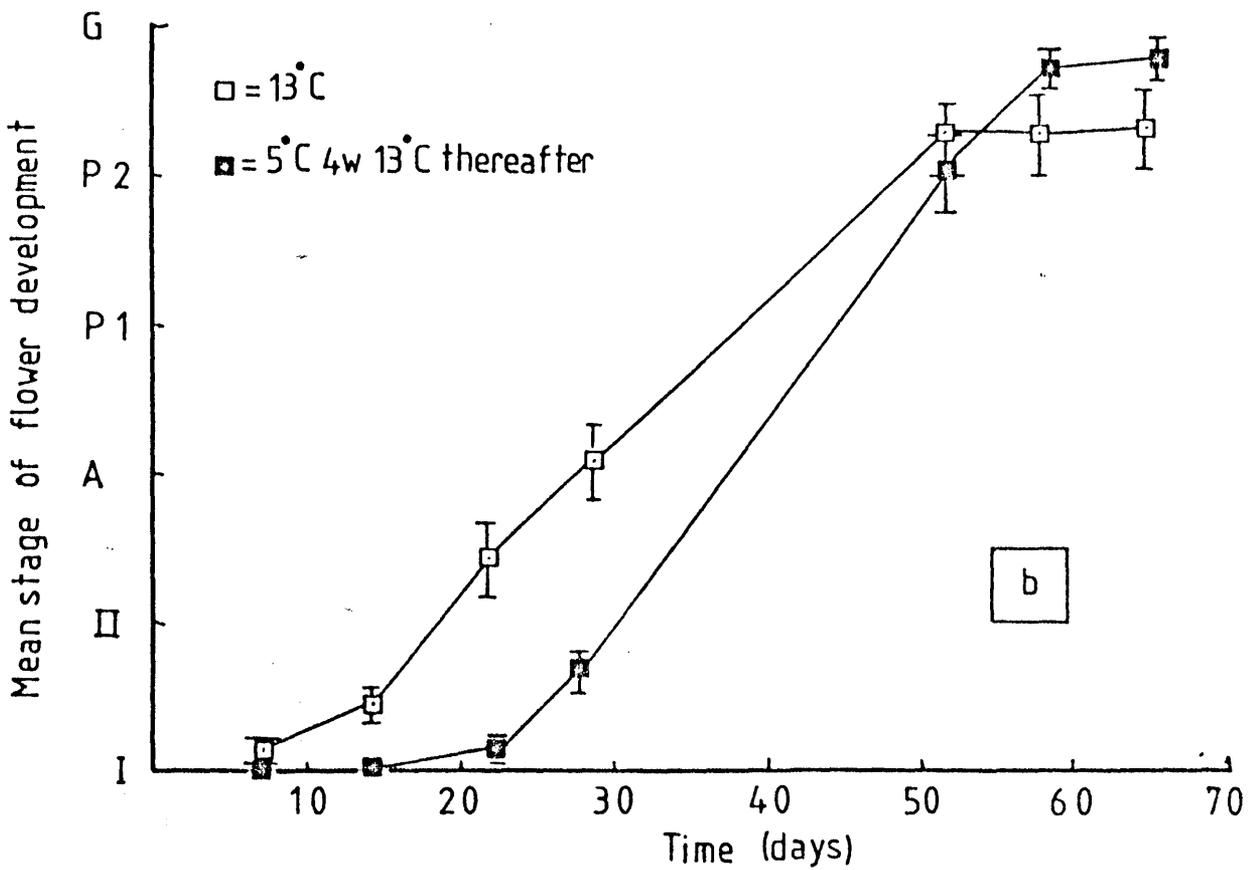
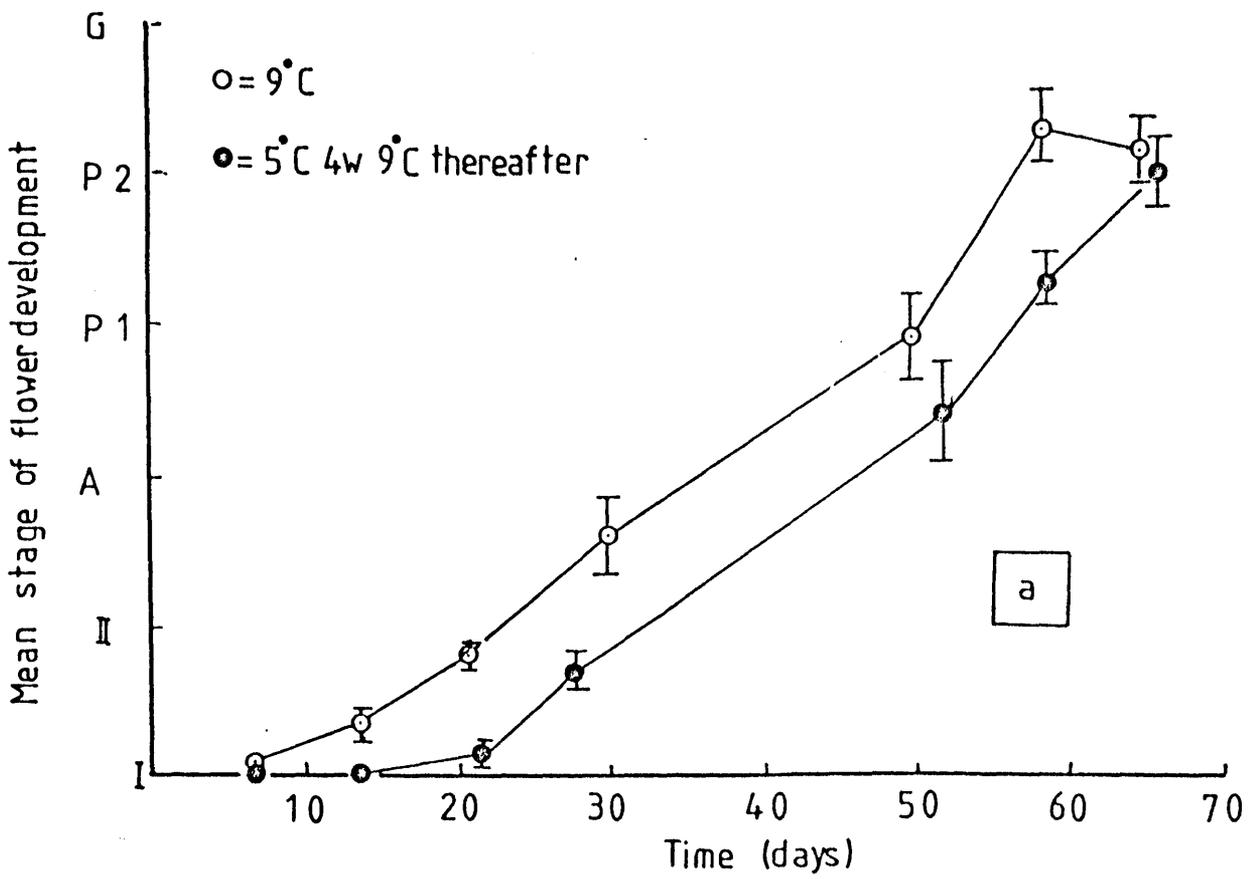
$$(b) \quad A = -0.154 + 0.00207 \theta_1$$

$$r^2 = 0.99 \quad (p < 0.001)$$

From table 4.4 it can be seen that a linear relationship was not proved to exist between rate of perianth initiation and temperature, in most cases, for temperatures in excess of T_0 to T_m . With the best fitting lines, however, T_m and T_0 were consistent. If the rate of perianth initiation had been determined at more temperatures between T_0 and T_m then a linear relationship might have been found. At 25°C no bulbs initiated perianths but at 20°C these were initiated. If the T_m could be more accurately determined, i.e. the temperature at which perianths are not initiated between 20 and 25°C , then a linear relationship might be shown to exist. The average base temperature for perianth initiation was $1.62 \pm 0.44^\circ\text{C}$.

Fig. 4.11 shows the mean stage of development of the first flower bud at various times under either constant temperatures of a) 9°C , b) 13°C and c) 17°C or after transfer to these temperatures following the first four weeks at 5°C . It can be seen that when 9°C follows the 5°C treatment, flower initiation is delayed by about 7 days compared to the constant 9°C treatment. However, once the flower is initiated the rate of development is similar, the two curves running almost parallel. When the 5°C treatment is followed by a 13°C treatment, however, although initiation seems to be delayed by an even longer period of time, about 11 days, both treatments reach the initiation of the second perianth whorl, (P2) at approximately the same time. When 17°C follows the 5°C treatment, the rate of flower organ differentiation following flower initiation, is even greater than at a constant 13°C . On average, bulbs under this transfer treatment, actually initiate the second perianth whorl and gynoecium sooner than bulbs stored at a constant 17°C - a typical vernalization response.

No blasting of flowers was observed until the flower was fully formed, then it was observed in bulbs stored at constant temperatures of 17°C or 20°C . No blasting was observed in bulbs transferred to or from these temperatures.



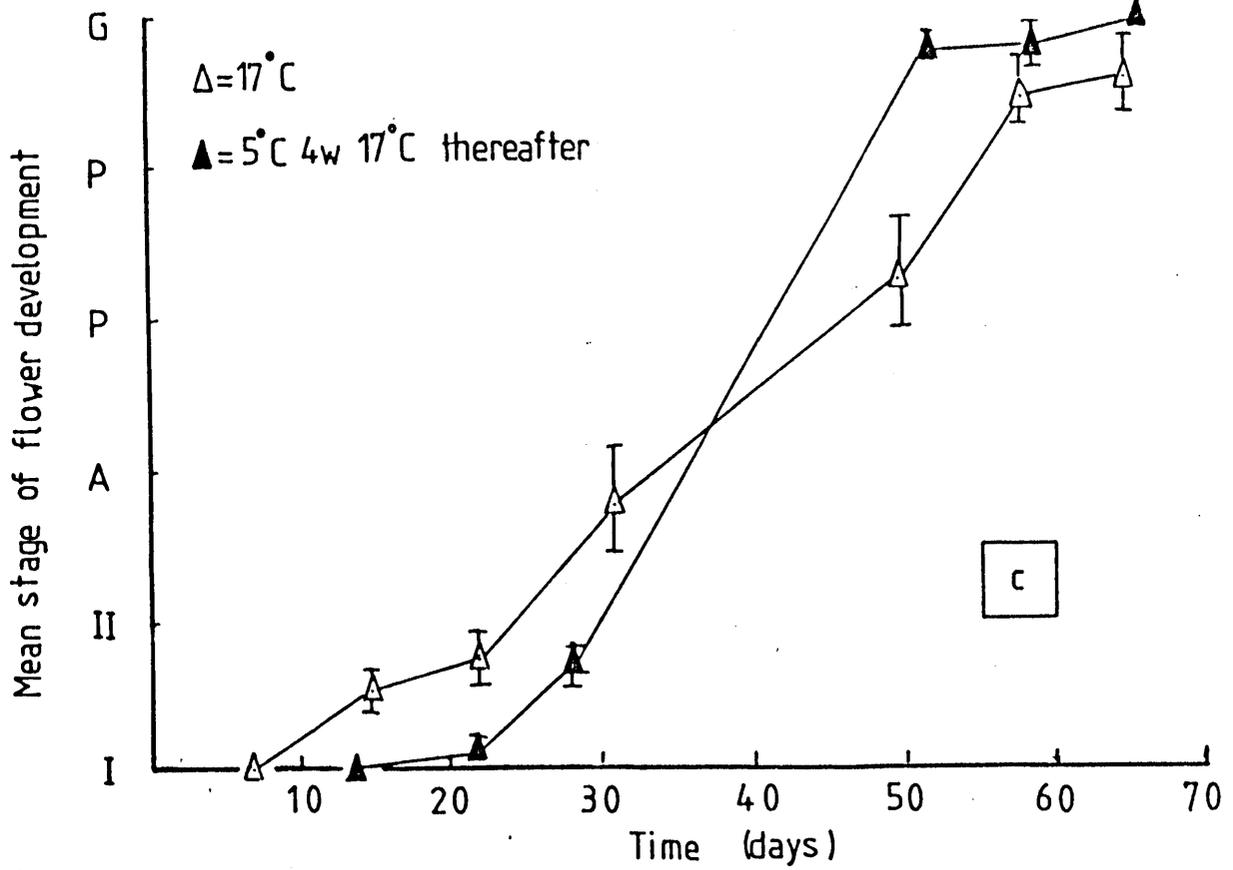


Fig 4.11

The mean stage of development of the first bud at various times, either when stored at constant temperature (open symbols) or after transfer to that temperature following 4 weeks at 5°C (closed symbols) a) 9°C : b) 13°C and c) 17°C

4.3. Discussion

The relationships between the rate of leaf, flower or anther initiation to constant temperatures, were shown to be adequately represented by two straight lines, one above and the other below the optimum temperature. Similar linear relationships have been reported for several physiological processes in many species. For example, the rate of seed germination, leaf initiation and flower initiation in pearl millet (Pennisetum typhoides), (Garcia-Huidobro et al., 1982; Ong, 1983), flower initiation in carrot (Daucus carota), (Basher, 1984) and leaf appearance, rate of branching and flowering in groundnut (Arachis hypogaea), (Leon and Ong, 1983).

From the intercept of the two straight lines representing the relationship between the rate of leaf initiation to constant temperatures, the optimum was defined as 13.1°C. This is in agreement with Blaauw (1941b) who found 13°C to be the optimum for leaf initiation in 'Imperator'. Little is known about the minimum temperature at which leaves can be initiated. It is possible, from the data of Blaauw, to calculate and plot the rate of leaf initiation against constant temperatures (Fig. 4.12). A linear relationship is again shown to exist and by extrapolation of this line obtained between 13 and 2°C, the base temperature (T_b) for 'Imperator' can be estimated as -0.4°C, the same T_b as obtained here for 'Ideal'. It is not possible to estimate the maximum temperature at which leaf initiation occurs from previous publications. Beijer (1952) noted that leaf primordia were initiated slowly at 25.5°C. The estimated maximum temperature for leaf initiation here was 26.7°C. Recent recommendations to retard growth and development are to store bulbs at 30°C (Schipper, 1982). At this temperature shoot and root growth are inhibited to a greater extent than at 25.5°C. There are no reports of leaf primordia being initiated at 30°C.

The form of flower induction is still uncertain. Sano (1974a,b) suggested that bulbs become 'ripe to flower' with increasing age. This term was not used to describe the end of the

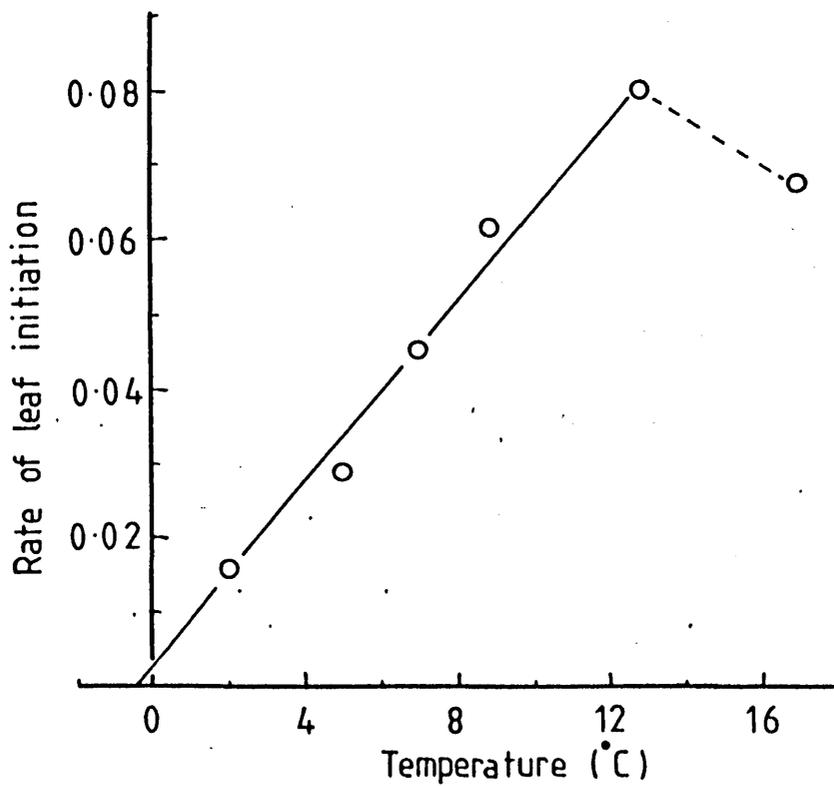


Fig 4.12

Rate of leaf initiation as a function of constant storage temperature for 'Imperator' Rates calculated from Blaauw (1941).

$$1/t = 0.00260 + 0.00611T$$

$$r^2 = 0.98 \text{ (} p < 0.001 \text{)}$$

juvenile phase, when plants are first able to perceive the conditions capable of bringing about induction, as used by Schwabe (1971). Instead, Sano uses the term 'ripe to flower' to explain autonomous induction such as that reported in Pisum sativum (Haupt, 1969). In peas induction appeared to be an effect of age, but was promoted by long days and vernalization and delayed by gibberellin.

Halevy (1984) claims that induction in iris is autonomous. Certainly photoperiod does not appear to affect induction, i.e. iris is a day neutral plant. At the time of induction the leaves, which would detect the photoperiod for induction, are enclosed within the scales of the mother bulb and hence are unable to sense the photoperiod.

There was a maximum leaf number when a plant initiated a flower regardless of the temperature within the range tested here. This is in agreement with Sano, in that flower induction occurs at a specific stage of development independent of the environmental conditions. It is known that bulbs stored continuously at 30°C will not initiate a flower, but at such temperatures all growth and development is reduced to a minimum, preventing the plant from reaching the stage of development when a flower could be initiated. Further evidence for flower induction occurring at a specific developmental stage rather than in response to environmental stimuli is the similarity of the optimum temperature for both flower and leaf initiation, that is the more rapid the vegetative development the earlier the flower initiation on a chronological time scale.

Induction occurring earlier in plant development would result in flower initiation at a lower leaf number. Plants grown at 2, 5 and 9°C had a lower average leaf number than plants grown at 13, 17 and 20°C. Other experiments here (see Table 5.6a), and by other workers (Blaauw, 1941b; Hartsema and Luyten, 1955; Sano, 1974a) found decreasing leaf number to flower initiation with lower constant storage temperatures from 25 to 2°C. In this experiment, with temperatures above 13°C there was no further increase in the leaf number. It is possible that a long storage period at high temperatures before the start of the cool period investigated,

allowed the production of a large number of leaf primordia so that plants were already at a late stage of development and were able to initiate a flower without the initiation of further leaves.

There are two possible explanations for low temperatures reducing the number of leaves produced before flower initiation:-

1. Low temperatures reduce the rate of leaf initiation, hence if induction occurs at a fixed age from the beginning of temperatures which allow growth and development, then those temperatures giving the lowest leaf initiation rates would also give the fewest leaves at the chronological time of flower initiation.
2. Low temperatures may accelerate and high temperatures delay induction. A cold treatment is then a quantitative thermoinduction causing evocation at the apex and initiation earlier in the plant's development.

If the first explanation was true then since the leaf initiation rate is also reduced by temperatures greater than 13°C, the leaf number below the flower would also be expected to fall with supra-optimal temperatures. This was not the case.

Is it possible that a combination of these two explanations causes the observed effects in that low temperatures reduce leaf initiation rates, but not to the same extent as the rate of flower induction while high temperatures delay the induction? Comparison of the respective base temperatures for leaf and flower initiation support this view. For flower initiation in 'Ideal', T_b was -2.7°C lower than that for leaf initiation. For flower initiation in 'Imperator' estimated from the results of Blaauw (1941b), T_b was -2.4°C, again lower than the base temperature for leaf initiation. Flower initiation could, therefore, occur at temperatures at which the leaf initiation rate was zero. From this argument it would appear feasible that low temperatures could reduce leaf initiation rates to a greater extent than flower induction, resulting in fewer leaves at the time of initiation.

Evidence strongly in favour of the second theory, i.e. low temperatures accelerating induction is derived from the thermal time model (equations 4.1, 4.2 and 4.3). Having established that a linear relationship adequately describes the rate of flower initiation and anther formation at constant temperatures, these equations can be used to predict the rates of flower initiation and differentiation when bulbs are transferred between temperatures. When measured and predicted rates were compared discrepancies were shown to exist. This was due to initial storage temperatures influencing the rates of induction and initiation of the flower at subsequent temperatures. The effect of temperature has, therefore, been shown not only to be a direct one, e.g. altering the relative rates of leaf and flower development, but as causing stable changes in development which are expressed even when the conditions which were responsible for their implementation are no longer applied to the bulbs.

Rodrigues Pereira (1964) suggested that low temperatures were responsible for the active transport of, or rendering the apex fit to respond to, three growth substances which were seen to move from the scales to the apex just before and during flower initiation. It appears that once such cold stimulation has occurred, development at the apex is not reversible by subsequent temperature treatments.

From the thermal-time model, results show the importance of thermal history in the initiation of the flower, it can be concluded that induction in iris is accelerated by a cold treatment, in terms of the development of the plant. At 13°C autonomous initiation occurs in the shortest chronological time, as a consequence of a combination of the most rapid vegetative development occurring at this temperature, so the plant attains a state at which autonomous induction can occur most rapidly, and slightly earlier induction in the development than occurs at higher temperatures. Lower temperature enables initiation at a lower leaf number, but as can be seen in Fig. 4.11, initiation in chronological time is delayed. Such cold treatments were seen to accelerate the rate of flower part differentiation at subsequent

warmer temperatures. Similar observations have been made with many other plants in that flower development is accelerated and flower numbers increased by the application of the optimum inductive treatment (Evans, 1969). Evidence for induction occurring within the first 4 weeks of low temperature treatment, after sufficient heat curing, is given in Chapter 5, where the optimum temperature for initiation is interrupted by high or low temperatures at different times.

The optimum temperature for flower part differentiation after flower initiation, was 17°C. This explains why the commonly practised reverse treatment, where 17°C follows 9°C, can reduce the time to anthesis by five to seven days (Durieux and Pagter, 1967). Abnormal flowers can be produced if bulbs are stored at very high temperatures, 31-38°C before the cool treatment, such a treatment can produce flowers with fewer flower parts than the normal two whorls of three perianths, three anthers and a tricarpellate ovary. Kamerbeek and de Munk (unpublished) have shown that quaternate, quinate or biternate flowers could be produced by storing at sub-zero temperatures during flower formation. No deviations in flower part numbers were observed in the 2°C treatment here, but dimerous flowers (5%) were observed when stored continuously at 20°C.

Once flowers were fully differentiated then prolonged storage at the higher temperatures resulted in the abortion of the flower (e.g. 23% at constant 17°C and 20% at 20°C).

Further work is necessary to investigate the initial heat treatment effects on the thermal time to initiation. The influence of smoke and ethylene on the thermal time to initiation should also be examined. The use of thermal time to initiation is not, at present, possible. The conditions of the model were not fulfilled, in that effects of temperature sequence were detected. Further work is required to investigate the factors involved in these sequence effects, before their influence can be accounted for in a thermal time model.

4.4 Summary

1. The rate of flower initiation (the reciprocal of time to flowering) in iris, was linearly related to the constant storage temperature following a fixed heat curing treatment. The rate increased linearly with increasing temperature to an optimum (shortest chronological time) at 13°C. The rate then decreased linearly with higher temperatures. By extrapolation of these straight lines the minimum, T_b , and maximum, T_m , temperatures for initiation were estimated as -2.7 and 26.5°C respectively.
2. Rates of leaf, second flower-bud and anther initiation were also linearly related to constant temperatures. Anther initiation rates were influenced by the rate of flower initiation per se. This influence was removed by considering rates of anther initiation from flower initiation rather than the beginning of the cool treatment. A shift in the optimum temperature following flower initiation was then detected.
3. Flower initiation rates were influenced by the thermal history, bulbs transferred between temperatures behaved differently to bulbs stored continuously under such temperatures. Initiation was slower when bulbs were transferred between temperatures, but initiation of the second bud and flower part differentiation were faster.
4. Induction occurs at a specific stage in the plant's development. The rate of leaf initiation and flower initiation both had the same optimum temperature. The more rapid the vegetative development, the sooner the state for induction was attained.
5. Low temperatures cause a quantitative thermoinduction resulting in flower initiation earlier in the plant's development. The treatment necessary for this thermoinduction delays flower initiation on a chronological time basis, but subsequent flower differentiation can occur at a faster rate.

5. TEMPERATURE AND POST-INITIATION
FLOWER DEVELOPMENT

5. TEMPERATURE AND POST-INITIATION FLOWER DEVELOPMENT

This chapter describes investigations into the influence of both pre-planting storage temperatures, and of conditions in the glasshouse during growth, on flower and whole-plant development. The aim was to elucidate the mechanism of flower-bud abortion.

5.1 Pre-planting temperature treatments

Introduction

Pre-planting temperature treatments influence post-initiation flower development (Fortanier and Zevenbergen, 1973). There are two possible mechanisms for such an influence:

1. A direct influence, by changing the way in which the flower is initiated. For example adverse temperatures during gynoecium initiation resulting in abnormal formation may affect subsequent flower development. We have observed the gynoecium to be the flower organ which shows the first signs of abortion when a bud fails, see Chapter 1. By removing parts of the flower and observing flower-stem extension Vonk and Ribot (1982) concluded that the gynoecium played an important role in the promotion of this extension.

2. Indirectly, by influencing aspects of development elsewhere in the plant, which in turn influence flower development. For example, low pre-planting temperatures induce daughter-bulbs and subsequent warmer temperatures favour their growth (Le Nard, 1973; Sano, 1974c). Such treatment will increase the competition between the daughter bulbs and the flower. The lower the storage temperature, the earlier flowering occurs in the plant's development i.e. after fewer leaves have been initiated (Blaauw, 1941; Sano, 1974a). Pre-planting temperatures also influence the rate of leaf growth and the final leaf length. Leaf growth is

stimulated by storing bulbs at 17°C before 9°C and is reduced by the reverse treatment (9 followed by 17°C) (Schipper, 1983). As storage temperatures increase from 8 to 20°C, final leaf length is increased (Sano, 1974b). Storage temperatures also affect the mobilization of reserves in the mother bulb and hence their availability for the flower and other sinks. Starch hydrolysis increases as temperatures fall from 25 to 10°C and then decreases again as temperatures are lowered below 10°C (Halevy, 1962).

The indirect influence is examined here. Previous reports have suggested that to allow a greater proportion of assimilates and reserves for flower growth and development, leaf growth should be reduced to a minimum (Fortanier and Zevenbergen, 1973; Kamerbeek, Durieux and Schipper, 1980; Schipper, 1981). Increased vegetative growth, encouraged by pre-planting treatments, would therefore be expected to result in an increased failure of flower development. This was tested by subjecting bulbs to a range of pre-planting cold treatments that would give rise to plants showing differing degrees of vegetative growth and various sink strengths of other organs in major competition with the flower.

5.1.1 Materials and Methods

'Wedgwood' 10-11cm grade bulbs were stored following cleaning and grading, at 25°C for 11 weeks to ensure sufficient heat curing before applying the pre-planting cool treatments. All treatments were applied for a total of 9 weeks, using constant temperatures of 5, 9, 13, 17 and 20°C and transfer treatments from 5°C to 9, 13, 17 or 20°C. Transfers were made from 5°C at 3 times: 1, 4 or 6 weeks. In addition, a 13°C treatment was interrupted after 1 week with 3 weeks at either 5 or 20°C or after 4 weeks with 2 weeks at 5 or 20°C, making a total of 21 treatments.

The treatments in which the proportion of time spent at 5°C was altered followed by different transfer temperatures were aimed to provide plants with flowers initiated at different stages of development. Interrupting the optimum temperature for leaf and flower initiation (13°C) with higher or lower temperatures was included to obtain bulbs at different stages of development which

had reserves which were adequately mobilized. All these treatments were applied to 'dry' bulbs as described in Chapter 3.

Following these treatments, bulbs were planted on 13 Feb 1984 at temperatures of 12.5°C at night and 15°C day temperatures, venting at 18°C. These conditions were used so that they would not, per se, cause flower blasting but would allow the influence of the pre-planting treatment to be assessed. Plants were harvested either when the first bud reached anthesis or, if this bud aborted, on the day when most plants from that treatment reached anthesis. The number of leaves, flower buds initiated, flower buds developing, scales, sheath leaves and length of the flower stem were recorded. The dry weights of the leaves, daughter bulbs, remaining mother bulb and flower were determined as described in the Chapter 3.

5.1.2 Results

5.1.2.1 Flower growth and development

The numbers of buds initiated in each treatment were counted. Table 5.1 shows the percentage of bulbs which had initiated two or more flowers.

Table 5.1 The percentage of bulbs with two or more flowers initiated following different transfer temperature treatments.

Weeks at 5°C	Weeks at Transfer temp	Transfer temperature (°C)				Time Means
		9	13	17	20	
9	0			100		100
6	3	88	83	73	80	82
4	5	100	100	93	62	90
1	8	100	100	100	100	100
0	9	100	100	100	100	100
Temperature means		97	96	92	88	94

Many plants were lost, the stock of bulbs being badly infected with iris mild mosaic virus and Penicillium corymbiferum. Only bulbs not showing symptoms of either of these diseases are included in the results, causing large differences in the number of replicates per treatment.

A binomial analysis was used to compare the proportion of bulbs with two or more buds initiated. The later the transfer from the 5°C treatment to the subsequent temperature occurred, within the 9 week treatment, the fewer the bulbs with two or more flowers initiated ($p < 0.001$). A single week at 5°C before transfer did not reduce the number of buds initiated; in fact these treatments were the only ones in which more than two flowers were initiated. Effects of the transfer temperature and the time x temperature interaction were not significant.

A similar analysis of the proportion of bulbs with two or more flower buds developing at harvest time again revealed an effect of time of transfer ($p < 0.001$). This could be due to the differences in number of buds which were initiated being reflected in the number which ultimately developed. There was also a significant time x temperature interaction in the proportion of bulbs with two or more flowers developing ($p < 0.001$), with fewer buds developing with later transfers to the higher temperatures, Table 5.2. There was a particularly high percentage of bulbs with two or more flowers developing in the constant 20°C treatment and the 5°C, 1 week followed by 20°C for 8 weeks treatment. This is possibly because of delayed initiation in these two treatments until after planting. The plants were then more influenced by the conditions in the glasshouse than by the pre-planting treatment itself.

Table 5.2 The percentage of bulbs with two or more flowers developing at the time of harvest, following different transfer temperature treatments.

Weeks at 5°C	Weeks at transfer temp	Transfer temperature (°C)				Time Means
		9	13	17	20	
9	0	75				75
6	3	81	44	0	10	40
4	5	60	67	60	31	55
1	8	67	89	67	95	82
0	9	75	73	73	96	79
Temperature means		71	68	52	66	66

The mean stem lengths of the flowers were calculated from all the bulbs in each treatment, regardless of whether they reached anthesis or not and separately for those bulbs in which at least the first bud reached anthesis, Table 5.3 a + b.

Table 5.3 The mean flower stem length (mm) following different transfer temperature treatments for (a) all bulbs (b) those in which the first bud reached anthesis.

a)		Transfer temperature (°C)				Time Means
Weeks at 5°C	Weeks at transfer temp.	9	13	17	20	
9	0	428				
6	3	433	396	332	148	349
4	5	366	359	317	251	326
1	8	443	403	353	410	407
0	9	409	352	450	593	453
Temperature means		419	396	383	347	390
b)		Transfer temperature (°C)				Time Means
Weeks at 5°C	Weeks at transfer temp.	9	13	17	20	
9	0	462				
6	3	433	403	368	264	392
4	5	458	400	345	325	382
1	8	447	403	430	-	424
0	9	409	393	450	593	467
Temperature means		439	416	412	444	426
a)	Time SED	= 22	b)		Time SED	= 16
	Temp SED	= 19			Temp SED	= 15
	Time.Temp SED	= 60			Time.Temp SED	= 38

Table 5.4 The mean number of days from planting to anthesis following different transfer temperature treatments.

Weeks at 5°C	Weeks at transfer temp	Transfer temperature (°C)				Time Means
		9	13	17	20	
9	0	83.6				
6	3	80.6	80.6	76.4	76.2	79.4
4	5	82.6	79.4	73.6	80.2	78.7
1	8	89.6	79.4	84.7	-	84.0
0	9	82.1	78.5	84.3	97.2	86.1
Temperature means		83.4	80.0	80.5	87.2	82.3

Time SED = 0.8
 Temp SED = 0.8
 Time.Temp.SED = 2.0

The shortest mean stem lengths were observed when bulbs were transferred at about the mid-point of the 9 week treatment i.e. after 4 weeks at 5°C, for both sets of results ($p < 0.001$). This suggests that the short stems were not due to the abortion of the flower but to a direct effect of transfer time on stem length. As the time of transfer from 5°C was shifted towards either end of the 9 week treatment, stems were longer than those obtained with the mid-point transfer. There were no significant differences between the average stem length for the constant 5°C treatment and the average for all the other constant temperature treatments (i.e. 9 weeks at 9, 12, 17 or 20°C). For both sets of results there was a significant interaction ($P < 0.001$) between the time of transfer and the temperature to which they were transferred, with later transfers and transfers to higher temperatures, producing the shortest stems. Temperature did not appear to affect the stem length of those plants in which the first bud developed to anthesis ($p > 0.05$) except at the late transfers, but if all bulbs were considered, the stem length decreased slightly with increasing transfer temperature from 419 to 347mm ($p < 0.01$).

The rate of development of the flower to anthesis was significantly increased with the later times of transfer and by particular transfer temperatures ($p < 0.001$), Table 5.4. The constant treatments of 5,9,8,17 and 20°C, resulted in slower development than when bulbs were transferred from 5°C to these temperatures after 4 or 6 weeks. This increased rate was not detectable after only one week at 5°C before transfer. When considering the temperatures independently of time of transfer, both 13 and 17°C gave faster rates of development than 9°C. There was, however, a significant interaction between time of transfer and temperature. The constant 20°C treatment produced the slowest rate of development. The constant 5,9 and 13 treatments reached anthesis at approximately the same time, whilst the 17°C treatment delayed anthesis but not as much as 20°C. Storage for 4 weeks at 5°C before transfer to 17°C gave the most rapid development. Similarly, after 6 weeks at 5°C the 17 and 20°C treatments gave the most rapid development. Transferring to 9 or 13°C after 6 weeks was no more effective than keeping the bulbs at a constant 5°C.

The dry weight of the flower stem and flower was significantly decreased by a later time of transfer ($p < 0.001$), the 13°C transfer temperature ($p < 0.01$) and there was an interaction between time of transfer and temperature ($p < 0.001$). Table 5.5 shows the dry weights of the flower and flower stem at harvest time.

Table 5.5 Effect of pre-planting temperature treatment on the dry weight of the flower plus stem (g) at anthesis

Weeks at 5°C	Weeks at transfer temp	Transfer temperature (°C)				Time means
		9	13	17	20	
		Dry weight of flower + stem (g)				
9	0	3.25				
6	3	3.07	2.44	1.85	1.32	2.44
4	5	3.49	2.40	2.11	2.12	2.51
1	8	3.00	2.47	3.11	-	2.78
0	9	2.44	2.63	3.24	4.47	3.27
Temperature means		3.00	2.63	2.75	3.15	2.85

Time SED = 0.16 Temp SED = 0.15 Time.temp SED = 0.38 - = no data

Heaviest flowers were produced by storing the bulbs at 20°C for the whole 9 weeks. It was apparent, when considering the time of transfer independently of the transfer temperature, that heavier flowers were produced by those treatments which did not involve a transfer between temperatures. If a transfer did occur, late transfers produced lighter flowers at high temperatures with little or no effect of transfer time at the lower temperatures.

5.1.2.2 Vegetative growth and development

The temperature treatments applied caused differences in the plants' development. The following results examine only those plants in which at least one bud developed to anthesis.

Table 5.6 shows the mean number of foliar leaves (not including the spathe leaves), the mean total leaf dry weight and the mean dry weight per leaf for the different transfer temperature treatments.

From the time means it can be seen that with an increase in the proportion of the 9 week treatment spent at 5°C, the average leaf number decreased ($p < 0.001$) up to 6 weeks with no difference between 6 weeks and 9 weeks at 5°C. When the transfer temperatures were considered independently of the time of transfer, leaf number increased with temperatures between 13 and 20°C. The constant temperature treatments of 5, 9, 13, 17 and 20°C produced increasing leaf number with increasing temperature, as observed in Chapter 4. Flower initiation in the plants development was delayed with increasing temperature. Following transfer to the various temperatures after 6 weeks at 5°C the leaf number no longer increased with increasing temperature, flower induction having already occurred while stored at 5°C. The dry weight of leaves follows a similar pattern to leaf number being influenced mainly by the number of leaves initiated.

When the average weight per leaf was considered, the temperature means were not significantly different. When considered separately, however, the weight per leaf increased as the temperature increased from 9 to 20°C in the no-transfer treatments (i.e. at constant temperatures) although the values for leaves given 13 and 17°C did not differ significantly. When transferred from 5°C there was no evidence that the weight per leaf was affected by the temperature except after 6 weeks at 5°C when increasing temperature reduced the weight per leaf, a trend exactly opposite to that observed in the absence of 5°C treatment.

The dry weight in the mother bulb at the time of anthesis of the first bud showed significant differences depending on the time stored at 5°C ($p < 0.01$), Table 5.7. There was no indication of either the transfer temperature or an interaction between time of transfer and temperature affecting the weight of motherbulb ($p < 0.05$).

Table 5.6 The effects of pre-planting temperature treatments on
a) leaf number b) leaf dry weight per plant and c) dry
weight per leaf.

Weeks at 5°C	Weeks at transfer temp.	Transfer temperature (°C)				Time means
		9	13	17	20	
(a) Leaf no.						
9	0	5.36				
6	3	5.44	5.31	5.25	5.40	5.36
4	5	5.91	5.77	5.54	5.60	5.70
1	8	6.79	5.94	7.00	-	6.44
0	9	5.92	6.00	7.00	8.20	6.87
Temperature means		5.91	5.67	6.09	6.57	5.99
(b) Leaf DW						
9	0	(g) 2.37				
6	3	2.36	1.67	1.76	1.12	1.87
4	5	2.27	1.86	2.00	1.88	2.00
1	8	2.73	1.96	2.50	-	2.33
0	9	1.71	2.44	2.77	4.30	2.88
Temperature means		2.29	2.05	2.30	2.87	2.31
(c) DW leaf⁻¹						
9	0	(g) 0.41				
6	3	0.43	0.32	0.33	0.21	0.35
4	5	0.39	0.32	0.36	0.33	0.35
1	8	0.38	0.33	0.36	-	0.35
0	9	0.29	0.41	0.40	0.52	0.41
Temperature means		0.38	0.36	0.38	0.42	0.38
a) Time SED = 0.14		b) Time SED = 0.14		c) Time SED = 0.02		
Temp SED = 0.13		Temp SED = 0.13		Temp SED = 0.02		
Time.Temp SED= 0.33		Time.Temp SED= 0.33		Time.Temp SED= 0.05		

Table 5.7 Effect of temperature treatments on the dry weight of the mother bulb at the time of anthesis of the first bud (g)

Weeks at 5°C	Weeks at transfer temp	Transfer Temperature (°C)				Time means
		9	13	17	20	
		Mother bulb Dry weight (g)				
9	0	1.50				
6	3	1.81	1.42	2.01	2.55	1.79
4	5	1.71	1.93	2.30	2.22	2.04
1	8	1.81	1.45	1.66	-	1.62
0	9	1.36	1.72	1.53	1.68	1.57
Temperature means		1.63	1.63	1.77	1.93	1.71

Time SED = 0.16

Temp SED = 0.15

Time.Temp SED = 0.38

The only evidence of an effect was the larger dry weight of mother bulb remaining when plants spent 4 weeks at 5°C before being transferred to the other temperatures. This result is probably connected with the rate at which the flowers reached anthesis. The fastest rates were observed following 4 week at 5°C.

Table 5.8 shows the dry weight of the daughter bulbs at harvest time. The later time of transfer and the moderate transfer temperature both caused significant reductions in the dry weight of daughter bulbs ($p < 0.001$) and there was a significant interaction ($p < 0.01$).

Table 5.8 Effect of pre-planting temperature treatment on the dry weight of daughter bulbs produced (g).

Weeks at 5°C	Weeks at transfer temp.	Transfer temperature °C				Time means
		9	13	17	20	
		Dry weight of Daughter bulbs (g)				
9	0	1.47				
6	3	1.29	1.10	0.89	0.60	1.07
4	5	1.51	1.20	0.70	0.54	0.99
1	8	0.90	0.77	0.61	-	0.78
0	9	0.99	0.85	0.74	1.20	0.94
Temperature means		1.20	1.06	0.83	1.05	1.04

Time SED = 0.11

Temp SED = 0.10

Time.Temp SED = 0.26

Not transferring the bulbs, but maintaining them at 5°C for the whole 9 weeks of the pre-planting treatment, resulted in the heaviest daughter bulbs. From the time means, as the time at 5°C decreased, the weight of daughter bulbs also decreased. When no 5°C treatment was applied, however, the daughter bulbs were heavier than following a single week at 5°C. Transferring to 17°C irrespective of duration at 5°C gave the lowest mean weight. The temperature mean weight for bulbs given any time at 20°C was heavily influenced by the heavy daughter bulbs produced by the constant 20°C treatment. This was because of the slower development to anthesis in this treatment, allowing more time for daughter bulb growth.

5.1.2.3 The relationship between vegetative and floral growth and development

As stated at the beginning of Section 5.1, the aim of this investigation was not only to determine the influence of pre-planting temperatures on whole-plant development but also to establish the relationship between flower growth and development and the growth of the various vegetative sinks in competition with the flower.

In order to establish the interrelation or association between the growth of the flower plus stem, number of buds developing to anthesis or the rate of development to anthesis and the growth of the various plant parts, correlation coefficients were calculated for these three flowering variables compared to the dry weights of other plant parts at harvest time (Table 5.9). A linear regression analysis was used to determine whether the evidence supported the existence of a relationship between the vegetative growth and flower growth and development.

Table 5.9 Correlation coefficients (r) for comparisons between flower and vegetative growth and development.

Comparisons	Logit (% bulbs with 2+ buds developing)	Flower + stem dry weight	Days to anthesis
1. Leaf dry weight	0.75	0.89	0.85
2. Daughter bulb DW	0.39(ns)	0.55	0.29(ns)
3. Daughter DW (1 + 2)	0.76	0.94	0.78
4. Motherbulb DW	-0.63	-0.56	-0.43(ns)
5. Days to anthesis	0.59	0.83	
6. Rate of veg. growth (3/5)	0.79	0.97	0.78
7. Stem length	0.82	0.96	0.83
8. Flower + stem dry weight	0.77		0.83

Comparisons marked (ns) were not significantly linearly related to each other ($p > 0.05$).

To compare the degree of successful flower development with the development of other plant parts, the percentage of bulbs with two or more buds developing at harvest, from Table 5.2, were used to calculate the logit flower development:-

$$\text{Logit} = \log_e \left(\frac{P}{100-P} \right) \quad \text{eq (5.1)}$$

where P = the percentage bulbs with more than two buds developing.

The total vegetative growth in competition with the developing flowers was considered as the sum of the dry weights of the foliar leaves and the daughter bulbs. The rate of vegetative growth was calculated by dividing this total by the mean number of days to anthesis.

The only negative correlation found was between mother-bulb dry weight at harvest and the number of buds developing or with flower plus stem dry weight. It appears, therefore that for successful flower development, mother-bulb reserves were utilized more than with treatments where fewer buds developed to anthesis. All other significant comparisons showed an increase in dry weight with increased flower development.

For flower development, the best fitting linear regression lines were obtained between logit flower development and the stem length (Fig. 5.1). Better flower development was associated with longer stem lengths. Similarly, but with greater variation about the fitted line, vegetative growth increased with increasing flower development ($r^2 = 61.7\%$ $p < 0.001$).

The dry weight of the flower plus stem gave a good indication as to the quality of the flower. The dry weight was highly correlated with the stem length ($r^2 = 91.0\%$ $p < 0.001$) and reasonably correlated with the percentage of bulbs with two or more buds developing ($r^2 = 56.3\%$ $p < 0.01$). Comparing the flower plus stem dry weight with the dry weight of leaves produced also gave a good correlation ($r^2 = 77.6\%$ $p < 0.001$), the dry weight of the flower plus stem increasing with increased leaf dry weight. Daughter bulb dry weight also increased with increased leaf production, although there was a large scatter about the fitted line ($r^2 = 26.7\%$ $p < 0.05$). When these two factors were combined, however to give the vegetative growth, the best fitting line accounted for 87.7 per cent of the variation (Fig. 5.2). The rate of this vegetative growth against flower and stem dry weight gave an even better fit (Fig. 5.3). As the rate of vegetative growth increased the dry weight of the flower and stem also increased.

No significant correlation existed between daughter bulb dry weights alone and flower development.

Fig. 5.1

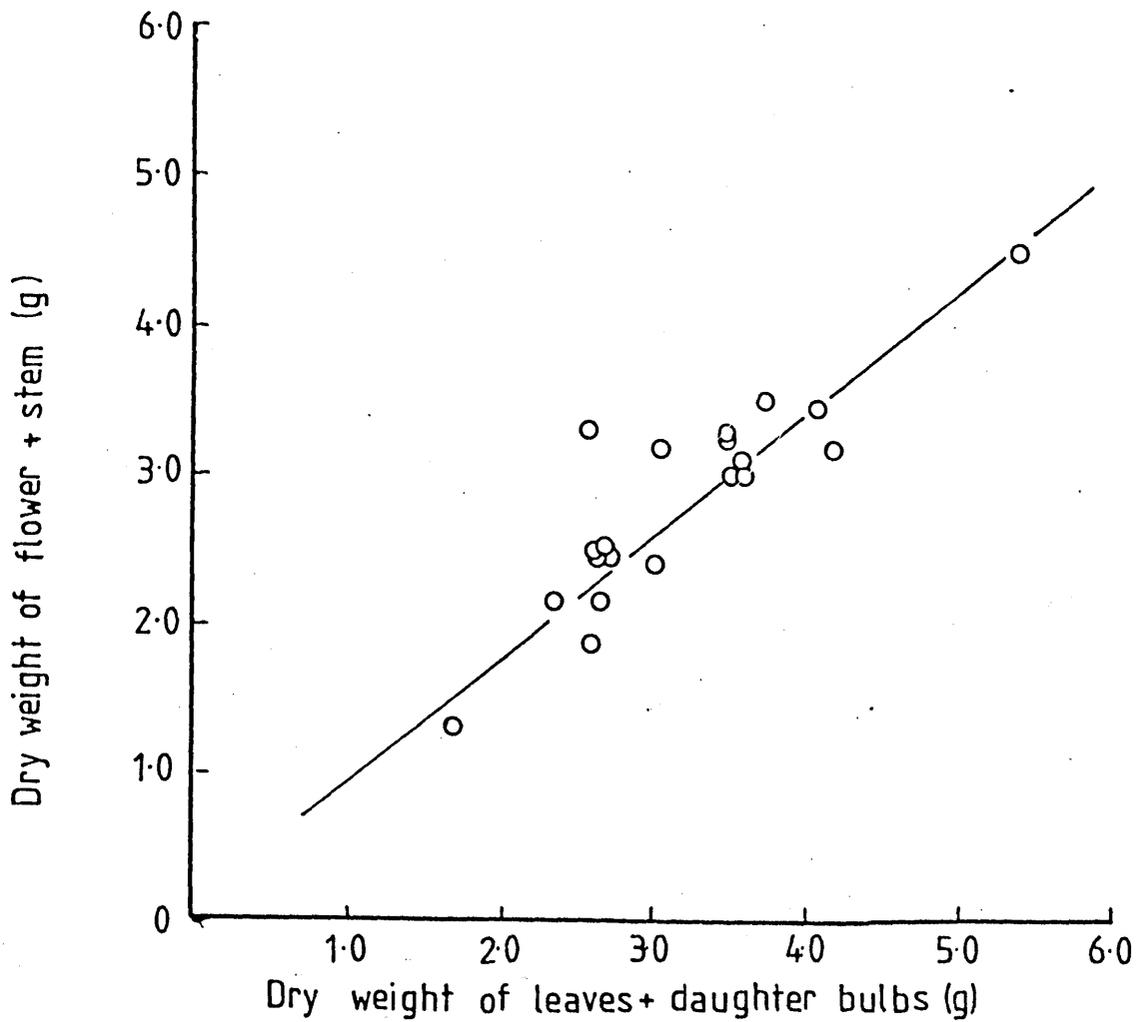
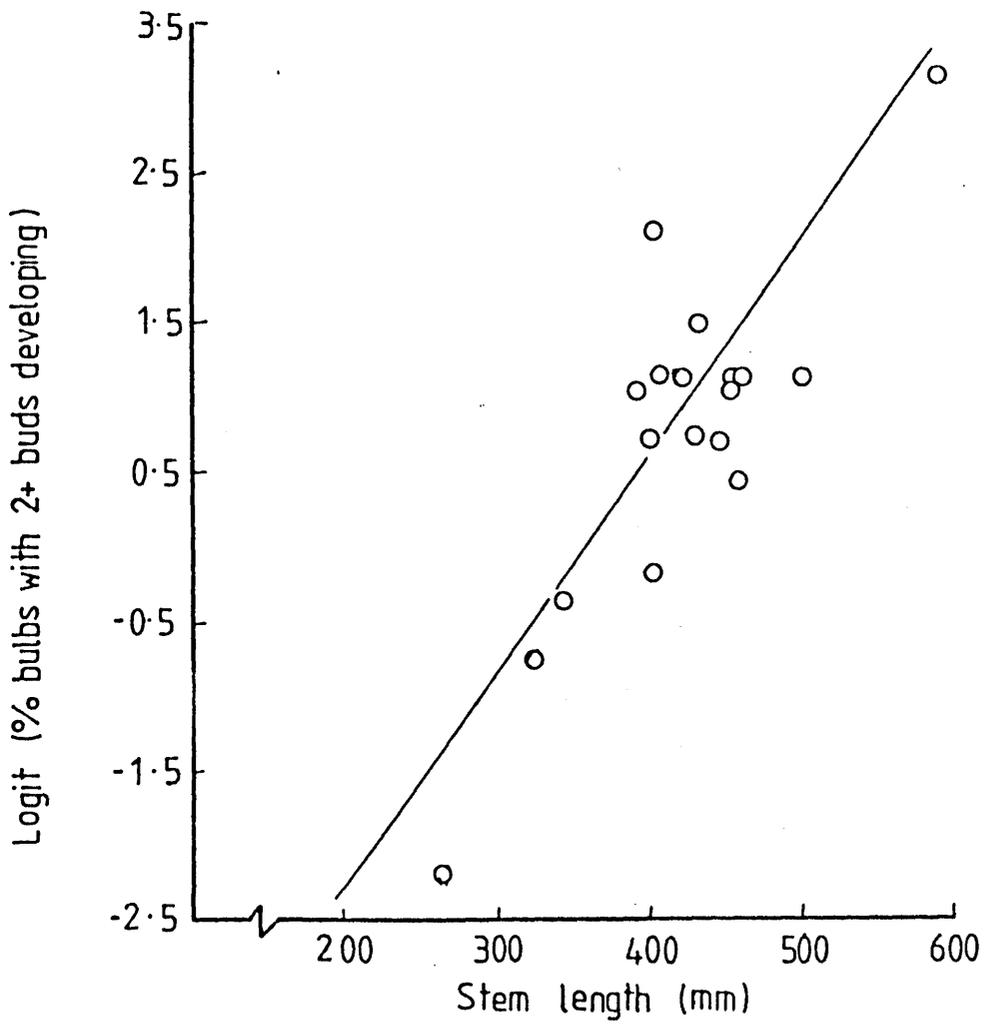
The relationship between logit transformation of the percentage bulbs with two or more bulbs developing (see equation 5.1) and the mean flower stem length (mm), following different pre-planting treatments.

$$\text{Ln } \frac{P}{(100-P)} = -4.72 + 0.013x$$
$$r^2 = 0.65 \text{ (} p < 0.001 \text{)}$$

Fig. 5.2

The relationship between flower plus stem dry weight and vegetative dry weight.

$$y = 0.121 + 0.808x$$
$$r^2 = 0.88 \text{ (} p < 0.001 \text{)}$$



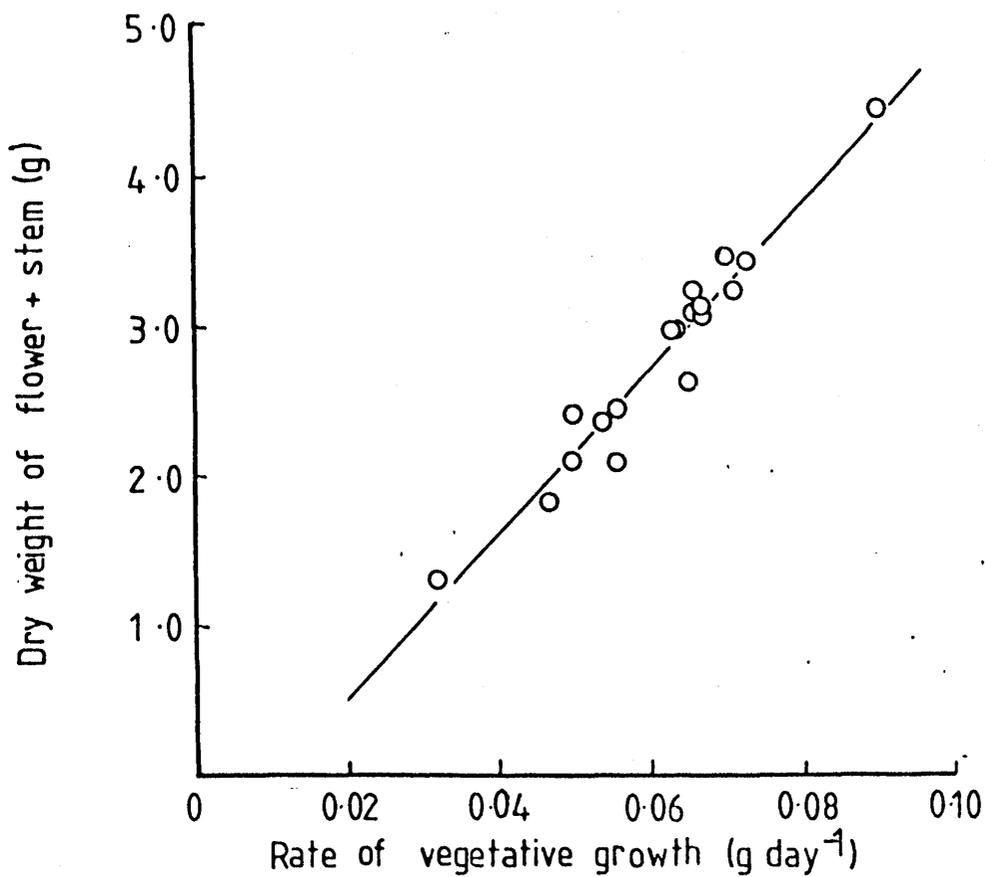


Fig 5.3

The relationship between flower and stem dry weight and rate of vegetative growth

$$y = -0.566 + 55.0x$$

$$r^2 = 0.94 \text{ (} p < 0.001 \text{)}$$

5.1.2.4 Interruption of the optimum pre-planting temperature treatment with higher or lower temperatures

To examine the effect of interrupting a 13°C treatment with either 20°C or 5°C a binomial analysis was again used to compare the percentage of bulbs with two or more buds initiated and then with two or more buds developing at harvest (Table 5.10).

Table 5.10 Effect of interrupting a 13°C treatment, with either 5 or 20°C, on flower growth and development

Treatment	Bulbs with 2+ buds initiated %	Bulbs with 2+ buds developing %	Days to anthesis (d)	Length of flower stem (mm)	Dry weight of flower stem (g)
Constant 13°C	100	73	78.5	393	2.63
1w13°C, 3w5°C, 5w13°C	93	80	79.7	434	2.49
1w13°C, 3w20°C, 5w13°C	100	95	86.0	530	3.41
4w13°C, 2w5°C, 3w13°C	79	64	81.3	420	2.61
4w13°C, 2w20°C, 3w13°C	95	79	79.1	405	2.75
SED MAX-MIN REP			1.1	31	0.25

When 5 or 20°C were interposed into a 13°C treatment there were no significant effects on the percentage of bulbs which had two or more buds initiated or with two or more buds developing at harvest time.

The rate of flower development to anthesis was slowest following the 1 week 13°C, 3 weeks 20°C, 5 weeks 13°C treatment (Table 5.10). Breaking the 13°C treatment after 4 weeks with 2 weeks at 5°C also reduced the rate of development, but the other treatments showed no significant differences to the uninterrupted 13°C treatment.

Splitting the 13°C after 1 week with 3 weeks at 20°C produced the flowers with the longest stems. Interruption after 1 week with both 5 and 20°C produced longer stems than when breaks were either not given or given after 4 weeks.

The heaviest flowers and stems were produced by the same treatment which gave the slowest rate of development. There was no evidence that any of the other treatments produced flowers and stems with different weights to those obtained with a constant 13°C.

The results of an interruption on the vegetative growth and development are shown in Table 5.11. There was little influence of interruption of the 13°C treatment, these treatments having no significant effects on mother bulb dry weight at the time of anthesis of the first bud, or daughter bulb dry weight at this time.

The average number of leaves was greater following the treatment in which 20°C for 3 weeks was interposed after 1 week at 13°C. Other treatments produced similar mean leaf numbers to the constant 13°C treatment but they did differ from other interrupted treatments. Following 4 weeks at 13°C a break with either 5 or 20°C for 2 weeks gave similar mean leaf numbers, this was higher than that obtained when 5°C for 3 weeks interrupted 13°C after 1 week, but lower than that obtained when 20°C was applied at this time. This suggests that induction had occurred by the time 4 weeks at 13°C had been given but not after a single week at 13°C.

Leaf dry weight was reduced by a 5°C treatment at any time, but a break with 20°C did not cause any difference in leaf dry weight compared to that obtained when no break was given. The same result was obtained when the dry weight per leaf was calculated, a 5°C interruption significantly reducing the mean dry weight per leaf compared to all other treatments.

Table 5.11 Vegetative growth and development following interruption of a 13°C treatment with 5 or 20°C.

Treatment	Mean leaf number	Dry weight of leaves (g)	Dry weight per leaf (g)	Mother bulb dry weight (g)	Daughter bulb dry weight (g)
Constant 13°C	6.00	2.44	0.41	1.72	0.85
1w13°C, 3w5°C, 5w13°C	5.67	1.81	0.32	1.92	1.13
1w13°C, 3w20°C, 5w13°C	6.84	2.62	0.39	1.76	0.95
4w13°C, 2w5°C, 3w13°C	6.11	1.93	0.31	1.73	1.01
4w13°C, 2w5°C, 3w13°C	6.17	2.39	0.39	1.74	0.61
SED MAX-MIN REP	0.20	0.21	0.03	0.26	0.20

Since the treatments did not cause differences in the degree of successful flower development, it would be expected that correlations between floral and vegetative development would not be significant. This, indeed, was the case, the only significant correlation existed between flower dry weight and the number of days to anthesis ($r^2 = 0.79$ $p < 0.05$). On examining the rate of vegetative growth (which correlated well with flower weight in section 5.1.2.3., Fig. 5.3) there was no significant difference between the rate of vegetative growth between the treatments.

5.2 Effect of post-planting temperature treatments

Introduction

Temperature affects the growth rate and development of the flower after planting. High temperatures (above 15-18°C), particularly during daylight hours, led to an increase in bud blasting (Kamberbeek, 1966; Fortanier and Zevenbergen, 1973; Sano, 1975a). Fortanier and Zevenbergen (1973), state that high temperatures cause rapid development with a reduced assimilate supply and insufficiently rapid mobilization and distribution of reserves. In general, temperatures which suppressed leaf growth

were thought to limit bud blasting (Fortanier & Zevenbergen, 1973). Having established a relationship between vegetative growth and floral development when influenced by pre-planting temperatures, post-planting treatments were investigated to determine whether the relationship still held.

5.2.1 Materials and Methods

Three grades of 'Wedgwood' (10+, 8-9 and 6-7cm) and 'Professor Blaauw' (10+ cm) bulbs were forced in four glasshouse compartments using different temperature regimes. Bulbs were first given adequate heat curing at 25.5°C for 5, 9 or 12 weeks. A pre-planting temperature treatment of 17°C for 2 weeks and 9°C for 6 weeks was then applied to all bulbs before planting on either 21 Jan, 18 Feb or 18 March, 1984 as described in Chapter 3. The temperature regimes in the four glasshouses following planting were:-

- 1) 12°C minimum with automatic venting at 20°C
- 2) 16°C minimum venting at 18°C
- 3) 18°C minimum venting at 20°C
- 4) 20°C minimum day temperature, venting at 24, 14°C minimum night temperature.

Three levels of shading were applied, unshaded and one or two layers of polythene netting (Rokolene kDA, Rokocontainers Nottingham). On average, a single layer of shading reduced the light by 46% and two layers by 70% compared to the unshaded treatment. There were 3 pots of each grade (15 bulbs) per treatment. Results recorded included: the number of days to anthesis; the number of bulbs which developed a flower to anthesis; the number of leaves produced per plant; dry weight of the flower and stem and dry weight of the leaves.

5.2.2 Results

This experiment was carried out in 4 different glasshouse compartments under natural conditions, therefore when drawing conclusions from the results two factors must first be considered:-

- 1) The rates of development differed with temperature regime causing plants to reach critical stages of development under different light conditions.
- 2) The temperature regimes applied were not replicated in different compartments, therefore the between compartment variation has not been taken into consideration.

The temperatures actually recorded in the glasshouses were always above the minimum temperature set during the day. Mean daily temperatures in the glasshouse for the 14 days before 'Wedgwood' 10+ bulbs (all shading treatments) reached anthesis are shown in Table 5.12.

Table 5.12 Mean daily glasshouse temperatures for the 14 days prior to anthesis for Wedgwood 10+ bulbs

Glasshouse	Planting date		
	21 Jan	18 Feb	18 March
	<u>Mean temperature °C</u>		
1	14.4 ± 0.2	15.1 ± 0.4	16.6 ± 0.3
2	16.7 ± 0.2	17.1 ± 0.2	18.0 ± 0.1
3	18.7 ± 0.1	18.8 ± 0.1	19.1 ± 0.1
4	18.8 ± 0.2	18.1 ± 0.2	19.1 ± 0.2

To investigate the rate of development to anthesis only the 'Wedgwood' 10+ grade bulb results are presented. These gave the greatest number of replicates, more bulbs developed a flower to anthesis than the other grades or than 'Professor Blaauw'. The

Fig 5.4

Effect of temperature regime and shading on the percentage bulbs with a flower developing to anthesis, for three planting dates (PD I, II, III) and two cultivars, 'Wedgwood' and 'Professor Blaauw'

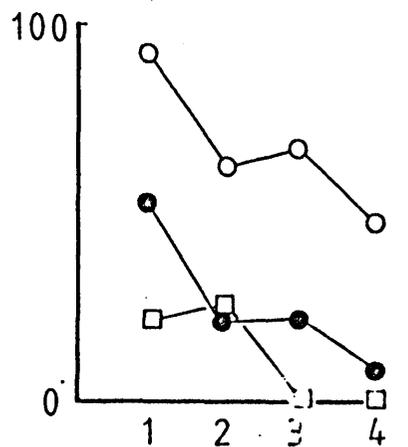
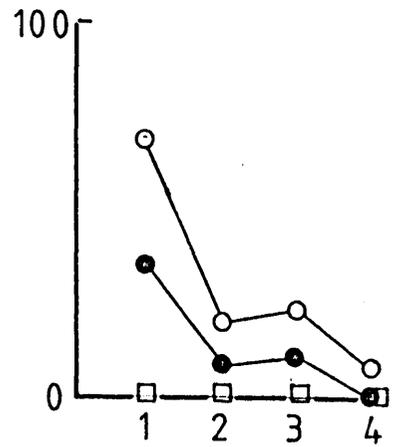
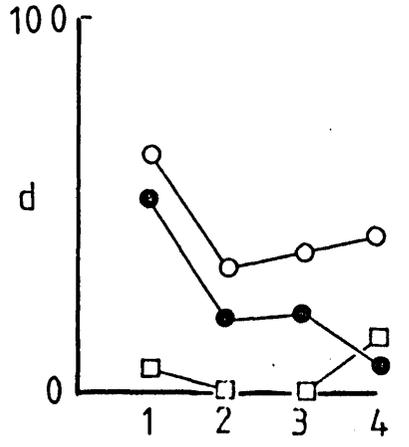
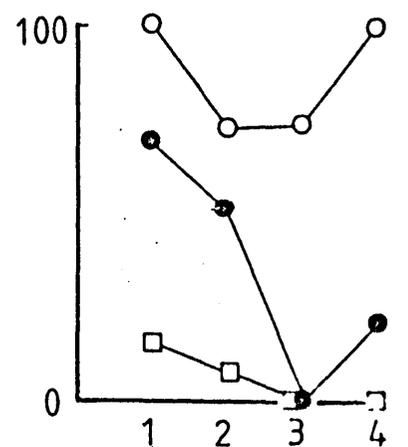
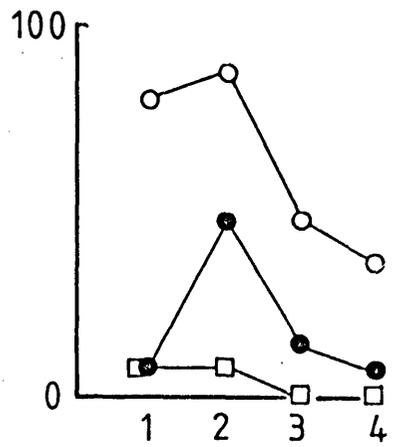
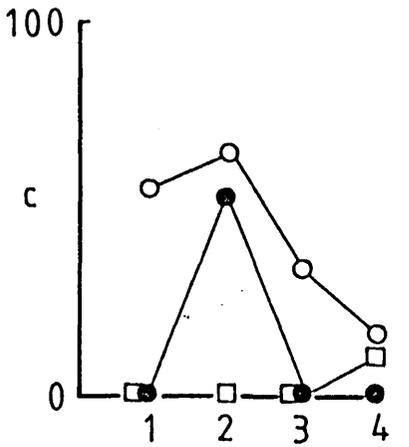
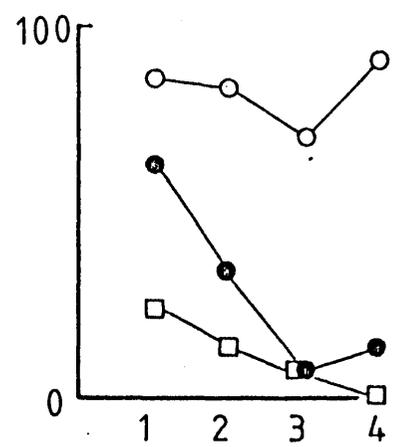
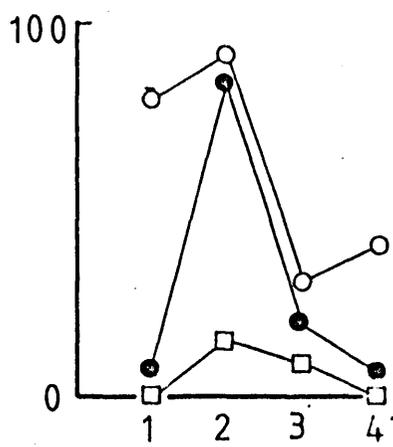
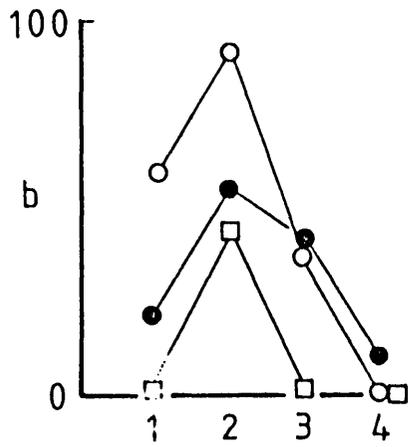
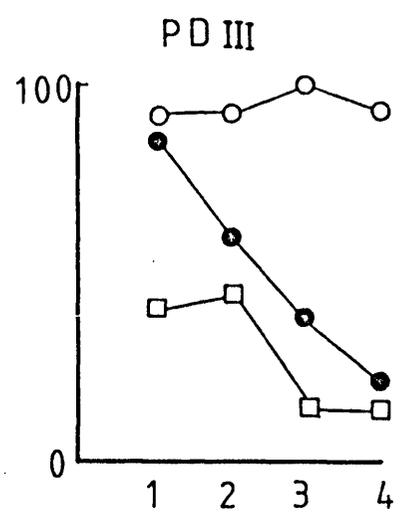
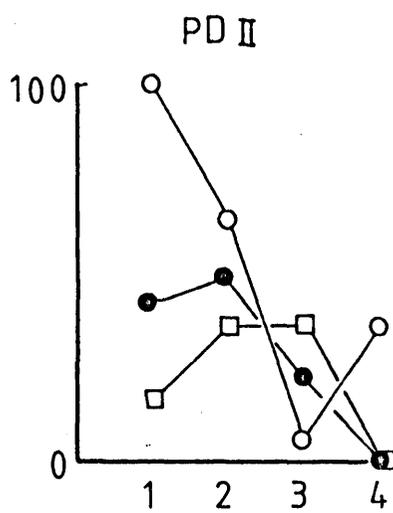
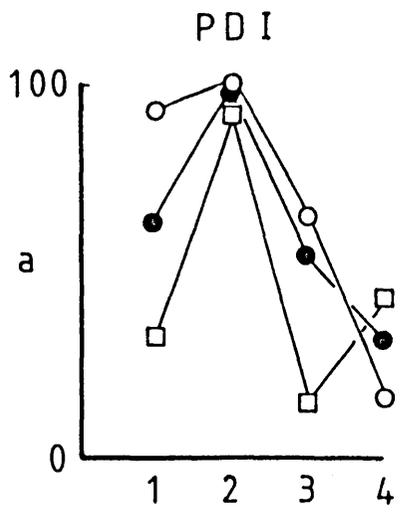
(○) = unshaded

(●) = 1 layer of shading material

(□) = 2 layers of shading material

a = 'Wedgwood' 10+cm; b = 'Wedgwood' 8-9cm; c = 'Wedgwood' 6-7cm

d = 'Professor Blaauw' 10+cm



Glasshouse

Whilst shading the bulbs delayed development ($p < 0.001$), this was not such a large effect as the effect of temperature. The longest delay occurring between no shade and two layers of shading material was about 4 days. The shading also greatly reduced the number of bulbs with flowers reaching anthesis.

The percentages of bulbs in which flowers developed to anthesis are shown in Fig 5.4. Each graph represents the results from one of the cultivars for one of the planting dates. There were differences between planting dates, the lowest percentage of flowers developing to anthesis occurred with the second planting date for 'Wedgwood' 10+ ($p < 0.05$) and with 'Professor Blaauw' 10+ ($p < 0.001$). For the smaller grades of 'Wedgwood' the 8-9 cm showed no significant differences between planting dates, while the 6-7 cm bulbs increased the percentage of flowers developing to anthesis with successive plantings from 19 to 43% overall treatments.

Mean daily temperatures above approximately 18°C significantly reduced the proportion of bulbs in which a flower reached anthesis ('Wedgwood' 10+ $p < 0.01$; 8-9 $p < 0.001$; 6-7 $p < 0.05$ and 'Professor Blaauw' $p < 0.001$). The percentage was reduced by about half of that obtained with mean daily temperature of around 16°C for 'Wedgwood'. With the low mean temperatures obtained in the first planting date (14.4°C) there was also evidence of poorer flower development with 'Wedgwood', but these conditions gave better flower development with 'Professor Blaauw'.

As the light integral increased with later planting dates, it can be seen that, by the third planting date the different temperatures influenced flower development less when the 'Wedgwood' plants were unshaded. The shaded plants still showed the decline in development with increasing temperature, as did the unshaded 'Professor Blaauw'.

Taking all planting dates and temperatures together, shading reduces flower development (Table 5.14), this reduction being greatest with smaller bulbs. Comparing the percentage of flower development obtained without shading with that obtained with two layers of shading material, flower development to anthesis was

about halved with 'Wedgwood' 10+cm ($p < 0.001$) while with the 8-9 cm grade development to anthesis was over seven times lower ($p < 0.001$) and seventeen times lower with the 6-7 cm grade. The reduction in flower development with 'Professor Blaauw' was over seven times when comparing the double shading treatment with the unshaded treatment.

Table 5.14 Effect of shading on flower development

Cultivar	Grade	Shading level		
		0	1	2
		% developing to anthesis		
Wedgwood	10+	70	46	31
	8-9	63	31	9
	6-7	64	23	4
Prof. Blaauw	10+	46	20	6

The effect of light on flower development will be examined in greater detail in Chapter 6.

Figure 5.5 shows the logit transformation of the percentage of bulbs with flowers developing to anthesis against the mean dry weight of the flower plus stem for 'Wedgwood' 10+cm and 8-9 cm grade bulbs. As the flower plus stem dry weight increased the percentage flower development also increased. The dry weight of the flower plus stem had an advantage as a measurement of flower development over the percentage bulbs with a flower developing to anthesis as this was a continuous, rather than a discrete, measurement.

When comparing flower dry weight across treatments an analysis of those bulbs in which the flower developed to anthesis was first investigated. For the two earlier planting dates there were generally no significant differences in the dry weights of the flower and stems. The later planting date, however, showed a decrease in the dry weight with temperatures above 18°C ($p < 0.001$), while shading also reduced flower dry weight ($p < 0.001$) for all grades of 'Wedgwood' and for 'Professor Blaauw'. This was

connected with the degree of development of the second flower bud, which always aborted while relatively small with the earlier planting dates. The results shown are those of 'Wedgwood' 10+ cm, which illustrate these trends adequately, Fig. 5.6.

When all bulbs from each treatment are considered, both high temperatures and shading reduced flower and stem dry weight ($p < 0.001$) (Fig 5.7). Increasing temperature, however, did not significantly reduce the flower weight of 'Professor Blaauw' (Fig 5.7 c), although shading the plants did ($p < 0.001$). In the glasshouse with low temperature regimes the flower weight of 'Professor Blaauw' remained low on this first planting date. This indicates a higher light requirement, even at low temperatures, for 'Professor Blaauw' than for 'Wedgwood'. There was frequently an interaction between temperature and shade for all grades and cultivars ($p < 0.05 - p < 0.001$). At high temperatures, flower weight was reduced to a level at which shading had very little influence (Fig. 5.7a). Where there was no interaction between temperature and shading, this could be attributed to light being insufficient for the production of heavy flowers even in the unshaded treatment. This was the case with 'Professor Blaauw' on the first planting date. Alternatively when light was sufficient for the production of heavy flowers, even at the high temperatures, again there was no significant interaction. This was the case with 'Wedgwood' on the third planting date (Fig 5.6 b & c).

To examine the relationship between vegetative growth and floral development, the dry weight of leaves and the dry weight of the flower plus stem produced were plotted for the different treatments. Fig 5.8 shows the plot for 'Wedgwood' 10+ and 8-9 cm grades for all three planting dates. There was a linear relationship between the weight of the flower plus stem and the leaf weight ($p < 0.001$). The number of leaves did not differ between treatments for the same planting dates, although the number did increase with successive plantings. To obtain the increase in flower plus stem weight associated with the increased weight of vegetation, it was unimportant, therefore, whether the latter was achieved by the initiation of a larger number of leaves

Fig 5.5

Logit percentage flower development against mean flower and stem dry weight for 'Wedgwood' 10+ and 8-9cm grades over three planting dates.

$$r^2 = 0.73 \text{ (p < 0.001)}$$

Fig 5.6

The effect of temperature regime and shading on the dry weight of the flower and stems in those plants in which flowers developed to anthesis

(○) = unshaded

(●) = 1 layer of shading material

(□) = 2 layers of shading material

Minimum glasshouse day temperatures were

1 = 12°C 2 = 16°C 3 = 18°C and 4 = 20°C

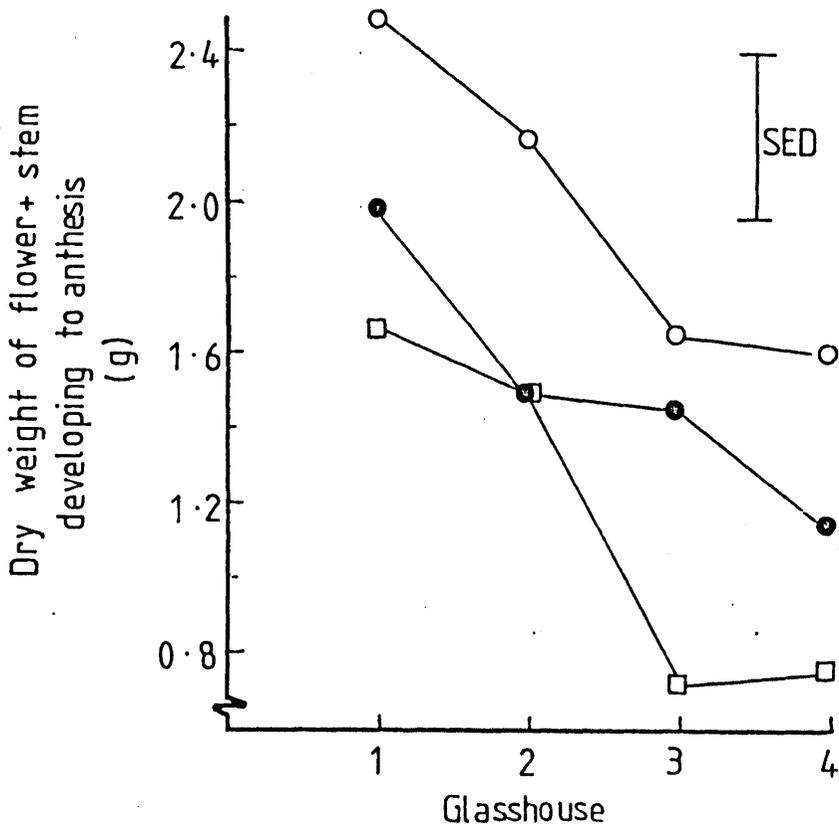
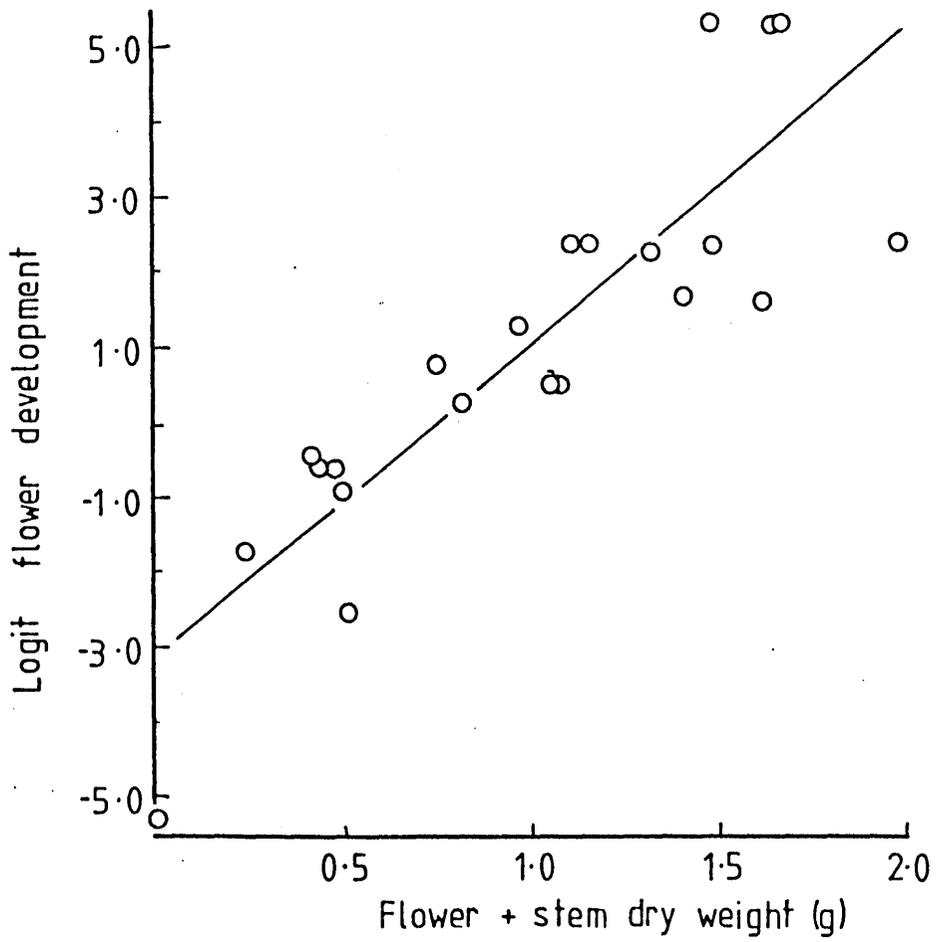
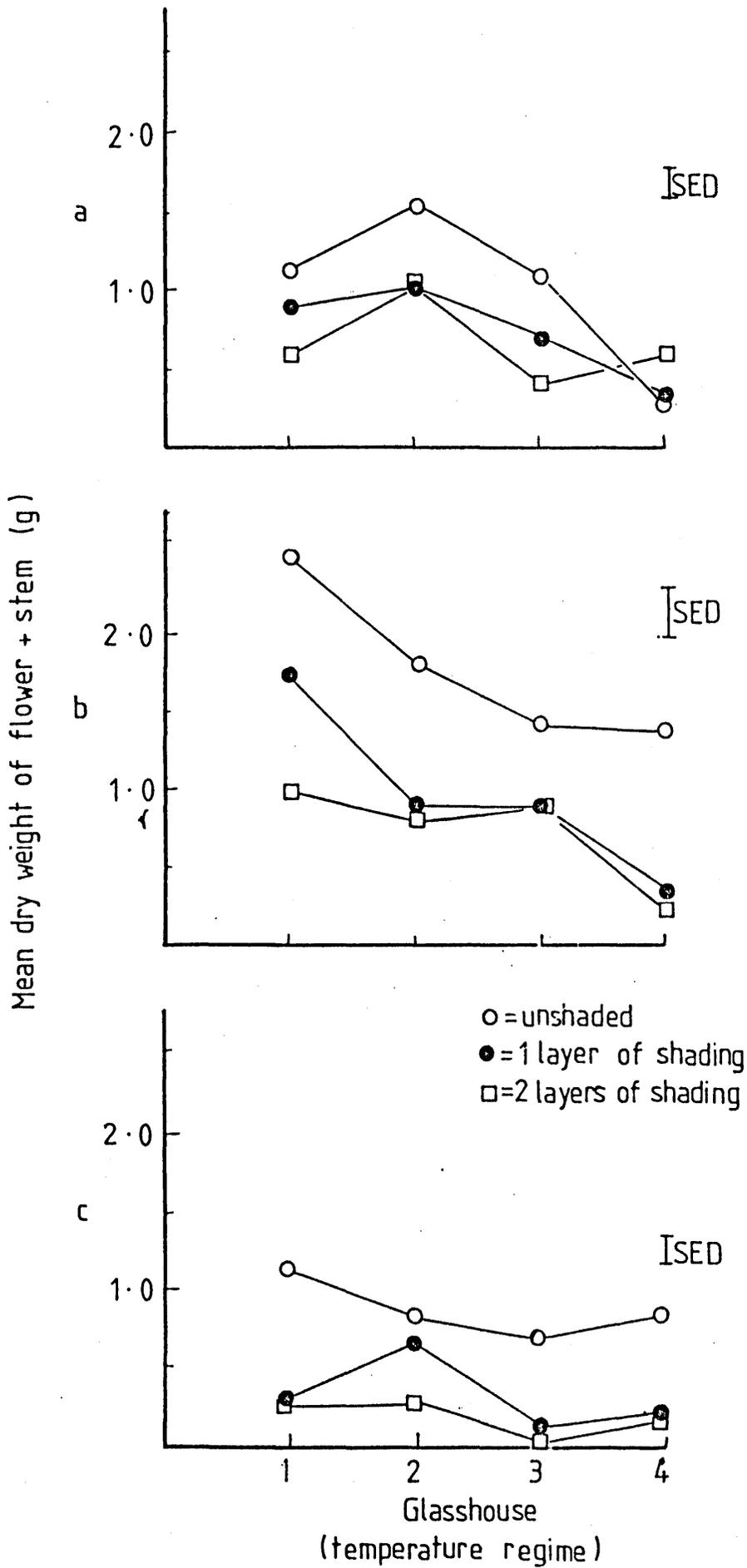


Fig 5.7

The effect of temperature and shade on flower and stem dry weight for a) 'Wedgwood' 10+cm, planting date I b) 'Wedgwood' 10+cm planting date III and c) 'Professor Blaauw' 10+cm planting date I. Minimum day temperatures of the glasshouses were 1 = 12°C, 2 = 16°C, 3 = 18°C and 4 = 20°C.



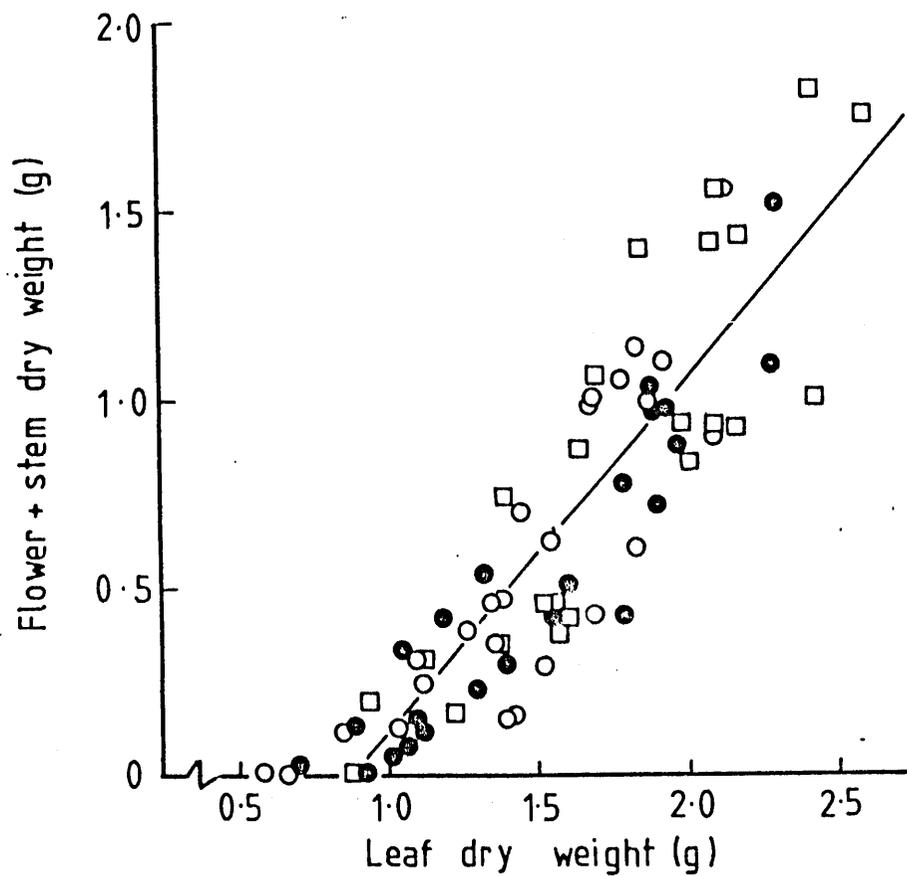


Fig 5.8

Relationship between the dry weight of the flower and stem and the dry weight of leaves

$$r^2 = 0.82 \text{ (} p < 0.001 \text{)}$$

- (O) = planting date I
- (●) = planting date II
- (□) = planting date III

or by greater leaf growth. 'Professor Blaauw' also showed a positive linear relationship between floral development and leaf dry weight ($p < 0.001$) but there was much greater variation in leaf weight for a given floral weight than was the case with 'Wedgwood'.

5.3 Discussion

The results obtained in these experiments confirm the effects of temperature reported by other workers, that both pre-planting and post-planting temperatures influence flower development (Kamerbeek and Beijer, 1964; Kamerbeek, 1965; and Fortanier and Zevenbergen, 1973). After planting, temperatures above 18°C caused flower failure due to 'blasting' but the response was modified by the light level. The greater the light intensity the higher the temperature at which floral development could still occur.

Temperature during storage of the dry bulbs caused differences in the rate of development. The most rapid flower development was obtained with the treatment involving 5°C for 4 weeks followed by 17°C for 5 weeks. The temperature during forcing had an even greater effect on the rate of development of the flower. Previous reports have suggested that the more rapid flower development, the greater the chances of flower bud failure due to an insufficiently rapid supply of assimilates or mobilization of reserves (Fortanier and Zevenbergen, 1973). This is supported here by the highly significant correlation which existed between the number of days to anthesis and the dry weight of the flower, or logit percentage bulbs with two or more buds developing (Table 5.9).

From this investigation flower development appeared more closely related to the rate of vegetative growth than to the number of days required to reach anthesis. With more rapid vegetative growth, influenced by the pre-planting treatment, the better the flower development. Similarly with glasshouse temperatures, the greater the weight of vegetative growth

produced, the better was the flower development (Figs 5.2 and 5.8). This conflicts with the common belief reported by Fortanier and Zevenbergen (1973), that suppression of leaf growth encouraged flower development. It is also inconsistent with work on other plants where competition between vegetative and reproductive growth has been shown to occur. In tomato, Kinet (1977), has shown that flower development improved with the removal of the three leaves below the inflorescence. Similarly, reducing vegetative growth by either restricting root growth with small pots or by water stressing the plants, promoted floral development. (Cooper and Hurd; 1968; De Koning and Hurd, 1983). Tse, Ramina, Hackett and Sachs (1974), demonstrated enhanced inflorescence development when young leaves were removed from Bougainvillea and competition between floral development and vegetative growth has been observed in Vitis vinifera (Mullins, 1968).

Walla and Kristoffersen (1969), investigated the effect of the length of a 9°C pre-planting treatment. As the length of the cold treatment increased from 2 to 12 weeks, leaf length decreased. The length of the flower stem increased with increasing cool treatment from 2 to 6 weeks, and then decreased as the cool period was extended from 6 to 12 weeks. The highest percentage flower development to anthesis coincided with the longest flower stems, following 6 weeks' cooling. This suggests that excessive leaf growth will reduce floral development, but also supports the conclusion drawn here, that reduced leaf growth results in fewer flowers developing to anthesis.

The relationship between vegetative and flower development for iris is inconsistent with that of other species. Differences in the development of iris compared to these other species could explain this disparity. In iris, all organs are initiated and continue their early development in the dark, within the bulb scales. Once planted the final stages of development are mainly concerned with the growth and extension of existing organs. The leaves elongate first consuming varying proportions of the stored reserves depending on the extent of leaf growth, pre-planting treatment, light intensity and temperature (Wassink and Wassink-van Lummel, 1952). All the leaves that the plant will

initiate, grow simultaneously and are dependent on the mobilization and supply of stored reserves from the bulb more than on current assimilates. The final developmental stage of the flower then occurs utilizing more and more of the assimilates produced and exported by the leaves, as stored reserves are depleted (Wassink and Wassink-van Lummel, 1952; Wassink 1969). At the time the flower requires most resources for stem extension, the sink strength of the leaves is diminishing.

In other species, where competition between vegetative and floral development occurs, there are not large specialized storage organs and the initiation and early development of the young leaves occurs shortly before or at the same time as the floral development. The leaves and the flower are both, therefore, competing for current assimilates, both organs being of similar strengths as simultaneously competing sinks. This point will be considered further in Chapter 6.

The morphology of the iris leaves suggests that they are poor interceptors of available light. When the leaves are short they remain erect and close to the flower stem, with a large degree of mutual shading. As leaf length increases they bend away from the flower stem exposing much more of the surface of the leaf at a suitable angle for the interception of the available light (Fig. 5.9). The greater the leaf growth, the greater the chances of supplying sufficient assimilates for flower development.

A discrepancy in this relationship between vegetative growth and reproductive development exists when bulbs are treated with ethylene or smoke. This treatment reduces leaf growth and promotes flower development (Schipper, 1981; Duineveld and Munk, 1983). Although overall leaf growth is reduced, treatment with ethylene does cause earlier sprouting and a faster rate of leaf growth (Imanishi and Fortanier, 1982). Hence, leaf growth must be completed earlier than in untreated bulbs, causing less drain on reserves and not competing for substrates at the same time as the flower.

The nature of the control mechanisms of the effects on iris described in this chapter are largely unknown. Hormones are known to play a major role in directing the movement of substrates and in establishing sinks in bulbous plants (Munk, 1981; Rees, 1972).

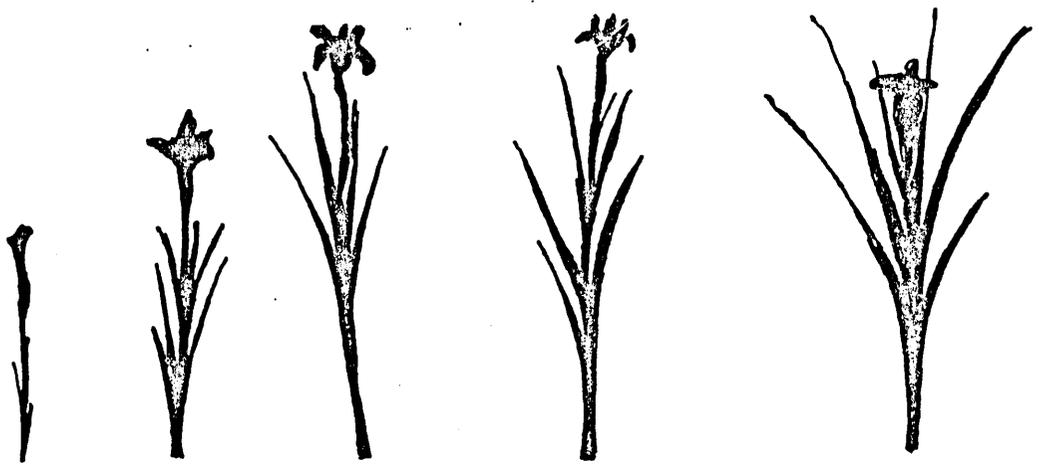


Fig. 5.9 Diagrammatic representation of irises with increasing leaf lengths illustrating how, as leaves become longer, they bend away from the flower stem exposing more leaf at a suitable angle for light interception.

Most work on growth promoters and inhibitors has been concerned with the breaking of dormancy by heat curing (Tsukamoto and Ando, 1973 a) or changes leading up to flower initiation during the cold period before planting (Rodrigues Pereira 1962, 1964, 1965, 1966, 1970). Alpi, Ceccarelli, Togmoni, and Gregorini (1976), investigated the gibberellin and inhibitor content of the different organs of iris during the pre-planting cool period (9°C for 6 weeks) and then during forcing in the higher temperatures and light intensities of the glasshouse following planting. During the pre-planting treatments both gibberellin-like-substances and inhibitors accumulated, although the inhibitors did not have the ability to re-induce dormancy. With different pre-planting temperature treatments, it is conceivable that the balance between inhibitors and promoters will alter, causing differences in the shoot growth. Inhibitors may increase due to water stress conditions occurring in the unplanted bulb, as observed in water stressed tomatoes (Aloni and Pressman, 1981). Future research could investigate these growth regulators from different cool treatments applied to planted and unplanted bulbs.

Once the bulbs were planted in the glasshouse Alpi et al. (1976) observed an increase in GA-type-activity in those parts of the plant showing active growth, following an initial decrease in all tissues which was possibly due to rehydration. The flower showed increased activity corresponding to the beginning of stem elongation. This was followed by a decline in the flower but considerable activity in the daughter bulbs, which also paralleled active growth. Three weeks before anthesis this activity had declined with the largest activity occurring in the flower organs. Inhibitor activity increased during forcing, in the stem, daughter bulbs and leaves. Three weeks before anthesis their activity started to decrease and no activity was detectable in these organs at anthesis. The reason for this decline is unknown. Activity in the roots remained high during the entire glasshouse period. Here, again, it appears conceivable that temperature, and stress caused by temperature effects, could cause differences in the balance of promoters and inhibitors. Low light has been shown to increase the ABA levels in flower buds, possibly decreasing their

sink strength, but this can be rectified by an injection of zeatin (Vonk and Ribot, 1982). This will be considered further in Chapter 6.

Halevy and Shoub (1964) examined the effect of exogenous GA when injected into the bulb or as a foliar spray, with the aim of substitution for the cold requirement for flower induction and development. They found the time of application was critical. Application soon after flower initiation accelerated emergence, leaf growth and development of the flower to anthesis, but had no effect on the final length of the leaves or the flower stem. Repeated applications of GA at later stages of development, however, did increase both leaf and flower stem length and reduced daughter-bulb yield. Temperature treatments were capable of producing similar responses, depending on whether they were pre-planting or post-planting treatments.

Differences in the responses of the two cultivars, 'Wedgwood' and 'Professor Blaauw' were observed. The relationship between vegetative growth and flower development was less clearly defined with 'Professor Blaauw' than with 'Wedgwood'. The vegetative growth is generally greater in 'Professor Blaauw' than that of 'Wedgwood'. More leaves are formed before flower initiation and these leaves are broader and longer than those of 'Wedgwood'. 'Professor Blaauw' also has a much slower rate of development to anthesis, this being the case it is subjected to the influence of many factors which could not be controlled in the glasshouse. It is possible that these uncontrolled factors caused the large variation around the significant positive correlation between the vegetative and floral development.

5.4 Summary

1. Growth and development of Dutch iris was influenced by the pre-planting dry-bulb temperature treatment. The effects were persistent when plants were subsequently grown under identical glasshouse conditions.

2. Transferring bulbs from 5°C to warmer temperatures late in the pre-planting treatment resulted in the initiation of fewer flowers and a lower number of flowers developing to anthesis. These reductions did not occur if stored at a constant 5°C. The slower the development of the flower, the heavier were the flower and stem produced.
3. The number of leaves formed prior to initiation of the flower was reduced with increasing time at 5°C up until 6 weeks. The heaviest daughter bulbs were produced following a constant 5°C treatment. As the time at 5°C decreased, the weight of daughter bulbs also decreased.
4. The relationship between vegetative and reproductive growth and development was investigated. A strong correlation was found between the promotion of leaf growth and successful flower and stem development. Encouragement of any form of vegetative growth did not reduce the chances of flower development to anthesis.
5. Post-planting temperature treatments showed a complicated interaction with light integral for successful flower development. For all temperature and light combinations, however, the growth and development of the flower was simply related to the degree of vegetative growth as observed with pre-planting temperature effects.

6. LIGHT AND POST-INITIATION FLOWER DEVELOPMENT

6. LIGHT AND POST-INITIATION FLOWER DEVELOPMENT

Introduction

Forcing iris during conditions of low light in winter produces problems due to blasting. The difficulty is in achieving a successful balance between rapid production to meet key markets (or achieve an economically viable turnover) and reliable flower development.

Light appears to influence flower development in two ways:-

- 1) Low light, particularly under high temperatures, is thought to result in rapid depletion of bulb reserves early in the plant's development (Hartsema and Luyten, 1960; Rees, 1972). Assimilates from current photosynthesis are insufficient to support the rapidly developing flower stem and flower.
- 2) Light affects the supply of assimilates to the developing bud via hormonal changes. Flowering under low light is improved when cytokinins are injected into the bud (Mae & Vonk, 1974). Low light is thought to decrease the sink strength of the flower for assimilates, possibly due to an increase in abscisic acid under stress conditions (Vonk and Ribot, 1982).

The importance of each of these influences is considered here in terms of the role of stored reserves and current assimilates; intra-plant competition between the developing flower and other organs; movement of carbohydrates to the bud and partitioning during the plants development.

6.1 Light integral required for flower development

Previous investigations attempting to establish the minimum light integral required to achieve satisfactory flower development have all been carried out under controlled environment conditions with artificial light (Hartsema and Luyten, 1955a); Kamerbeek,

1969; Fortanier and Zevenbergen, 1973). No previous attempts are known to have been made to determine the critical irradiance level under natural light conditions in the glasshouse environment. This would seem particularly relevant since generally iris is forced under glass and secondly the forcing of iris under artificial light is unsatisfactory and is not recommended (Anon, 1970). In addition, under glass there is a natural link between increased light intensity and increased temperature by solar gain.

6.1.1. Materials and Methods

The Materials and Methods are as described in Section 5.2.1. Light integrals were calculated from the transmission into the glasshouses and under the shading material, measured at regular intervals during the time course of the experiment. The total short-wave radiation was measured with a Kipp solarimeter.

6.1.2. Results

A regression analysis of the proportion of first flower buds developing to anthesis and the light incident on the crop showed a highly significant fit for the 10+cm bulbs. A single curve fitted data for all three planting dates of those plants grown in the lowest temperature glasshouse (14-17°C) (Fig. 6.1). At these temperatures about 130 MJm^{-2} total short-wave radiation was required during the last 14 days before anthesis to ensure maximum flower numbers reaching anthesis. Similar curves were fitted to data for the smaller graded bulbs, there was no significant difference between the curves produced by the different grade, a single line represented the relationship for all grades adequately, Fig. 6.2. In addition, similar relationships existed if the total light integral from 50% emergence to anthesis, or the mean daily irradiance were used.

Fig. 6.1 The relationship between the light integral for 14 days before plants developed to anthesis and the percentage of bulbs in which flowers developed to anthesis for 'Wedgwood' (10+cm) at 14-17°C.

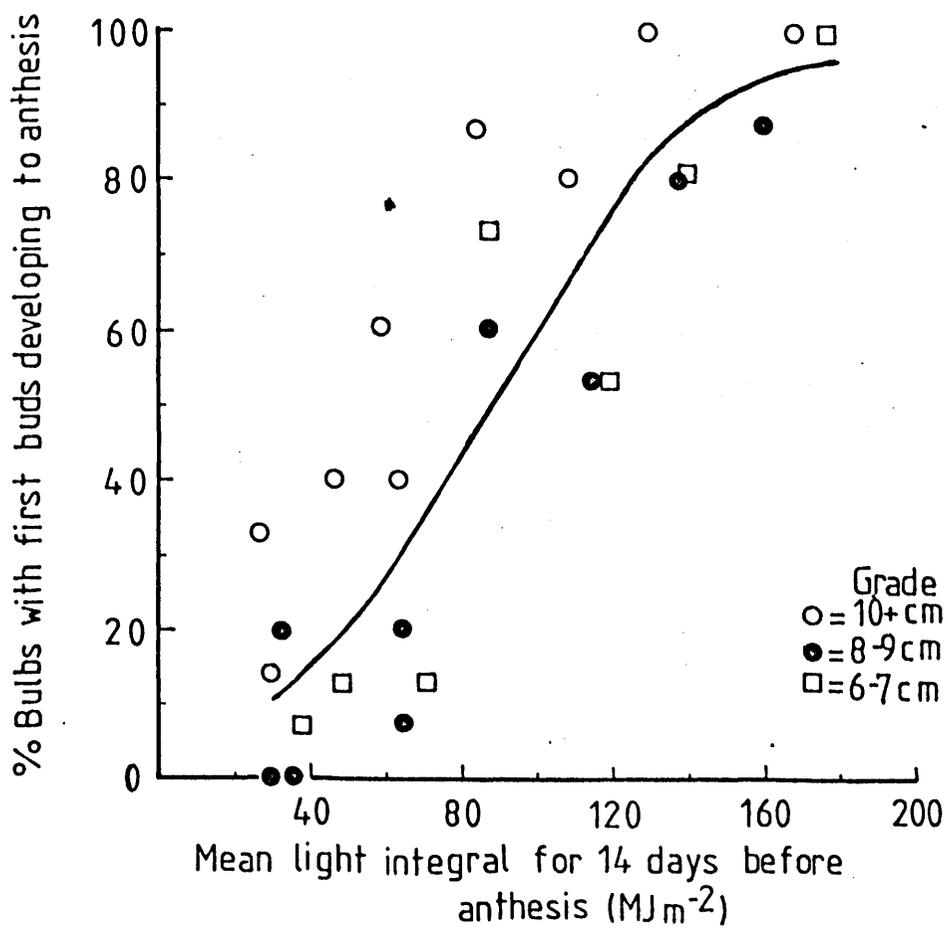
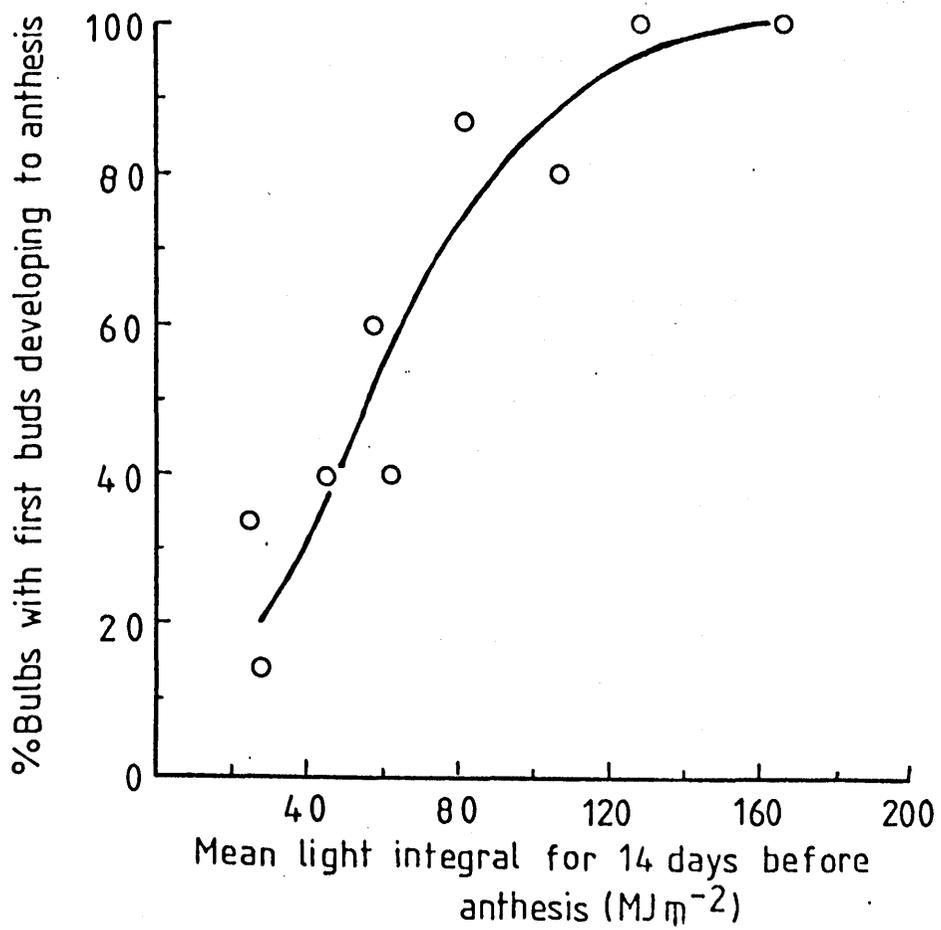
$$\text{Log}_e \left(\frac{y}{100-y} \right) = -2.774 + 0.0467x$$

Deviance accounted for = 89.3% (p < 0.001)

Fig. 6.2 The single relationship between the light integral for 14 days before plants developed to anthesis and the percentage of bulbs in which flowers developed to anthesis for three grades of 'Wedgwood' grown at temperatures between 14-17°C.

$$\text{Log}_e \left(\frac{y}{100-y} \right) = -3.69 + 0.0487x$$

Deviance accounted for = 62.7% (p < 0.001)



In the glasshouse at slightly higher temperatures (17-18°C), the 10+ grade showed two distinct responses to the incident light (Fig. 6.3). Those planted 21 Jan. were capable of developing flowers at lower light integrals than bulbs planted later. For the 8-9cm grade both the first and second planting dates required similar but lower light integrals than the last planting date, for the same flower development (Fig. 6.4). The smallest grade of bulbs, 6-7cm, could be represented adequately by a single line for all three planting dates, but the scatter about this line was large. Temperature appears to have the overriding effect on flower development of these small bulbs and not the time of planting (Fig. 6.5).

In the two glasshouses where temperatures were between 18 and 19°C, the temperature also had the major effect with the larger grade of bulbs, the proportion of bulbs with a flower developing to anthesis being low at high as well as low light integrals.

The change in photoperiod with later planting dates was considered responsible for differences in the number of flowers developing successfully, rather than increased time at retarding temperatures. This is supported by the fact that there were two distinct responses rather than three for the different planting dates; one response under short and the other for long photoperiods. Secondly, the smaller grade bulbs did not display poorer flower development until later plantings; whereas if reserve depletion during high temperature storage was responsible, these smaller bulbs would be expected to show reduced development of the flower before the larger bulbs.

Under glasshouse conditions, with progressively longer days, the same light integral is achieved at lower light intensities. The theory that photoperiod was involved in flower development was therefore tested next.

Fig. 6.3 The relationship between the light integral for 14 days before plants developed to anthesis and the percentage of bulbs in which flowers develop to anthesis for 'Wedgwood' (10+cm) at 17-18°C. Different planting dates responded differently to the incident light.

$$\bullet = \text{planted 21 Jan } \text{Log}_e \left(\frac{y}{(100-y)} \right) = 3.051 + 0.0179x$$

$$\circ = \text{planted 18 Feb and 18 March } \text{Log}_e \left(\frac{y}{(100-y)} \right) = -1.016 + 0.0179x$$

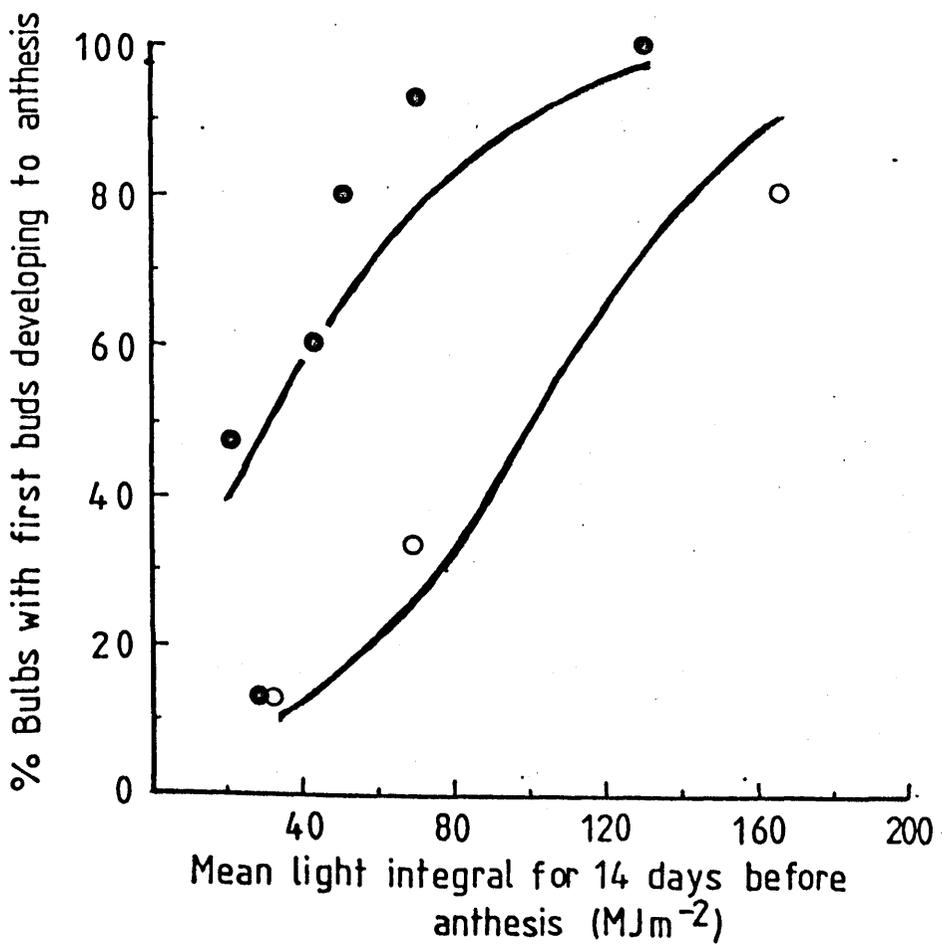
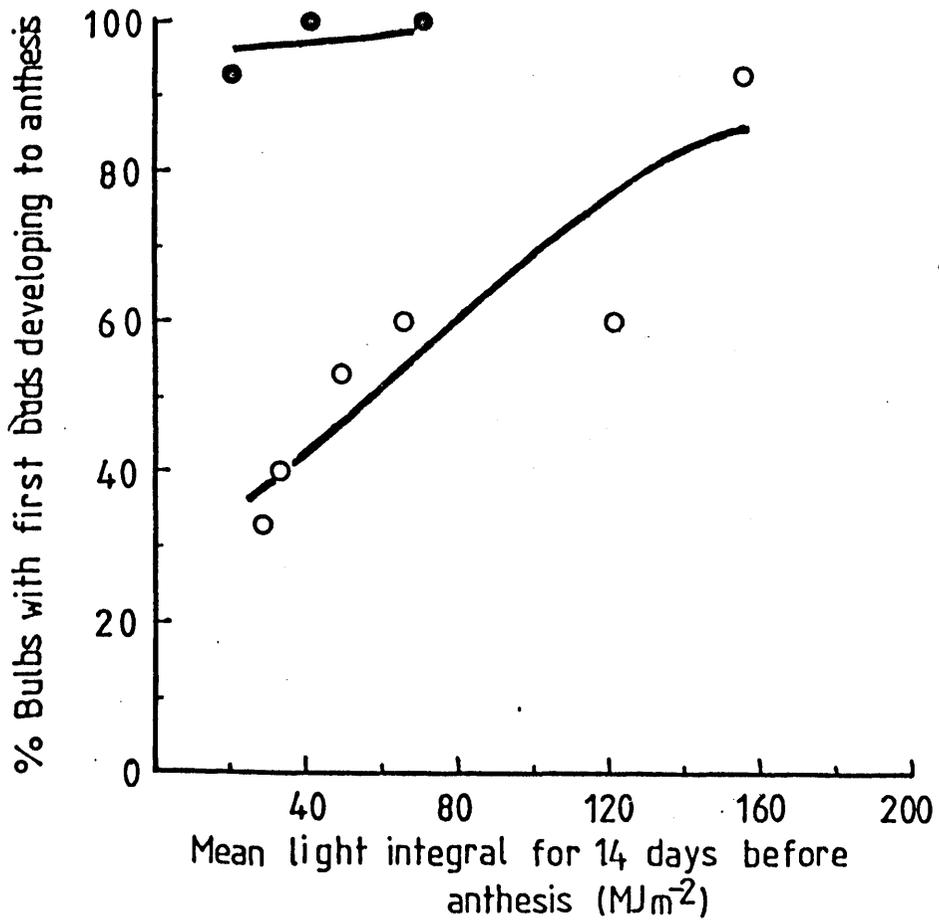
Deviance accounted for = 92.0% (p < 0.05)

Fig. 6.4 The relationship between the light integral for 14 days before plants developed to anthesis and the percentage of bulbs in which flowers developed to anthesis for 'Wedgwood' (8-9 cm) at 17-18°C. First two planting dates respond differently to the incident light than last planting date.

$$\bullet = \text{planted 21 Jan and 18 Feb } \text{Log}_e \left(\frac{y}{(100-y)} \right) = -1.031 + 0.0341x$$

$$\circ = \text{planted 18 March } \text{Log}_e \left(\frac{y}{(100-y)} \right) = -3.374 + 0.0341x$$

Deviance accounted for = 78.6% (p < 0.05)



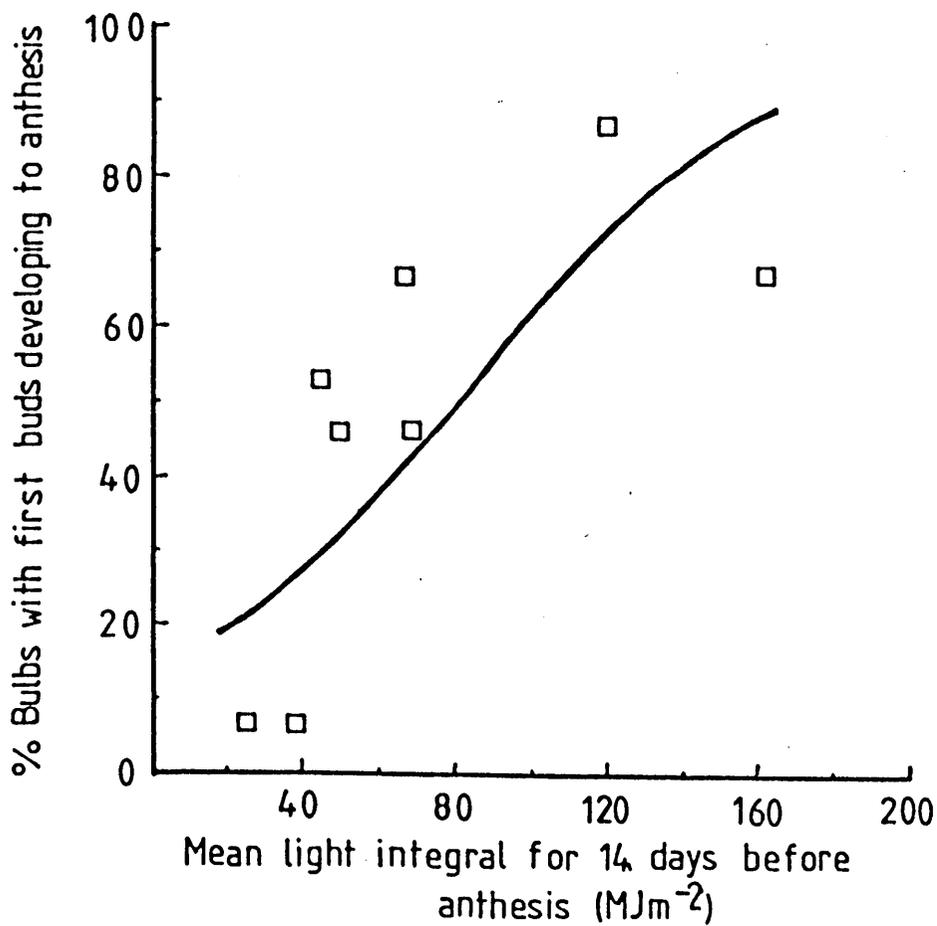


Fig. 6.5 The relationship between the light integral for 14 days before anthesis and the percentage of bulbs in which a flower developed to anthesis for 'Wedgwood' (6-7cm) at 17-18°C. A time of planting influence was not detected.

$$\text{Log}_e \left(\frac{y}{(100-y)} \right) = -1.904 + 0.0237x$$

Deviance accounted for = 48.6% (p < 0.05)

6.2. Photoperiodic effect on flower development

Hartsema and Luyten (1955a) reported a photoperiodic effect on blasting in 'Imperator'. The light energy required for flower development was significantly higher under long photoperiods than short. Fortanier and Zevenbergen (1973), however, tentatively concluded that there was no influence of photoperiod on flower development. They concluded that the discrepancy between the two studies was probably due to the low light intensity used by Hartsema and Luyten.

This investigation examines the proportion of bulbs in which a flower develops to anthesis and the intra-plant competition under different photoperiods.

6.2.1. Materials and Methods

'Wedgwood' bulbs of 10-11, 8-9 and 6-7 cm diameter were treated for early forcing (35°C for 2 weeks, 40°C for 3 days, 17°C for 2 weeks and 9°C for 6 weeks) and planted in Universal potting compost (Fisons, UK) as described in General Materials and Methods, on 28 November 1983. They were then forced in Saxcil growth cabinets at 14 or 16°C and two photoperiods, 8h or 24h. Warm white fluorescent tubes provided light for 8h, the photoperiod was extended in the 24h treatment with six 40W incandescent lamps. The light intensity, measured with a cosine corrected quantum meter with a selenium sensor, was $85 \pm 5 \text{ W m}^{-2}$ (PAR) at pot level. This was hoped to be sufficiently high to avoid at least the early abortion of the flower due to a low intensity effect rather than a photoperiod effect.

Samples of 5 plants were taken at intervals and 10 plants at the final harvest, 10 weeks after planting. The number of buds initiated and the number developing were recorded. Plants were divided into leaves, mother-bulb, daughter-bulbs, roots and flower with stem and their dry weight determined as described in Chapter 3.

6.2.2. Results

All 10-11cm bulbs initiated a flower, approximately half of the 8-9 cm, but none of the 6-7 cm grades did so. More flowers blasted at 16°C than at 14°C ($p < 0.001$) (Table 6.1). For the largest grade of bulbs there was no effect of photoperiod on the number of buds blasting at 14°C but at 16°C fewer buds developed to anthesis under long than short photoperiods, ($p < 0.05$). For the 8-9 cm grade which did initiate a flower, there was an increase in the number of buds aborting with long photoperiods for both temperatures, but abortion was greater under long photoperiods at 16°C than 14°C ($p < 0.05$).

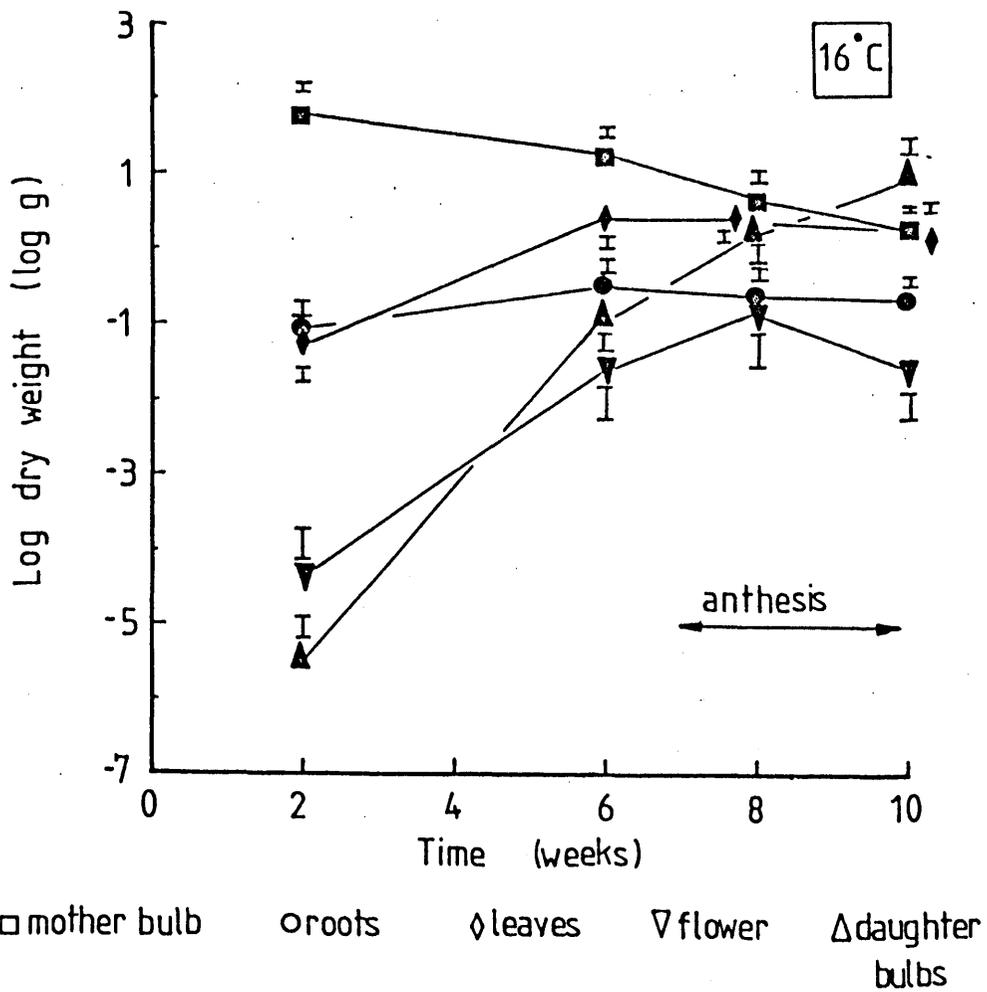
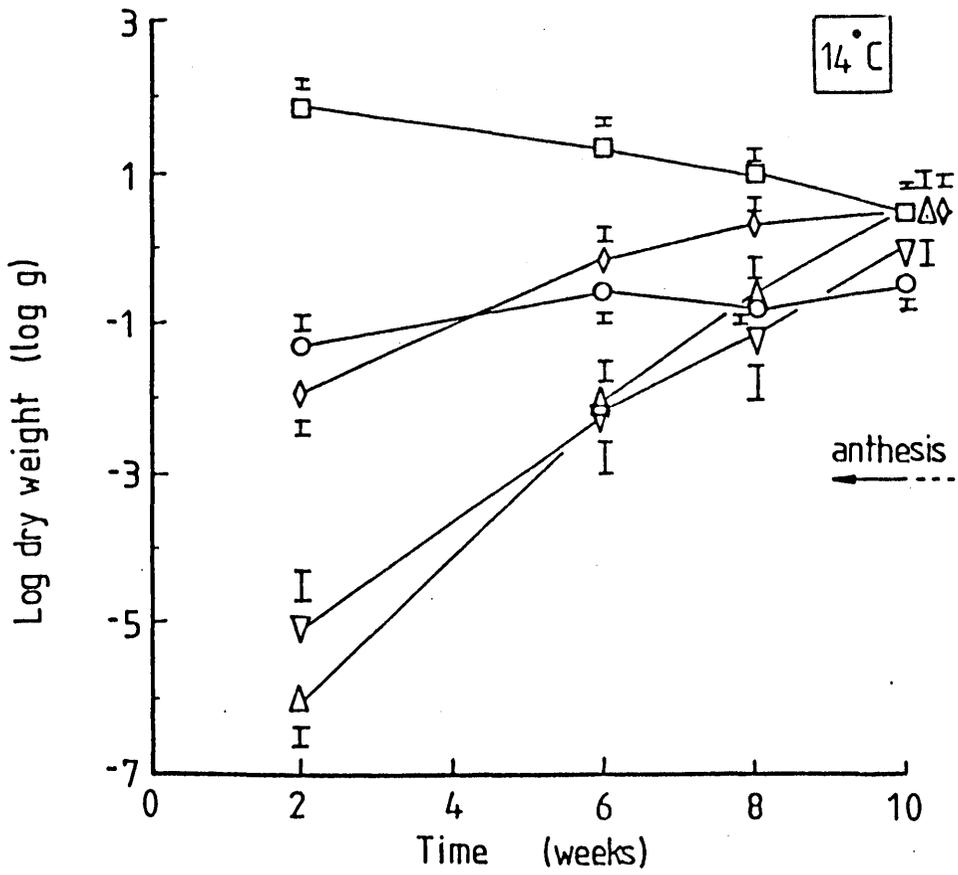
Table 6.1 The percentage of flowers initiated which had aborted at the final harvest when forced under a fixed light intensity of 85 W m^{-2} , 2 photoperiods and 2 temperatures. (20 plants per treatment).

Grade (cm)	Temperature °C	Percentage buds aborted	
		Short photoperiod	Long photoperiod
10-11	14	37	30
	16	75	88
8-9	14	50	70
	16	56	73

The growth curves for the Wedgwood 10-11 cm plant parts measured at both 14 and 16°C are shown in Fig. 6.6 and 6.7. These show that during the 14 days prior to anthesis the daughter bulbs are the only other organs in major competition with the flower and stem. The roots and leaves have reached their full weight by this stage of plant development. This is also supported by the results obtained in Chapter 5. Roots only showed a significant increase in weight between week 2 and 6, ($p < 0.001$). The leaves also only showed a significant increase between week 2 and 6 ($p < 0.001$) with no differences in leaf weight between the two temperatures

Fig 6.6 Changes in the dry weight of various plant parts after planting at 14°C. (Vertical bars = Standard errors of differences of means) 'Wedgwood' 10+cm bulbs - all initiated flowers.

Fig 6.7 Changes in the dry weight of various plant parts after planting at 16°C (Vertical bars = standard errors of differences of means). 'Wedgwood' 10+cm bulbs - all initiated flowers.



except at week 10; the weight of leaves was significantly lower at 16°C than 14°C ($p < 0.05$), but by this time some of the plants at 16°C were beyond the stage of anthesis and the leaves were starting to senesce.

Flower dry weight did not show any significant differences with temperature or photoperiod except at the time of the last harvest, when the flower was heavier at 14 than at 16°C. This, again, was probably due to differences in the rate of development of the flower, the mean flower weight was at a maximum at week 8 at 16°C, but at 14°C the weight was still increasing at week 10. At this time the weight of the flower grown at 14°C was greater than the maximum weight achieved at 16°C ($p < 0.01$). Hence, if the bulbs had all been harvested at the same stage of development e.g. when most flowers reached anthesis in each treatment, the relationship between flower development and flower and stem dry weight observed in Chapter 5 would still hold.

At the time of flower stem extension, when plants are most susceptible to blasting, there are two main competing sinks, the flower and the daughter bulbs. In short photoperiods, there were generally no significant differences between the weight of the daughter bulbs produced at 14°C and those produced at 16°C. In long photoperiods, however, bulbs produced at 16°C were heavier than those at 14°C for harvests at week 6, 8 and 10 (Fig. 6.8). Hence, this increased daughter bulb weight was apparent before any visual signs of bud blasting. Fig 6.9 shows the percentage of the total plant weight accounted for by the daughter bulbs for all harvests after angular transformation. The largest percentage of total weight accounted for by daughter bulbs occurred under long photo-periods at 16°C, ($p < 0.05$) but short photoperiods at 16°C also produced daughter bulbs which accounted for a larger percentage of the total plant weight than in plants grown at 14°C, ($p < 0.001$).

In order to confirm that the increase in daughter bulb weight was a cause rather than an effect of flower bud blasting, it was necessary to examine daughter bulb growth under these conditions in the 6-7 cm grade bulbs which did not initiate a flower. The growth curves for these smaller grade bulbs shows heavier daughter bulbs under long photoperiods at 16°C than other treatments at

Fig. 6.8 Growth curves for daughter bulbs at 14°C (circles) and 16°C (squares) for long (open) and short (closed) photoperiods. 'Wedgwood' 10+cm bulbs all initiated flowers.

Fig. 6.9 Angular transformation of daughter-bulb dry weight as a proportion of total plant dry weight as a mean for all four harvests for treatments of 14 or 16°C and short (SP) or long photoperiods (LP). 'Wedgwood' 10+cm bulbs - all initiated flowers.

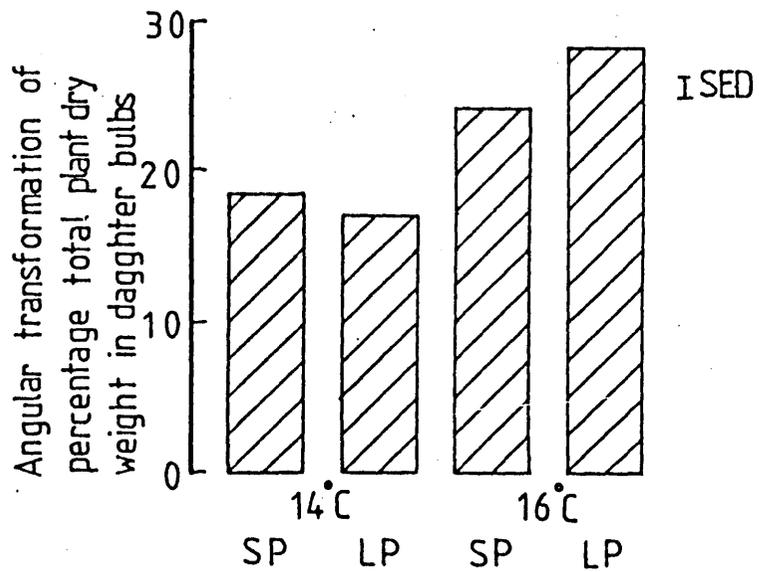
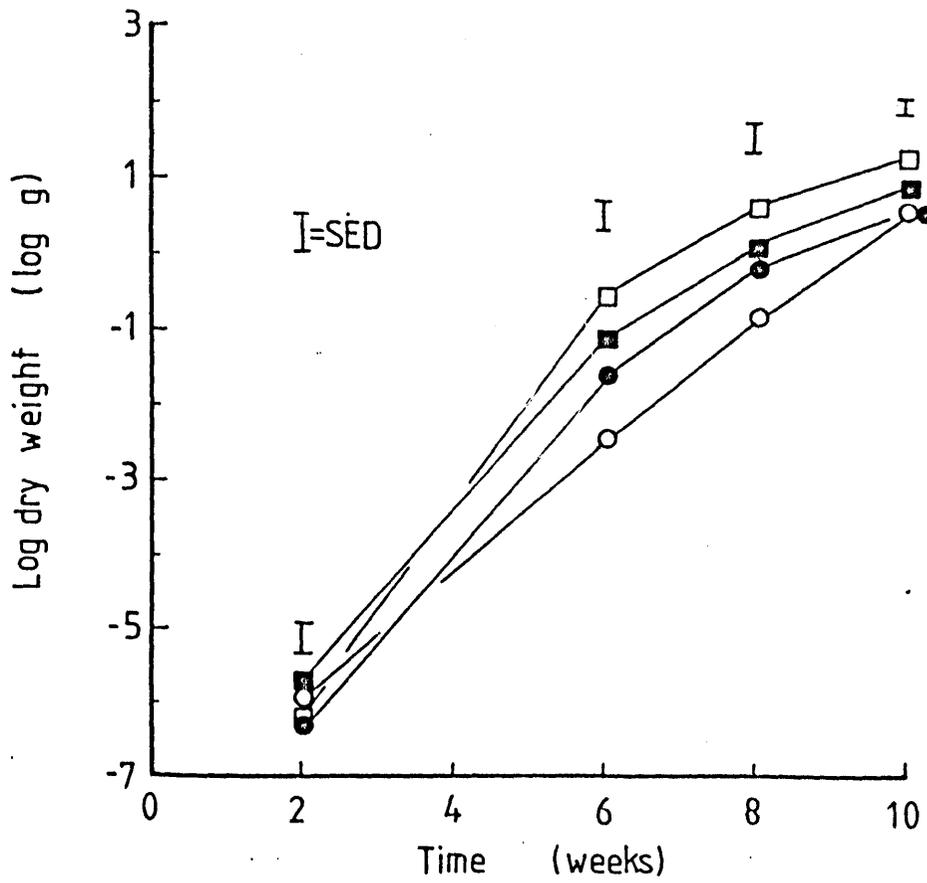
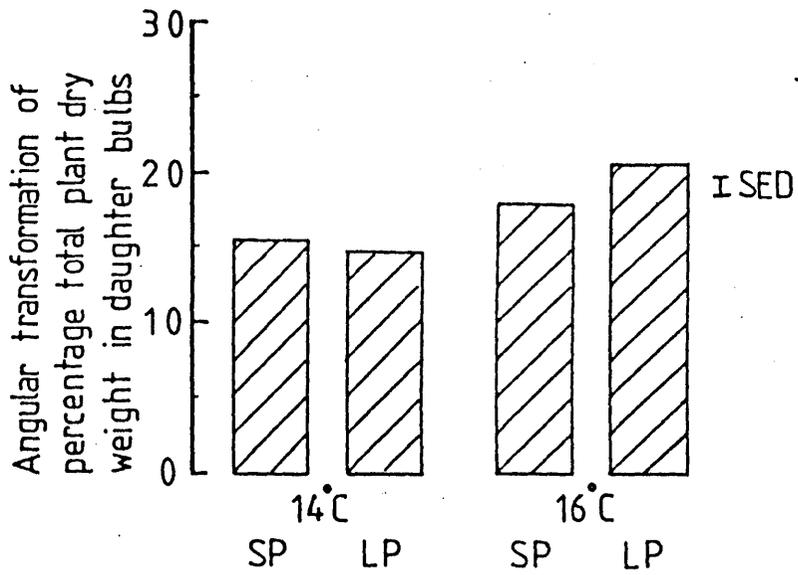
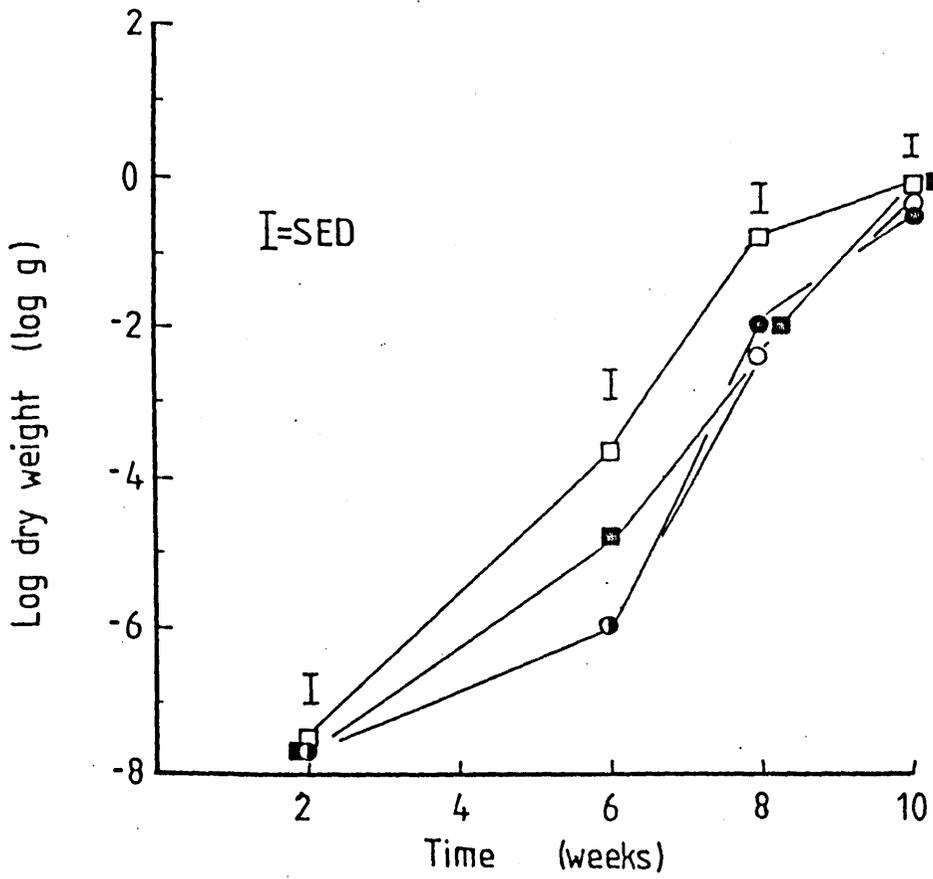


Fig. 6.10 Growth curves of daughter bulbs at 14°C (circles) and 16°C (squares) for long (open) and short (closed) photoperiods. 'Wedgwood' 6-7cm bulbs - no flowers were initiated.

Fig. 6.11 Angular transformation of daughter-bulb dry weight as a proportion of total plant dry weight as a mean for all four harvests for treatments of 14 or 16°C and short (SP) or long photoperiods (LP). 'Wedgwood' 6-7cm bulbs - no flowers were initiated.



week 6 and 8 ($p < 0.05$) (Fig. 6.10). At week 6, the short photoperiods at 16°C also gave heavier daughter bulbs than either of the photoperiods at 14°C, ($p < 0.01$). The photoperiod has no significant effects on daughter bulb growth at 14°C. By week 10 there is no significant difference between any of the treatments suggesting that it is only the initial growth rate of the daughter bulbs which is affected by temperature and photoperiod.

The percentage of total plant dry weight recovered in the daughter bulbs shows an identical response to the treatments as the larger 10-11cm grade bulbs. The only difference was that the daughter bulb weight, over all treatments, was lower in these smaller bulbs (Fig. 6.11).

Total plant dry weight for the largest grade of bulb was not significantly different between harvest dates. This suggests that most of the carbohydrates for growth were supplied by the mother bulb. For the smaller grades, the total plant weight increased with successive harvests ($p < 0.001$); hence, current assimilates play a more important role in the growth of plants from smaller bulbs.

6.3. Localized shading, defoliation and flower development

This experiment examined whether light affected flower development, either by influencing the rate of photosynthesis and, hence, the level of assimilates in the plant or by establishing the flower as a sink of sufficient strength to enable completion of its development.

6.3.1. Materials and Methods

Bulbs of 'Wedgwood' (10+ cm grade) were retarded at 25.5°C then given a standard pre-planting treatment of 2 weeks at 17°C followed by 6 weeks at 9°C before planting on 2 March 1984, as described in Chapter 3. Treatments applied 4 April 1984 were:-

- 1) All foliar leaves were removed
- 2) The lamina of the leaves were covered with aluminium foil (plate 6.1)
- 3) The buds and spathe leaves were covered with a foil cap
- 4) Controls left unshaded

On average the controls reached anthesis 21 days after the application of the other treatments. The bulbs were forced in a glasshouse at a constant 16°C, with automatic venting at 18°C. There were eight pots of each treatment (40 bulbs) and these were arranged into four blocks each containing two pots of each treatment.

At the time of application of the treatment eight pots of bulbs were harvested so as to obtain the dry weights of the daughter bulbs, leaves, mother bulb and flower before treatments began. The remaining bulbs were harvested when the first bud had developed to anthesis or on the day the controls reached anthesis if judged to have aborted by gently squeezing, (the so-called 'pinch test') the bud (Vonk and Ribot, 1982). The number of buds developing and the dry weights were recorded.

6.3.2 Results

There were no significant differences between the blocks, therefore results were analysed as a completely randomised design.

The number of plants in which the first and second buds developed to anthesis, blasted, diseased or were not initiated are shown in Table 6.2.

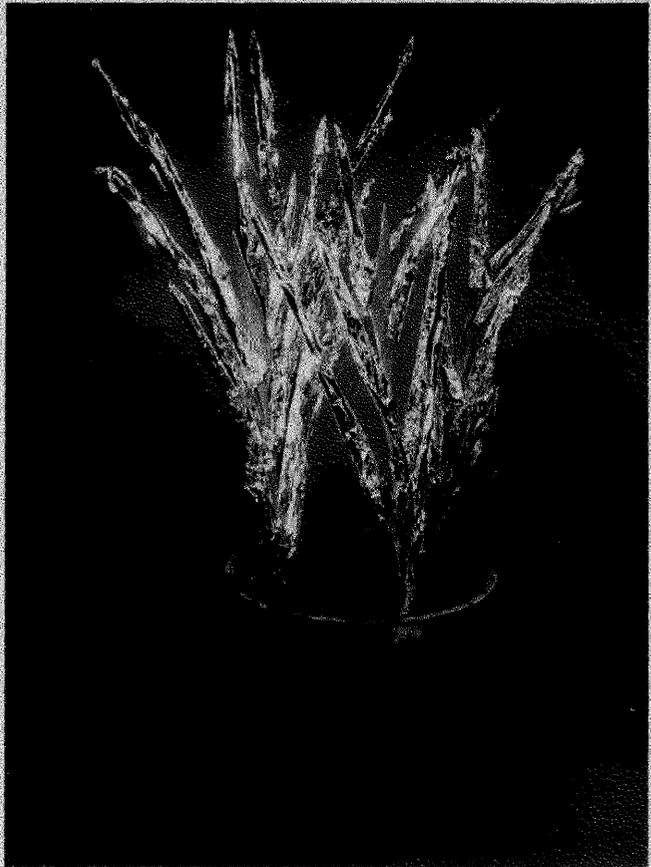


Plate 6.1 The leaf shade treatment. Foliar leaves were covered with aluminium foil. The spathe leaves and buds were left uncovered.

Table 6.2. The effect of shading different plant parts or defoliation on the number of buds developing to anthesis

Treatment	Number developing to anthesis	Number blasted	Number not initiated/diseased
<u>First bud</u>			
Control	37	2	1
Leaves shaded	34 ns	3 ns	3
Leaves removed	32 ns	6 ns	2
Buds shaded	31 ns	5 ns	4
<u>Second bud</u>			
Control	28	11	1
Leaves shaded	17 *	19 *	4
Leaves removed	15 **	21 **	4
Bud shaded	24 ns	12 ns	4

ns = not significantly different from control

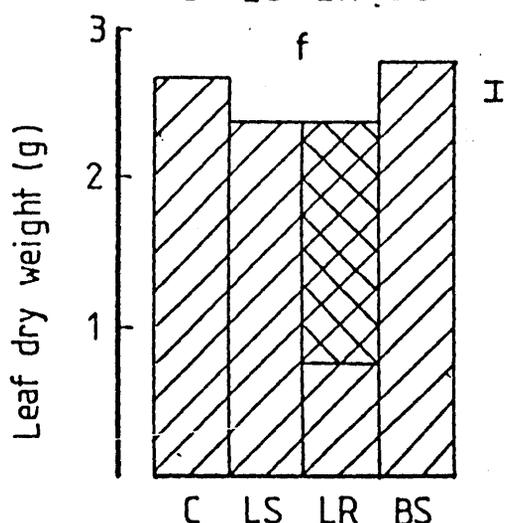
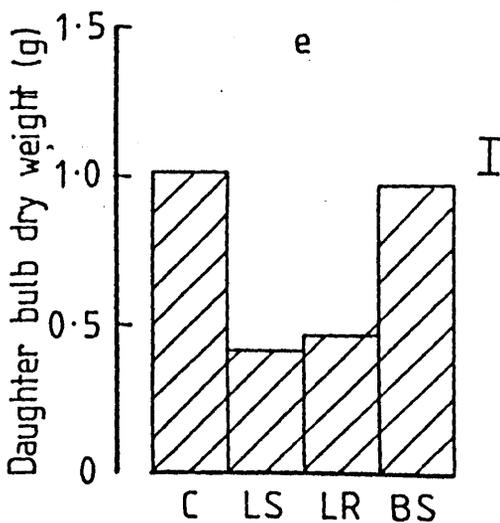
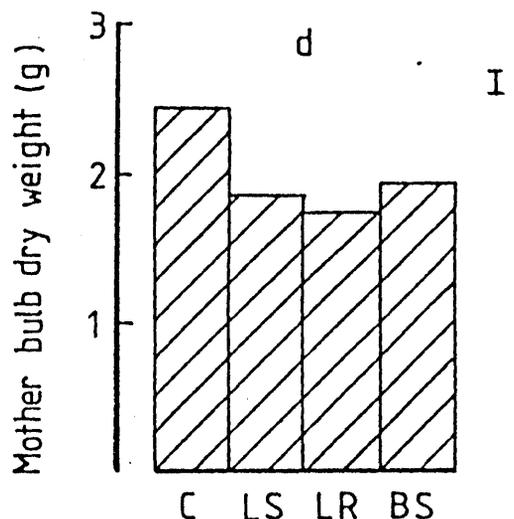
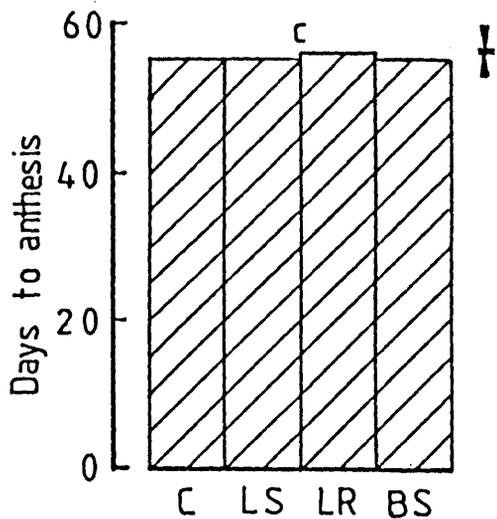
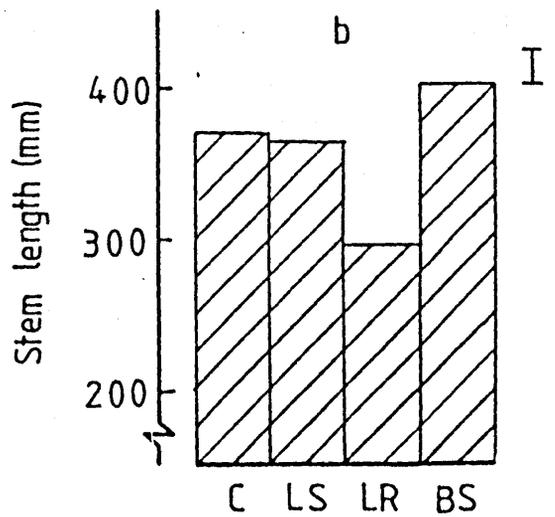
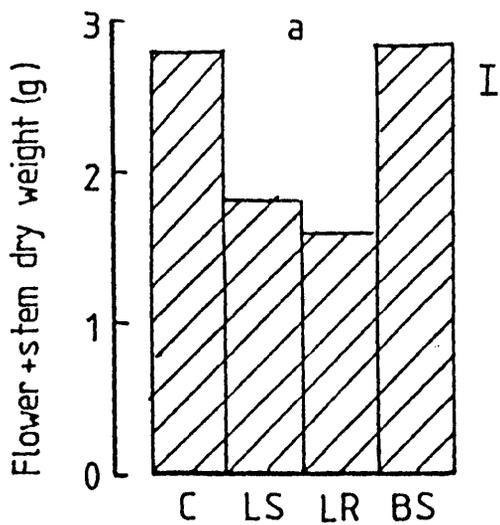
* = significantly different from control ($p < 0.05$)

** = highly significant different from control ($p < 0.01$)

A chi-squared test showed that there was no significant difference in the number of first buds developing to anthesis when comparing the controls with any of the other treatments. The development of the second bud, however, was significantly reduced when the leaves were removed ($p < 0.01$) or the leaves were shaded ($p < 0.05$). Shading the buds did not reduce the number of flowers developing compared to the controls.

The dry weights of the various plant parts and the flower stem length are shown in Fig. 6.12 a-f for the four treatments. Between the start of treatments and the controls developing to anthesis (on average 21 days) the dry weight of the flower and stem increased from 0.18g to 2.78g. A similar increase in weight was observed when the flower buds were shaded, (Fig. 6.12 (a)). Removing or shading the leaves reduced the increase in flower and stem dry weight ($p < 0.001$), the increase from the start of the

Fig. 6.12. The effect of defoliation and shading the leaves or the bud on a) flower and stem dry weight b) stem length c) days to anthesis d) mother-bulb dry weight e) daughter-bulb dry weight f) leaf dry weight.



C= Control
 LS= Leaves shaded
 LR= Leaves removed
 BS= Bud shaded

 = Leaf weight removed

I = Standard error of difference of means

treatment to harvest was only 8-10 fold. There was no significant difference between shading or removing the leaves.

Leaf removal reduced the stem length ($p < 0.01$), while shading the leaves produced stems of similar length to the controls (Fig. 6.12(b)). It is possible that the bud detects light and the flower stem extends until the bud emerges from the leaves. Removing the leaves also removes their shading effect on the bud and stems remain shorter. Shading the buds alone, however, did not produce stems significantly longer than those of the controls.

The rate of flower development to anthesis was delayed by leaf removal but only by an average of 1 day (Fig 6.12(c)). The treatments were remarkably similar in the rate at which they developed to anthesis suggesting that the most important factor governing this was the temperature.

The mother-bulb dry weight remaining at harvest was lower in all the treatments than in the controls ($p < 0.001$), (Fig. 6.12(d)). Daughter bulb growth was reduced by shading or removing the leaves ($p < 0.001$), but shading the buds had no significant effect compared to untreated controls (Fig 6.12(e)). Similarly, leaf dry weight was reduced by shading or removing the leaves, but shading the bud had no significant effect compared to the untreated controls.

These results suggest that the major cause of blasting is a reduction in the carbohydrate status of the plant due to reduced photosynthesis which causes reductions in the dry weight of all plant parts. When the buds alone were shaded, there was a similar reduction in mother-bulb reserves as when leaves were removed or shaded, but shading the bud did not affect its development while the latter two treatments did. It is possible that the light influence on transport of carbohydrates to the bud is detected in the leaves and not at the bud itself.

6.4. Establishment of the relationship between transpiration rate and flower development.

Previous reports and work here, where leaves have been covered or plants placed in the dark, have not taken into account

the effect such treatments have on the rate of transpiration. There are two likely effects of transpiration rate which could influence flower development. First, the cooling effect due to the movement of water past the apex and evaporation and, secondly, the movement of minerals, growth regulators and carbohydrates in the transpiration stream.

All the factors which lead to bud abortion can also cause stomata closure and reduced transpiration, i.e. low light, high temperatures and water stress. Pazourek (1970) showed reduced light intensity lowered the stomatal frequency in 'Wedgwood' leaves.

The relationship between leaf growth and flower development has been established here. The morphology of the iris plant with longer leaves would be expected to allow greater transpiration than a short leaved plant.

The relationship between transpiration rate and flower development was investigated.

6.4.1. Materials and Methods

Retarded 'Ideal', 10 cm, bulbs were cool treated with 2 weeks at 17°C and 6 weeks at 9°C before planting, as described in Chapter 3, on 2 August 1984. They were forced in a glasshouse at a constant temperature of 16°C, venting at 18°C. A week after planting each pot was covered with black polythene to reduce evaporation from the compost surface. The pots were placed on saucers and watered under the polythene until pot capacity was achieved. The pots were then weighed at this maximum and again later that day or 24h later. The rate of water loss was calculated from the reduction in weight between successive weighings. Pots were restored to pot capacity when it was considered necessary for those with the greatest water loss rates.

The pots were placed either side of two tubes of polythene. These tubes were inflated by continuously blowing air into the north end with a small electric fan. Opposite half of the pots holes were cut into the polythene tube to produce a stream of air over the plants (Plate 6.2). The tube was left intact opposite



Plate 6.2 Method of ducting air to create greater air movement over selected pots than others.

Fig. 6.13 The number of first buds per pot which developed to anthesis against the transpiration rate (g of water per plant per day).

$$y = -28.4 + 3.67x - 0.101x^2$$

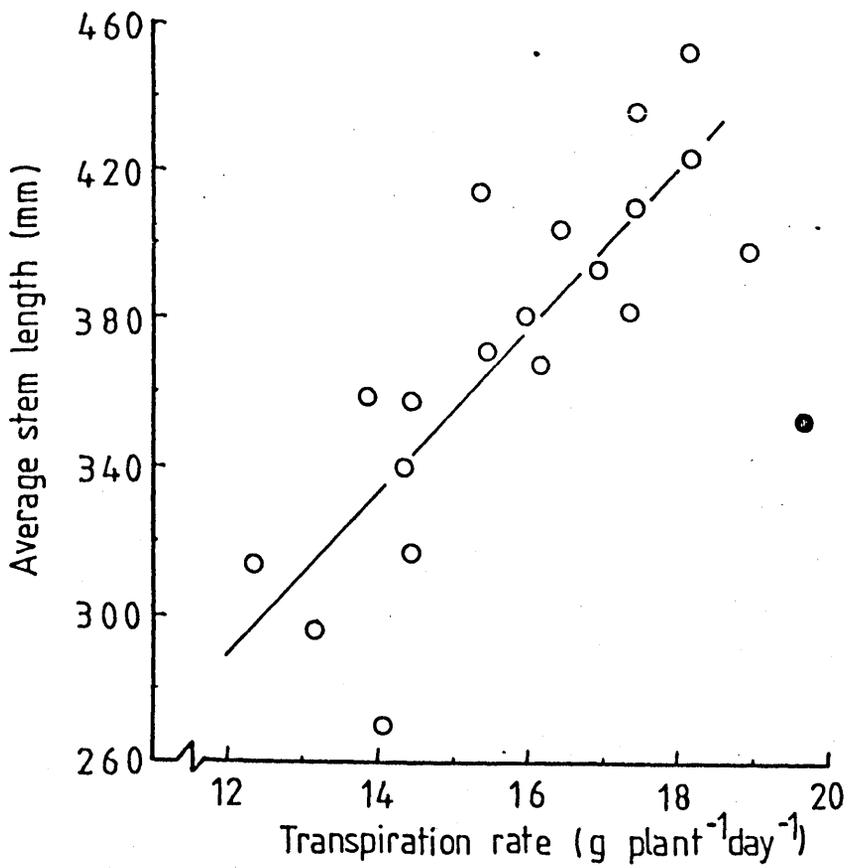
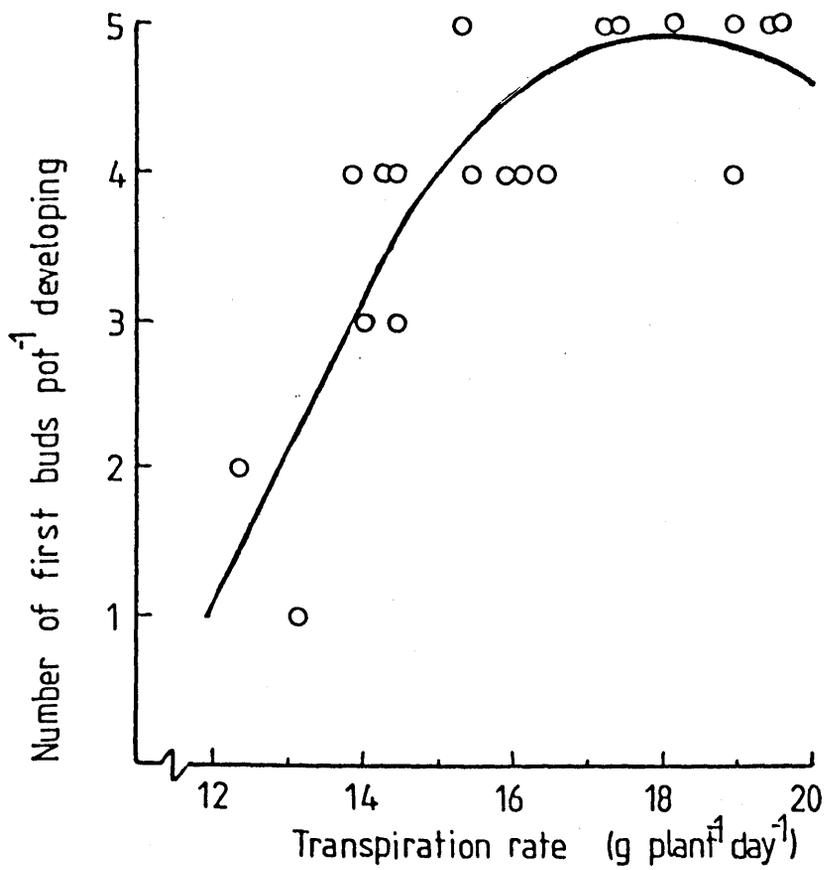
$$r^2 = 68.5\% \quad p < 0.001$$

Fig. 6.14 The average stem length per pot against the transpiration rate (g of water lost per plant per day).

$$y = 29.5 + 21.7x$$

$$r^2 = 66.1\% \quad p < 0.001$$

Closed circle not included in regression (see text).



other pots. This experiment was totally randomised, a range of transpiration rates were achieved.

All 20 pots were shaded, reducing the light by approximately 50%, producing conditions which could cause flower abortion.

All plants were harvested five weeks after planting, the number of buds initiated, the number developing and the flower stalk length were recorded.

6.4.2. Results

As the transpiration rate increased the proportion of first buds developing also increased (Fig 6.13). This proportion of bulbs with a developing first bud is a crude method of measuring the degree of flower development, as there were only five bulbs per pot. The length of the flower stem reflects the time of abortion and is a better quantitative measure than the proportion developing (Fortanier and Zevenbergen, 1973). The relationship between average stem length and the transpiration rate shows clearly that as transpiration increases, flower development increases, (Fig. 6.14). At very high rates of transpiration, however, there is some evidence of a reduction in flower development. These very high rates, indicated by the shaded symbol in Fig 6.14, have not been included in the regression.

6.5. The role of light in the partitioning of carbohydrates

Cytokinins reduce flower blasting following a dark treatment (Mae and Vonk, 1974). A prolonged dark treatment has been shown to lead to an increase in ABA in the bud, and cytokinin binds with the ABA causing its activity to fall (Vonk and Ribot, 1982).

The aims of these experiments were, firstly, to determine the sugars present in the flower bud and how light and cytokinin affect the proportions of these sugars in the developing bud. Secondly, with ¹⁴C radio-isotope tracer, the movement of current photosynthate and stored carbohydrates from the mother bulb, under high and low light was examined.

6.5.1. Materials and Methods

Carbohydrate determination

Wedgwood 10+ cm bulbs were retarded for 9 weeks at 25.5°C before pre-treating for forcing with 2 weeks at 17°C and 6 weeks at 9°C. Bulbs were planted on 26 Jan 1984 as described in Chapter 3.

Treatments were applied on 13 March, 1984, ten pots were covered with two layers of polyethylene shading and ten pots were left unshaded. Half of the flower buds in each light treatment were injected with 1ml of 10^{-4} M BA (N^6 -benzylaminopurine) dissolved in 0.5% DMSO into the gap between the two spathe leaves. The other half of the buds were injected with 0.5% DMSO only.

Between 27 and 29 March, a representative sample of 16 plants were harvested from each treatment. Only those bulbs in which there were no visible signs of abortion were analysed. The buds were removed at the last node, weighed and divided into half. One half of each of the buds were extracted, clarified and analysed for carbohydrates as described in Chapter 3. Sugars present in the buds were identified using HPLC with 80% acetonitrile as a solvent.

The brown outer scales of the remaining mother bulb were removed and these divided into mother-bulb scales and base plate and daughter bulbs. These were then dried along with the remaining half of the flower buds for dry weight determination.

14 C distribution

Mother bulbs labelled with 14 C were stored for 7 weeks at 25.5°C. These were then graded, taking bulbs as large as possible for the treatments. Those bulbs selected were graded at 6-7cm.

To assess the movement of current photosynthesis 8-9cm grade bulbs were used. These had been retarded at 25.5°C for 27 weeks, but they received the same pre-planting cool treatment as the labelled mother bulbs at 17°C for 4 weeks followed by 9°C for 4 weeks. All bulbs were planted 21 May 1985.

Two pots were placed under two layers of shading, one contained labelled bulbs and the second contained bulbs to be labelled to monitor ^{14}C -assimilate movement. These were surrounded by guard pots. The remainder of the pots were left unshaded. After 2 weeks, an unlabelled pot from each light treatment was fed $^{14}\text{CO}_2$ as described in Chapter 3. These were then replaced under the treatments for a further day. For the first harvest, a second pot of bulbs from each treatment was also fed, and this was harvested 5 days later.

This procedure was repeated twice during the time of bulb forcing. All shaded bulbs were, therefore, only in low light for 15 days or in the first harvest, the second pot for 20 days. This permitted the effect of shading to be observed at different stages of development without being influenced by previous depletion of reserves.

Harvests were taken on day 15, 20, 28 and 42 from planting. The plants were divided into roots, scales, fed leaf, other leaves, flower and stem, daughter bulbs and base plate. These were frozen in liquid nitrogen before freeze drying, weighing and counting as described in Chapter 3.

6.5.2 Results

Sugars present in the alcohol soluble extract from buds were identified as mainly glucose, fructose and sucrose. The time required for soxhlet extraction was established as 6 hours (Fig. 6.15).

The hexose sugars, sucrose and starch extracted from the flower buds of each treatment are shown in Fig. 6.16. The results are expressed on a dry weight basis and as the content of a bud of average weight for each treatment.

When results were expressed per gram dry weight of bud, hexoses were slightly higher in those buds grown in reduced light ($p < 0.05$), while those grown in high light contained about twice the content of sucrose per gram than those grown in low light. The starch content did not show any significant differences with

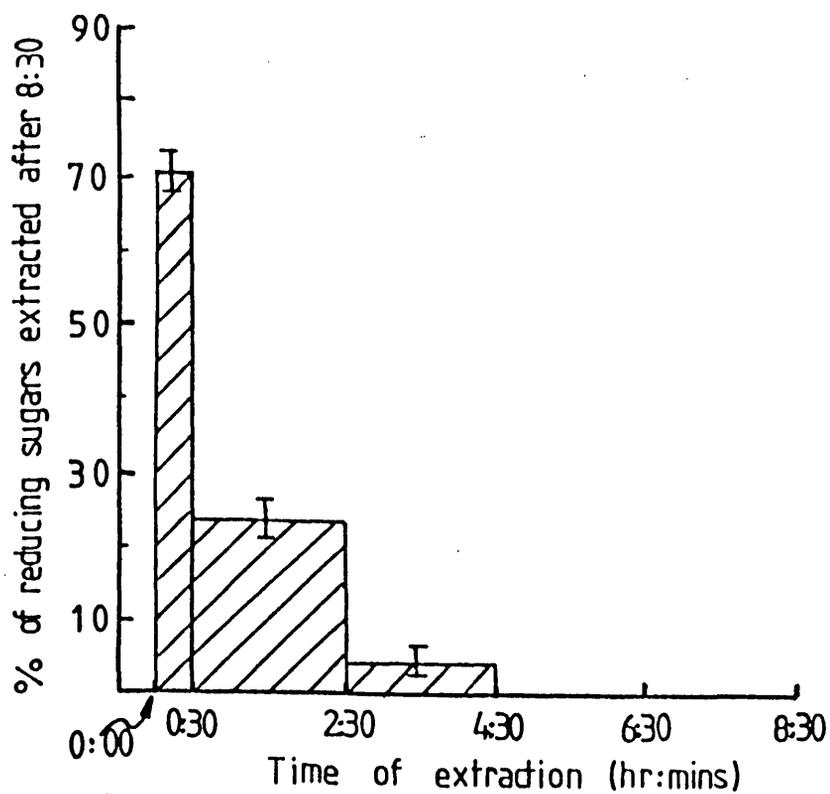


Fig. 6.15 Determination of extraction time necessary for alcohol soluble carbohydrate analysis of flower buds. (Vertical bars = standard error).

Fig. 6.16a Sugar content (mg equivalent of glucose) in each gram of dried bud material from plants grown under unshaded or shaded conditions, with or without cytokinin application.

Fig. 6.16b Sugar content (mg equivalent of glucose) extracted from a bud of average dry weight for each of the treatments; unshaded and shaded and with or without cytokinin.

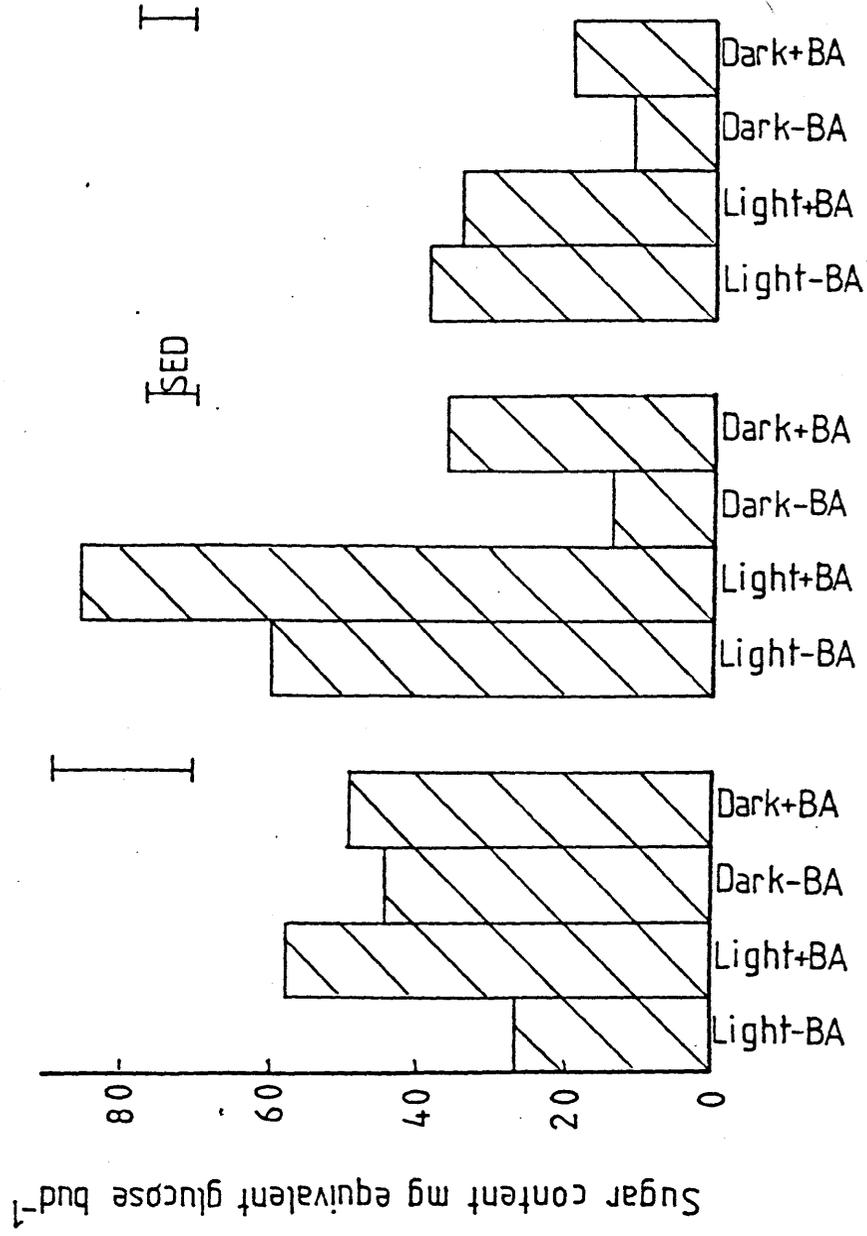
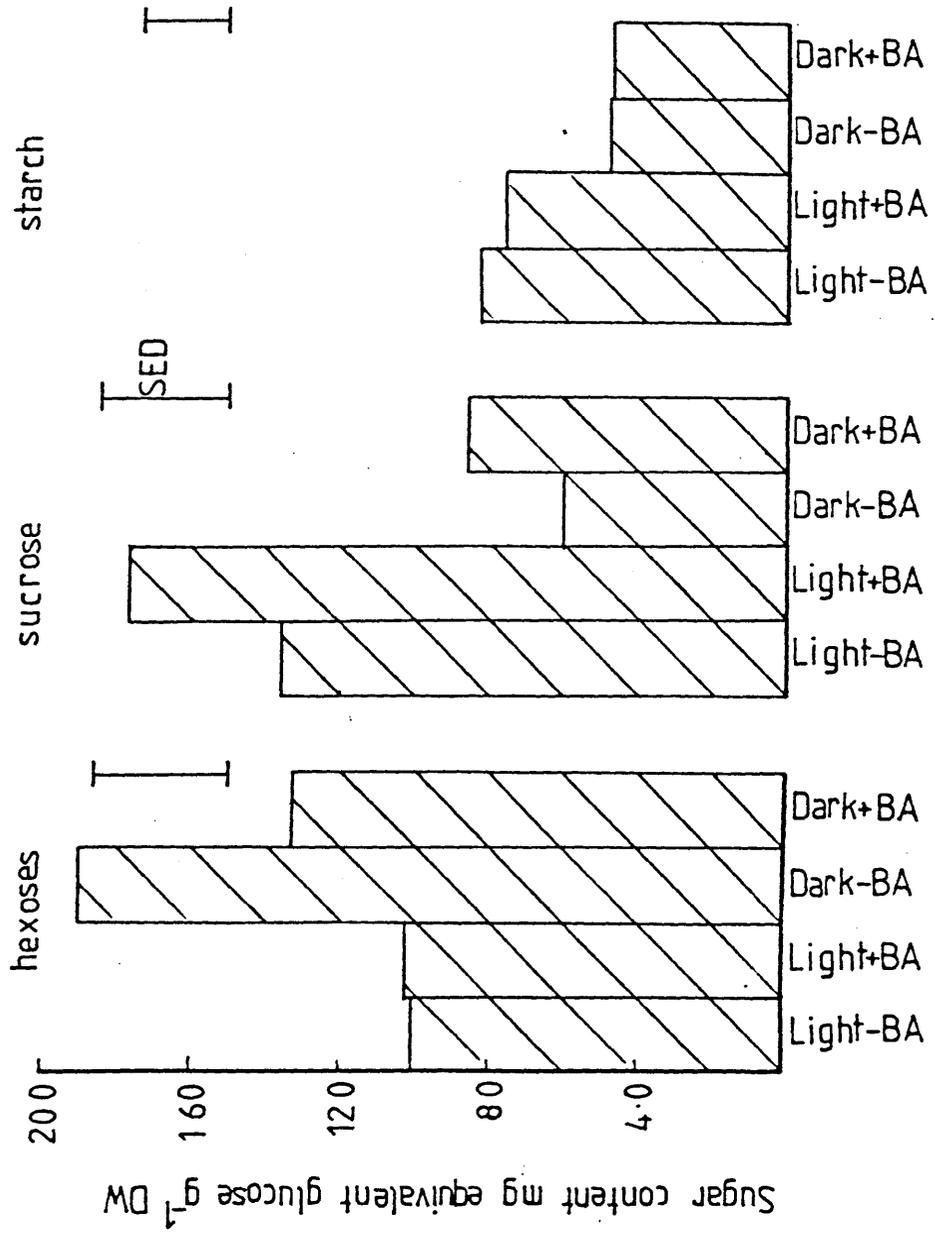
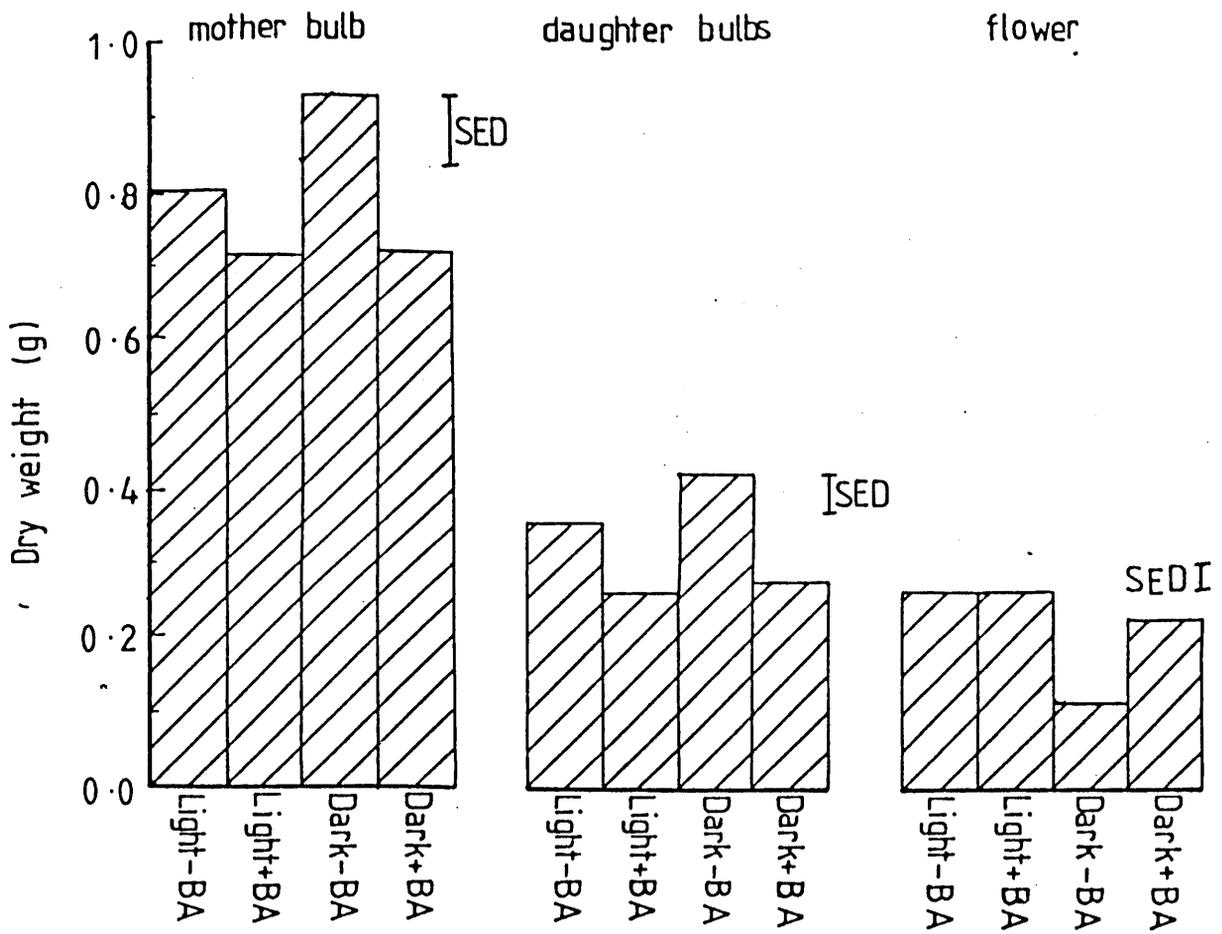


Fig. 6.17 The dry weight of flower buds, daughter bulbs and mother bulb remaining when forced unshaded or shaded and with or without the application of cytokinin.



irradiance level. There was no significant effect of BA on hexose, sucrose or starch present in each gram of bud.

When the sugar content of an average weight bud was calculated, sucrose levels were increased by both increased light and cytokinin ($p < 0.001$) but there was no difference in the hexose content. The cytokinin was as effective at increasing the sucrose content in high light as in low. Only light increased the starch levels in a bud, high light buds containing about twice as much starch as low light ones, ($p < 0.01$).

Cytokinin applied to the bud decreased both the weight of daughter bulbs ($p < 0.01$) and the weights of the remaining mother bulb ($p < 0.05$) at the time of harvest (Fig. 6.17). The irradiance level had no significant effect on these dry weights. The dry weight of the flower bud was not significantly affected by cytokinin, but it was heavier under high than low light, ($p < 0.01$). Cytokinins applied to the bud appear to decrease the relative sink strength of the daughter bulbs and cause a greater depletion of mother-bulb reserves, they did not cause increases in flower weight, whereas increased irradiance did.

The results of plant growth and partitioning under high and low irradiances can be expressed in three ways. Dry weights provide the actual mass changes between harvests. Distribution of the exported ^{14}C assimilates is described by the percentage of the exported radioactivity, from the source leaf, recovered in the various plant parts 24h after labelling. Finally, the relative specific activity, RSA, provides a method of calculating the relative sink strength of a given organ independently of the size of the organ. The RSA is calculated according to the method of Mor and Halevy, (1979):-

$$\text{RSA} = \frac{\text{dpm g}^{-1} \text{ in a specified plant part}}{\text{dpm g}^{-1} \text{ of the whole plant excluding the source leaf}}$$

where dpm g^{-1} is the disintegrations per minute for each gram of dry matter.

The sequence of demands for assimilates by the various plant parts during forcing were examined. The dry weight changes and log dry weight changes for rapidly growing plant parts are shown in Fig. 6.18 and 6.19 respectively.

6.5.2.1 Flower plus stem changes during forcing

The dry weight of the flower plus stem increased by approximately 80 fold between day 15 and day 42 from planting under both high and low irradiances. The flower plus stem, however, were significantly heavier under high irradiances but only late in the flower development, i.e. the harvests at day 28 and 42 ($p < 0.001$). Under high irradiances, the percentage of the exported ^{14}C -assimilates increased with later harvests ($p < 0.001$). The percentage of the exported assimilates distributed to the flower was lower under reduced light than in the unshaded plants, on day 15 and 42, ($p < 0.01$), but there was no significant difference on day 28 (Fig. 6.20). Under low light there was a decrease in the percentage of assimilates which were exported to the flower between day 28 and day 42 ($p < 0.001$).

The RSA of the flower and stem followed a similar pattern to the percentage distribution of radioactivity (Fig. 6.21). The RSA was higher under high irradiances at the first and final harvest ($p < 0.01$). Under high light the flower RSA was high for the first two harvests and decreased at the final harvest. Under low irradiances the RSA was lower at the first harvest, equivalent at the second, but lower again at the third harvest.

6.5.2.2 Changes in leaves during forcing

The dry weight of the leaves increased between day 15 and 28 from planting, but there was no further significant increase between day 28 and 42. At the time of the last harvest, however, the high light plant and leaves were heavier than the low light leaves ($p < 0.05$). The dry weight of the single leaf, which was the source leaf fed with $^{14}\text{CO}_2$, also only showed differences in weight at the final harvest ($p < 0.01$).

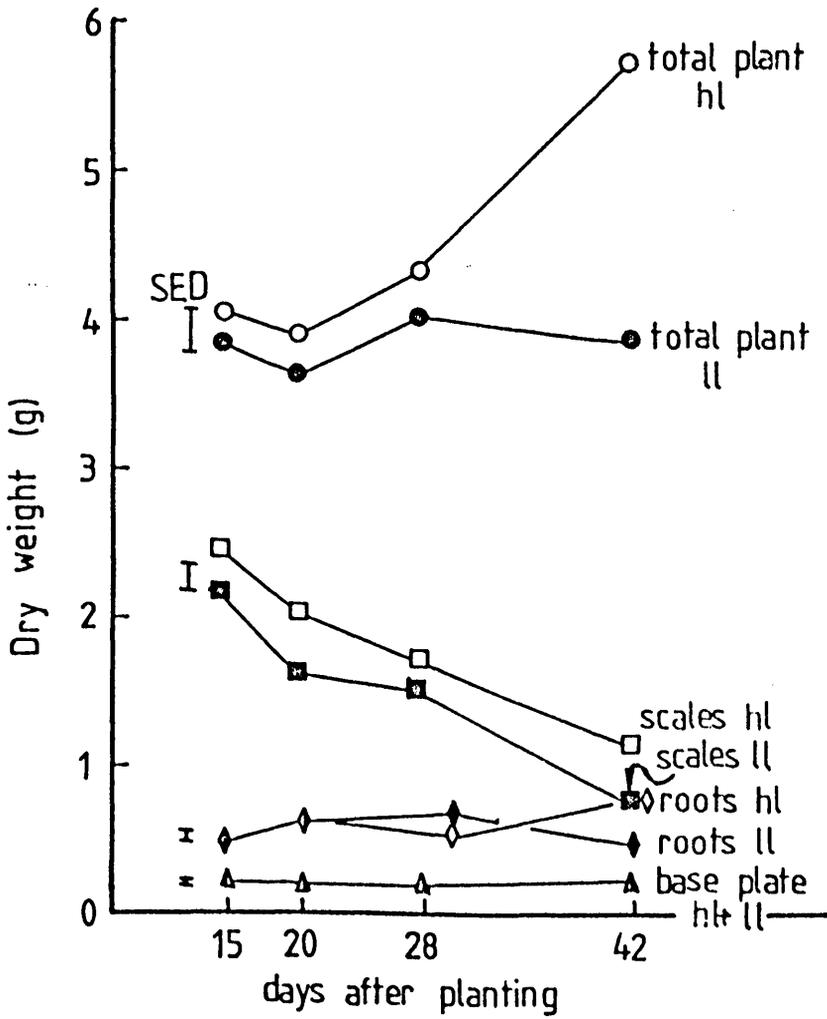


Fig. 6.18 The dry weights of scales, roots and base plate and total plant dry weight when forced under high or low light for the 15 days before harvest.

hl = high light (open symbols)
 ll = low light (closed symbols)

Planting date = 21 May 1985

Fig. 6.19 Log dry weights of exponentially growing plant parts after planting:- leaves, flower and daughter bulbs forced under high or low light for the 15 days before harvest.

High light = open symbols

Low light = closed symbols

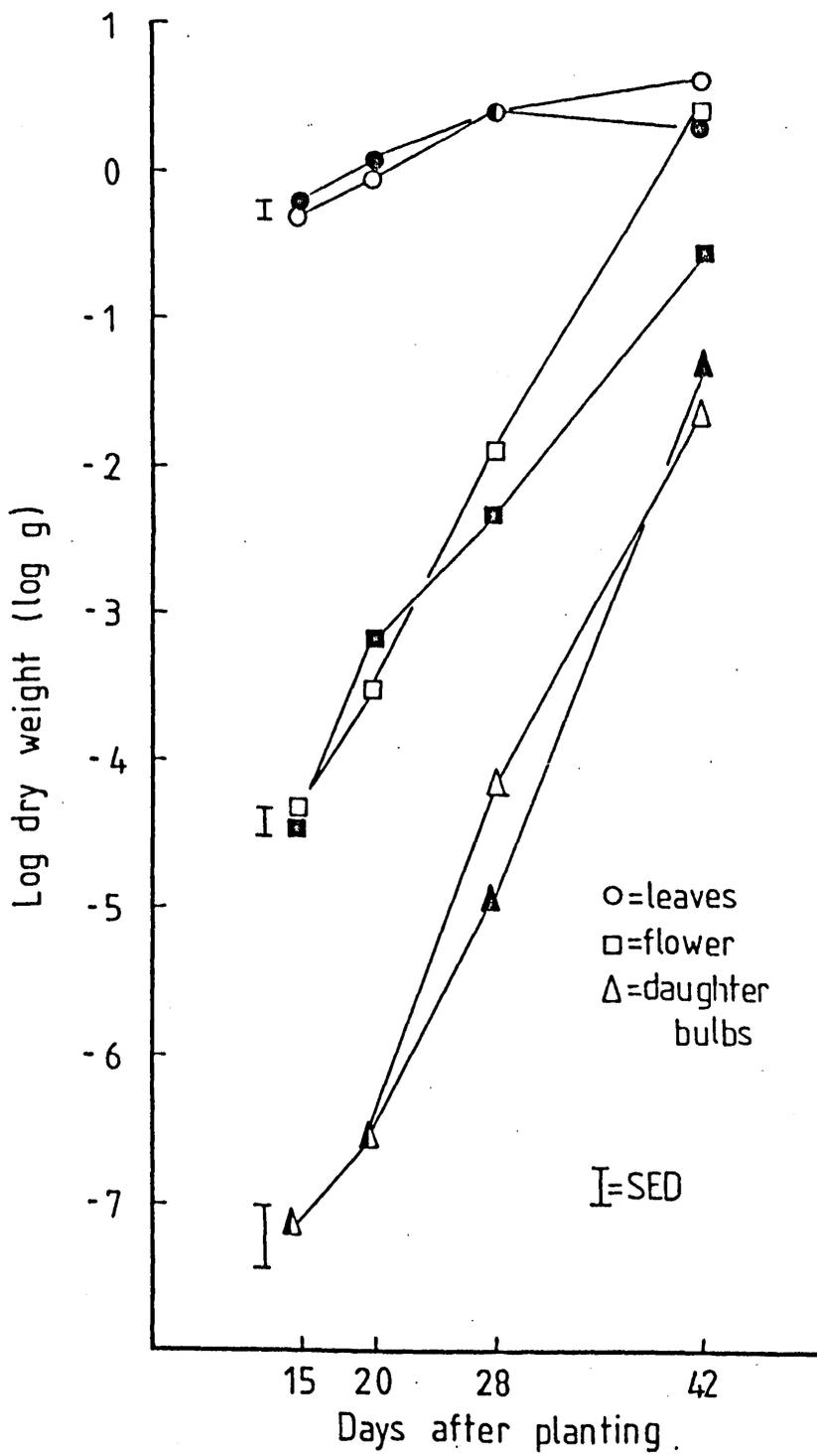
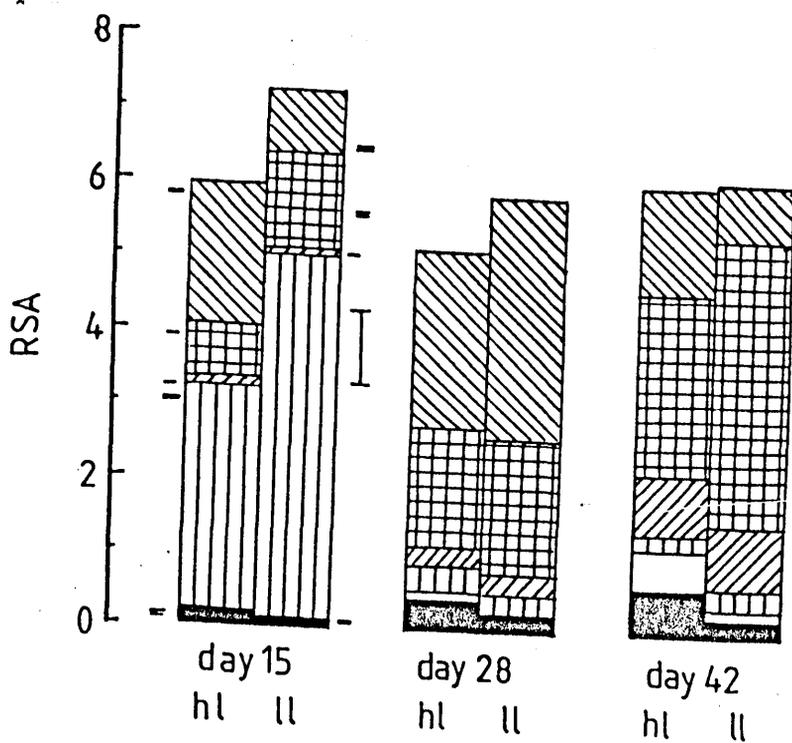
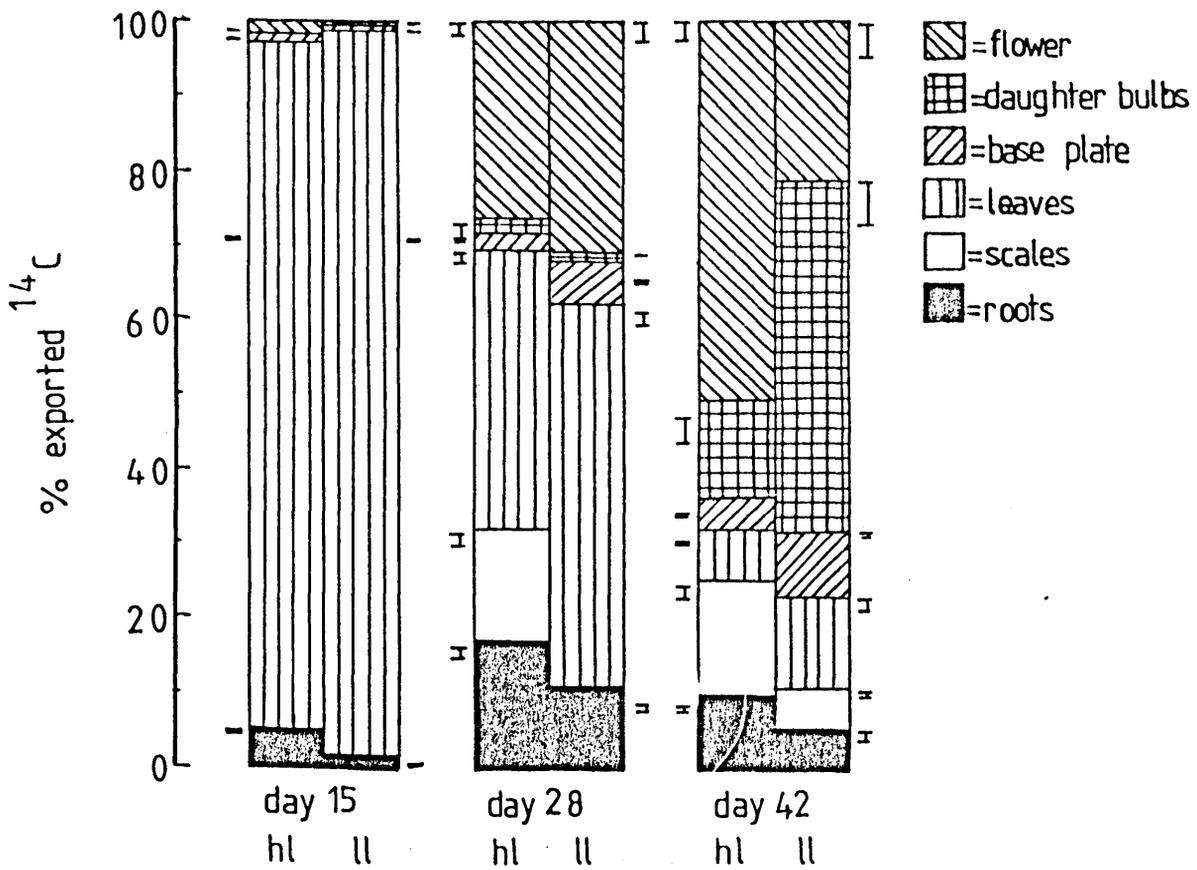


Fig. 6.20 The percentage of exported ^{14}C distributed to the various plant parts under high (HL) or low (LL) light on three harvest dates from planting.

Fig. 6.21 The relative specific activity (RSA) of the various plant parts under high (HL) or low (LL) light for three harvests from planting (key as 6.20)



Of the radioactivity assimilated by the second basal leaf, most of that exported was distributed to other leaves early in the plants growth. For high light plants 92% and for low light 97% of the exported assimilate was exported to the other leaves at the first harvest. This percentage declined with successive harvests, very little being distributed to other leaves by day 42. The proportion distributed to the leaves was always significantly higher for the low light plants than the high ($p < 0.001$). The leaf RSA declined between day 15 and 28 and then remained steady, the irradiance level had no significant effect on this RSA.

6.5.2.3 Daughter bulb changes during forcing

The dry weight of daughter bulbs increased by approximately 260 fold between day 15 and 42. Their weight was not significantly affected by high or low irradiance. Similarly, the irradiance level had no effect on the percentage of the exported assimilates recovered from the daughter bulbs, except at the last harvest, when the percentage found in daughter bulbs growing under low irradiances was greater than that from high irradiance level plants ($p < 0.001$). The RSA of the daughter bulbs was always greater under low light than high, ($p < 0.001$). This was particularly the case at the first and last harvest ($p < 0.001$).

6.5.2.4 Changes in the roots during forcing

The roots had similar dry weights under low and high light until the final harvest when those in high light had heavier roots than those in low, ($p < 0.001$). The proportion of ^{14}C -assimilates exported to the roots and the RSA was always significantly higher in high light ($p < 0.001$).

6.5.2.5 Changes in the mother-bulb scales during forcing

The dry weight of the mother bulb scales steadily declined with time from planting, this decline was generally greater under low than high light. At day 15 no radioactivity could be detected in the scale material. This was expected since at this time the

scales are a major source for growth, rather than a sink for current assimilates. By day 28, however, radioactivity was detected in those plants grown in high but not in those grown in low light. At the final harvest, there was still a considerably larger percentage of the exported radioactivity in the scales of high light than low ($p < 0.001$) but for the first time radioactivity was detectable in the low light scales.

The bulbs fed previously, so as to determine the movement of stored carbohydrates from the mother bulb, unfortunately, did not initiate a flower. There was no significant effect on the distribution of ^{14}C between the high and low light grown plants. The activity of the scales fell between day 15 and day 28 ($p < 0.05$) but not between day 28 and 42. There was no significant difference with irradiance levels. The proportion of the ^{14}C exported, which was recovered from the leaves did not change with high or low light or with the time of harvest.

6.5.2.6 Whole plant changes and assimilate distribution at different times after feeding

The total plant dry weight showed a large difference between the high and low light, the high irradiance plant weight increased by approximately half the total weight of that again at day 15. There was no evidence of such an increase in the low light plants, even though these were in reduced light for 14 days. A comparison between those plants harvested 1 day after feeding and those harvested 5 days after feeding (15 and 20 days after planting) is shown in Fig 6.22. As the time between labelling and harvesting was increased, the percentage of the radioactivity recovered from the flower increased, this increase was greater in the low light than high ($p < 0.001$).

After 5 days, less ^{14}C was present in the leaves ($p < 0.001$) than after a single day. Light levels had no effect on the amount found in the leaves at this early stage. ^{14}C was only detected in scales of the high light treatment after 5 days. The percentage of the exported radioactivity recovered from the roots was greater after 5 days than 1 day ($p < 0.001$) and was always greater in high light treatments than low, but the difference between the two was reduced after 5 days ($p < 0.001$).

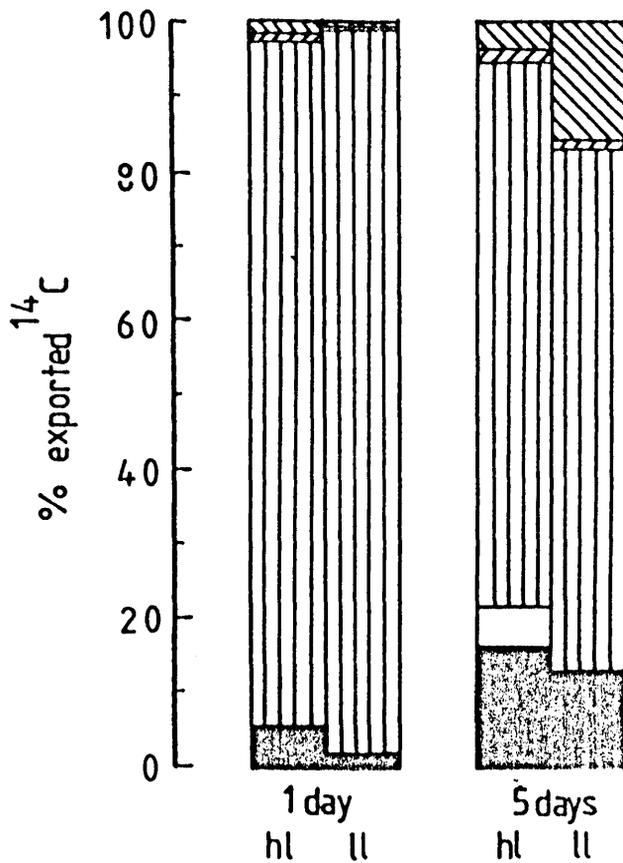


Fig. 6.22 The percentage of exported ¹⁴C distributed to the various plant parts (see key Fig. 6.20) under high and low light (HL and LL) when labelled on day 14 from planting and harvested one or five days later.

6.6 Discussion

Low irradiance, particularly under high temperatures, has been observed to increase flower-bud blasting. This is in agreement with many previous reports (Hartsema and Luyten, 1955 a+b; Kamerbeek, 1966, 1969; Fortanier and Zevenbergen, 1973). The irradiance required to achieve the maximum number of first buds developing to anthesis in 'Wedgwood' (10+cm) bulbs was approximately 130 MJ m^{-2} over the last 14 days, at temperatures between 14 and 17°C. This was a much higher light requirement than expected when compared to the results of previous workers using phytotrons (Hartsema and Luyten, 1961; Kamerbeek, 1969; Fortanier and Zevenbergen, 1973). The lowest light requirement observed was with the first planting date where plants were forced at temperatures between 16 and 17°C and even these had a higher light requirement than expected from previous reports. For comparison of the light required Fortanier and Zevenbergen estimated the results of earlier reports converted to $\text{MJ m}^{-2} \text{ day}^{-1}$. Hartsema and Luyten (1961) had a range of light energies from 0.34 to 1.97 MJ m^{-2} necessary for forcing 'Imperator' between 15 and 17°C. The light requirement was dependent on the pre-planting temperature treatments applied and on the daylength

during forcing. Hartsema and Luyten (1955a and 1962) and Kamerbeek (1966), generally recommended $30 \text{ cal cm}^{-2} \text{ day}^{-1}$ for successful flower development, which was converted to $1.26 \text{ MJ m}^{-2} \text{ day}^{-1}$. Fortanier and Zevenbergen (1973) considered 0.84 MJ m^{-2} sufficient at 15°C for all the flowers to develop. The light measured here was the total short-wave radiation whereas previous results were from experiments using only the wave length of fluorescent lighting. Approximately half of the daylength recorded by a Kipp solarimeter is PAR. From the glasshouse experiment an average irradiance of $4.64 \text{ MJ m}^{-2} \text{ day}$ PAR can be considered necessary for all flowers to develop. Assuming the light requirements given by Fortanier and Zevenbergen to be PAR, the value obtained here is five times as large as their recommended irradiance. Even with the lowest light requirement obtained with the first planting at the slightly higher temperatures, the light requirement was about three fold greater in the glasshouse. There are two reasons for these differences:-

- 1) The light measured here was that incident on the crop. Shading by other plants in the crop will increase the irradiance necessary above that of a single or well spaced plants.

- 2) In phytotrons, light given was at a constant flux density and temperature can be well controlled. In the glasshouse the light intensity can vary to a large extent through the day. There are peaks in irradiance which, because of other limiting factors, the plant cannot use efficiently. The glasshouse temperature will also alter, due to solar gain. High temperatures occur which cannot be avoided by ventilation alone. These may lead to bud abortion despite the average glasshouse air temperature and light incident on the crop appearing adequate for flower-bud development.

At the time of flower stem extension, when plants are most susceptible to blasting (Fortanier and Zevenbergen, 1973) there are two main competing sinks in Dutch iris; the flower and the daughter bulbs. This can be seen from the growth curves of both the photoperiod experiment and ^{14}C experiments (Fig. 6.6, 6.7,

6.18 and 6.19). Conditions which cause the sink strength of the daughter bulbs to increase relative to that of the flower will cause a decrease in the proportion of successfully developing flowers. At high temperatures long photoperiods resulted in increased daughter bulbs sink strength with the consequence that higher light integrals were required to obtain the same number of flowers developing as at shorter photoperiods or lower temperatures. The higher light integrals compensate by either increasing the sink strength of the flower or the available assimilates because of an increased rate of photosynthesis.

Shillo and Halevy (1981) found a similar response to flower and corm development in Gladiolus with photoperiod. Long photoperiods checked corm growth and flowers developed at an enhanced rate compared to short photoperiods. This check in corm growth also occurred in smaller corms when grown in long photoperiods even though they did not have a competing flower. Similarly, iris bulbs the weight increase obtained with long photoperiods, was greater than that of short photoperiods, even without a competing flower sink. This indicated the weight increase to be a cause rather than an effect of flower abortion with the larger bulbs. It was observed, however, that it was only the initial growth that was significantly affected by photoperiod or temperature in these smaller bulbs. This can be explained by the mother bulb reserves becoming depleted at this stage and the supply of assimilates was then the factor controlling growth rate.

Smaller bulbs which did initiate a flower, appear to require longer photoperiods than larger bulbs before flower development was affected; the requirement for a higher light integral with 8-9cm bulbs was not detected until the third planting date, while with the larger 10+cm bulbs, this was evident at the second planting date. It appears that bulbing in iris is similar to that in onion. In onion bulbing is promoted by long photoperiods and the critical daylength for bulbing decreases with increased temperature (Brewster, 1977). Similarly, small plants do not respond as rapidly to the bulbing stimulus as larger ones.

Further evidence for daughter bulb competition causing flower blasting can be seen by considering the relative specific activity (a measure of relative sink strength) of the daughter bulbs. The

RSA of the daughter bulbs under low light was greater than that under high light at the critical stage for flower development. Cytokinin injection into the bud, which increased the number of flowers developing to anthesis, as observed by Mae and Vonk (1974), also caused a reduction in the dry weight of the daughter bulbs at the time of harvest. The relative sink strength of the flower was increased by injection with BA. The leaves do not compete with the flower and stem at the critical time of maximum stem extension (Fig 6.6, 6.7 and 6.19). The percentage ¹⁴C-assimilate exported from the fed second leaf to the other leaves was low by this stage of development, Fig 6.20 (day 42). The RSA of the leaves was low in both high and low light by day 28 from planting, Fig 6.21. There was, however, competition between the leaves and the flower soon after planting, i.e. day 15. The percentage exported ¹⁴C found in the flower was smaller under low than high light, as was the flowers' RSA, at day 15. There was no significant increase in leaf weight beyond day 28 from planting, under either treatment.

The flower, therefore, competes for current assimilates with various other plant parts during the course of its development to anthesis. Since a critical stage, when flowers are more likely to abort, has been shown to exist (Hartsema and Luyten; 1961; Fortanier and Zevenbergen, 1973; Mae and Vonk, 1974, the competition during the later stages of growth of the stem and flower was more likely to lead to blasting than that earlier in their development. This is probably because the weight increase of both the stem and flower and the daughter bulbs are exponentially related to time, their demand for assimilates increasing likewise. Earlier in the flowers' development the leaves competed for assimilates more in low light than high light, when these plants from low light conditions were transferred to high light ones after 15 days, all the buds were still able to develop to anthesis. The competition for assimilates at this stage of flower development is not as determined as at later stages.

Ho and Rees (1975, 1976 and 1977) found that with tulip, current assimilates contributed mainly to the growth of the stem and flower, and only after anthesis are they diverted to the growth of the daughter bulbs. This was despite the daughter bulbs

having a higher sink strength than the flower. The scales of the mother bulb were able to satisfy the demands of the daughter bulbs until the flower had developed to anthesis. Hence with tulip, in contrast to iris, if the flower blasts, the weight of daughter bulbs increases as a consequence, rather than the increased daughter bulb demand for assimilates causing flower bud blasting. With iris, however, the reserves of the scales become exhausted earlier in the flower's development, than with tulip, and a delicate balance exists between the demands of other growing sinks and the flower. Factors which upset this balance in iris, even to a small extent, such as the effect of photoperiod on the daughter bulb growth, can lead to the abortion of the flower.

The conclusion from Chapter 5 - that the dry weight of the flower and stem is closely related to the dry weight of the leaves - is supported further by the results from the localized shading and defoliation experiment. Both the number of buds developing and the dry weight of the stem and flower followed a similar pattern to the dry weight of the leaves produced, rather than to the degree of depletion of mother bulb reserves. This suggests that the growth of the various plant organs was allometric. Therefore, the greater the vegetative growth, the greater the availability of assimilates for floral growth and development. This is further supported by the total plant weight increase which occurred in the high light plants but not in those given low light. The flower buds failed to develop to anthesis in those plants where a weight increase was not detected.

At the critical time of flower development, ¹⁴C- assimilates were detected in the scales of the mother bulb, particularly when grown under high light. Mae and Vonk (1974) also observed activity in the scales of the mother bulb during this late stage of flower development. The old scales clearly switch from being a source to being a sink. The purpose of this assimilate accumulation in the old scales is unknown. They could permit an even supply of carbohydrates to the developing buds which Sano (1974c) considered necessary to avoid blasting, i.e. the mother bulb scales could act as a temporary store which could be remobilized during the night or on days of lower light intensity.

The carbohydrate contents of buds showed large variation within treatments making it difficult to draw conclusions, (Fig. 6.16). This was possibly the result of the wide range of developmental stages obtained from a single harvest, this variation might have been reduced if buds of equivalent developmental stage had been compared.

Hexose content per gram dry weight of bud was greater in low light-treated buds than those from high light. As the buds from the high light had a greater dry weight than those from low light, the hexose content of buds of similar weight were not significantly different between treatments. There was no significant difference in the fresh weight or size of buds between treatments (results not shown). The water content of the buds in low light was greater than that of the buds grown in high light. The higher proportion of hexoses in the dry weight material could have had a role in maintaining a higher osmotic potential to support this greater water content.

The proportion of sucrose, on both a dry weight and per-bud basis, was greater under high light than low. With the greater photosynthetic rate under high light the gradient of carbohydrate between source and sink would also be steeper than under low light. Hence, the movement of sucrose, the major translocated sugar, would be greater. Cytokinin application increased the sucrose content of the bud as a whole but did not significantly affect the proportion of sucrose per gram dry matter. Therefore, the supply of sucrose to the bud was increased by cytokinin but the ratio of sucrose to other dried material was not changed by cytokinin treatment. The starch content of flower buds was greater in high light than low light. The content of starch was related more to light intensity increasing photosynthesis than to the ability of cytokinins to increase the sink strength of the bud.

These results suggest that cytokinin increases the turnover and metabolism of sucrose, causing greater concentration gradients between the source (leaf) and the sink (bud).^{*} Alternatively, light may increase the availability of carbohydrates but not necessarily the turnover, resulting in the storage of carbohydrates as starch. Low carbohydrate production by the leaves, even if the turnover of sucrose at the bud is increased with cytokinin, did not result in heavier buds. The production of carbohydrates was then the limiting factor. Further work is required to clarify the metabolism and turnover of carbohydrates at the bud.

* Cytokinin increased sucrose metabolism has been demonstrated in Chinese cabbage (Berridge and Ralph, 1971) and in radish cotyledons (Howard and Witham, 1963).

As the transpiration rate increased a corresponding increase in flower development was observed, both in terms of number of buds developing and the average length of the flower stem. It is difficult to distinguish if the increased transpiration was a cause or effect of improved flower development. Since successful flower development is related to stem extension then leaves will be more open and separate whilst flower failure is associated with a short stem with leaves overlapping in a tight structure, creating pockets of humid air around the stomata, thus reducing transpiration.

As many factors which lead to bud abortion also reduce transpiration the possibility that this is the cause of poor development cannot be overlooked. Mayak and Halevy (1971), showed that cut flowers which failed to open, a mild case of blasting, was due to xylem vessels being blocked by mucilageous material. As mentioned previously high temperature, low light and water stress can all lead to stomatal closure and low light intensity reduces stomatal frequency.

Increased transpiration could improve flower development due to a lower temperature at the apex during flower development. Watts (1972) with maize and Adams and Thompson (1973) with sorghum have both suggested that water flowing through the xylem vessels when transpiration was fast enough, could reduce meristem temperature.

Flower blasting produces symptoms expected with calcium shortage and Doss, Christian and Paul (1980) observed an increase in flower abortion when plants were grown in a calcium deficient nutrient solution. Calcium is carried in the xylem in the transpiration stream, therefore low transpiration rates will cause poor movement of calcium to the developing bud. Sugars may be moved up the plant in the transpiration stream, organic substances have been found in xylem sap, particularly during the spring (Anderssen, 1929). Roots are a major source of cytokinins and these too are moved in the xylem, therefore reduced transpiration would lead to a shortage of cytokinin in the bud. When sunflowers were water stressed Itai and Vaadia (1965 and 1971) observed a reduced supply of cytokinins to the shoot. If this was the case with iris, then the supply of cytokinins to the daughter-bulbs would be expected to be increased resulting in an increased sucrose turnover and sink strength.

6.7 Summary

1. High temperatures ($>17^{\circ}\text{C}$) and low light integrals were confirmed to increase flower-bud abortion. At temperatures between 16 and 18°C long photoperiods increased the number of buds aborting than observed in short ones. At higher temperatures, this photoperiodic effect was not detected because such temperatures were unsuitable for flower development even in short photoperiods.
2. The major cause of flower-bud abortion appears to be preferential distribution of carbohydrate to the daughter bulbs rather than to the flower. Long photoperiods increased the relative sink strength of the daughter bulbs, whilst an application of cytokinin to the bud resulted in a decreased relative sink strength of the daughter bulbs.
3. Restriction of current photosynthesis by shading or removing leaves resulted in a decrease in both flower and daughter-bulb dry weight and an increase in bud abortion.
4. Increasing the transpiration rate resulted in a decrease in bud abortion.
5. The leaves were shown not to be in major competition with the flower bud at the critical stage of development when it is most prone to abortion. Instead they are the source of assimilates essential to support the development of both the flower and daughter bulbs.
6. Light promotion of flower development appears to be mainly a result of a greater supply of assimilates as a consequence of increased photosynthesis, whereas cytokinin applied to the bud appears to increase sucrose metabolism thereby increasing the flowers sink strength for sucrose.

7. GENERAL DISCUSSION

7. GENERAL DISCUSSION

The purpose of this brief general discussion is to consolidate the major results of the previous chapters, which have already been fully discussed in isolation. Findings which are in agreement throughout the thesis are highlighted and where discrepancies exist possible explanations are offered. In this manner an attempt is made to answer the questions formulated in the introduction. The two major objectives of the study were to determine; first, the factors which lead to flower initiation and secondly the control and processes involved in flower development to anthesis. The potential for alterations to commercial treatments for improved production and the possible physiological mechanisms involved in initiation and development of Dutch iris flowers are discussed.

The results obtained in Chapter 4 indicated that flower induction leading to initiation occurred at a specific stage in the vegetative development, regardless of the environmental conditions, providing they were suitable for growth. A low temperature treatment was seen to be responsible for a quantitative thermoinduction, causing earlier flower initiation in the development of the plant and faster differentiation of the flower. Similarly, Chapter 5 results showed the rate of development to anthesis to be more rapid when bulbs were transferred from 5°C to other temperatures, than when kept at constant temperatures (Table 5.4). An exception was a constant 13°C treatment which was as rapid as any of the 5°C transfer treatments. This was in agreement with the conclusions drawn in Chapter 4. At 13°C vegetative development occurs so rapidly that the stage of autonomous induction is achieved at a sooner chronological time than if given a treatment for earlier induction in terms of plant development. Autonomous induction with a quantitative thermoinduction is further supported by the leaf numbers obtained with the various pre-planting treatments in Chapter 5 (Table 5.6).

The commercially applied standard treatments for forcing iris were derived empirically. From the results of Chapter 4, a better physiological understanding of these standard treatments was

achieved. In particular, the improvements claimed to be achieved by the reverse treatment (Kamerbeek and Beijer, 1964; Durieux and Pagter, 1967), were explained in terms of a shift in the optimum temperature for the most rapid flower development following flower initiation. With the further understanding of factors involved in flower initiation and development gained from this study, it is still not feasible to suggest alterations which would improve the production of cut-flowers compared to the current standard treatments. This is despite the discovery of treatments which enable a more rapid initiation, differentiation and development to anthesis of the flower. The problem is that such treatments which are optimal for the attainment of a specific stage (e.g. flower initiation), when looked at in isolation, also cause complicated interactions with other whole-plant developmental processes (e.g. the number of leaves, leaf growth and stem extension). Commercially used treatments result in a "well balanced" plant, with the flower emerged well above the leaves. Rees (1972) commented "There is method, therefore, in empirical work which produces a regime which gives a sensible commercial end-product." In the light of some of the findings in this study, further developmental work is required to obtain suitable treatments which will also give a suitable end-product.

In Chapter 6, leaves were shown not to be in competition for assimilates with the flower at the critical stage of development. The suggestion by previous workers that leaf growth should be kept to a minimum appears unfounded. Leaf removal or shading and ¹⁴C-assimilate movement all showed leaves to contribute an important proportion of assimilates necessary for flower development.

It has been suggested here that the preferential distribution of carbohydrates to the daughter bulbs rather than the flower, was the cause of flower abortion. In Chapter 5, however, heavier daughter bulbs produced as a result of the pre-planting treatment did not cause differences in the number of successfully developing flower buds. Hence, the results of Chapter 5 and 6 appear to be in disagreement.

These conflicting results can be explained by considering whether treatments caused differences in the overall level of assimilates or in the relative sink strengths of the daughter bulbs and the flowers. In Chapter 5 it was proposed that variation in the degree of leaf growth ultimately caused disparity between the treatments in the availability of assimilates for the growth and development of the flower. The treatments giving rise to the plants capable of producing the greatest quantity of assimilates at the crucial stage for flower development, permitted the largest number of buds to develop to anthesis and the growth of the heaviest daughter bulbs. When this competition for the assimilates existed, environmental factors which altered the relative sink strengths of the two organs become the critical factor in determining whether a flower will develop or abort.

Ethylene has been reported to accelerate initiation and reduce the occurrence of flower abortion (Kamerbeek, Durieux and Schipper, 1980; Stuart, Asen, Could, 1966; Imanish and Fortanier, 1982; Stuart and Schipper, 1982). Such a treatment has largely overcome the problem of flower abortion in commercially grown Dutch iris. A smoke treatment (which contains ethylene) can be given to bulbs during their storage/pre-planting treatments. Many reports conclude that the improved flower development following ethylene treatment is due to the reduced leaf growth resulting in less within and between plant competition for assimilates and light (Kamerbeek, Durieux and Schipper, 1980; Schipper, 1981; Duineveld and Munk, 1983). The results presented here provide no evidence for reduced leaf growth allowing for greater flower development due to less within plant competition. Similarly, increased planting densities following a smoke or ethylene treatment did not result in fewer flowers developing to anthesis (Schipper, 1983b), suggesting that the between plant competition is not the important factor.

The effect of ethylene may be explained in terms of reduced daughter bulb growth, resulting in less competition for assimilates with the flower. If ethylene delayed the initiation of the daughter bulbs, or their growth then the earlier initiation of the flower could be explained in terms of Sachs' hypothesis. Carbohydrates which would otherwise have been utilized by the

daughter bulbs would then be available for distribution to the apex. Similarly, if daughter bulb competition for assimilates was reduced by ethylene, the development of the flower buds to anthesis would also be improved. Further work is necessary to determine if ethylene effects daughter bulb initiation and growth.

Previous studies, as with the experiments here, where both flower initiation and development have been investigated have involved the destructive sampling of a large number of plants to try to understand what occurs in individual plants under various environmental conditions. As a result the average of this sample is used to describe how these conditions effect the developmental stages. This appears particularly inappropriate when plants either abort or do not abort giving, in effect, two populations. A method for continuous monitoring of individual plants would be a tremendous asset to such a study as this.

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