CONTROL OF RHIZOME GROWTH

IN *ALSTROEMERIA*

by

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Abstract

Increases of temperature in the range from 8 to 18°C *in vivo*, significantly enhanced the dry weights of lateral rhizomes, roots and shoots. At the higher temperatures dry weight production was often seen to decrease. Decreases in irradiance from 100 to 50 per cent *in vivo*, produced significant decreases in the dry weights of lateral rhizomes, roots and shoots. In contrast, the numbers of lateral rhizomes, tubers and shoots were largely unaffected by temperature and irradiance treatments. Daylength treatments of 8 to 16 hours light in a 24 hour period *in vivo* produced few significant changes in either the dry weights or numbers of plant parts produced. However, daylength had a strong influence over the time of flowering. For maximum rhizome production a temperature of between 13 and 18°C, a high irradiance and a short daylength were required.

Increases of temperature in the range 8 to 18°C *in vitro*, caused significant increases in the number of lateral rhizomes and shoots produced. At the highest temperature the numbers produced often decreased. The number of roots produced was unaffected by temperature. Decreases in irradiance from 100 to 25 per cent and increases in daylength from 8 to 20 hours light in a 24 hour period, produced no significant changes in the number of lateral rhizomes, roots or shoots produced *in vitro*. Low irradiance, however, caused etiolation of the shoots. For a good multiplication rate the requirements for the culture environment were a temperature of 15°C, an irradiance of 5 $W\,m^{-2}$ with a daylength of 8 hours of light in a 24 hour period.

The presence or absence of tubers and damage suffered by 'splits' prior to planting were found to be important factors in the establishment of plants *in vivo*.

Subculture of rhizome explants without aerial shoot or rhizome apices and of rhizome explants divided into single internodes with or without aerial shoots, enhanced the rhizome multiplication rate. Addition of the plant growth regulators triiodobenzoic acid, thidiazuron, α-naphthaleneacetic acid, gibberellic acid and paclobutrazol to culture media, with and without BAP, caused no significant changes in the numbers of lateral rhizomes, shoots or roots produced. However, paclobutrazol produced changes in explant morphology, i.e. shoot size was reduced and the diameter of roots was increased.

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List of Abbreviations

ABA	-	Abscisic acid
BAP	-	6-Benzylaminopurine
GA_3	-	Gibberellic acid
IAA	-	Indole-3-acetic acid
NAA	-	α-Naphthaleneacetic acid
TIBA	-	2,3,5-Triiodobenzoic acid
MS	-	Murashige and Skoog revised medium (Appendix A)
PGR	-	Plant growth regulator

Chapter 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION AND AIMS OF THE STUDY

Alstroemeria, the 'Peruvian Lily', is grown as a cut flower crop and as such has become increasingly popular in the last few years. In terms of crop area it is now the second most important cut flower grown in the United Kingdom (Deen, 1986). It is grown extensively in the Netherlands and Colombia and there is a great deal of interest in it also in the United States of America.

A limited number of papers have been produced detailing the effects of temperature and light on the growth of *Alstroemeria in vivo*. These papers focus mainly on the effects of time of planting and environmental factors on the time to flowering and the length of the flowering period. Very little reference is made to the effects of these parameters on the growth of the rhizome rootstock. This is surprising as most *Alstroemeria* cultivars are vegetatively propagated by division of the rhizome *in vivo* and by rhizome culture *in vitro*.

Studies on *Alstroemeria in vitro*, which have taken place over the last two decades, have yielded only a few publications. These have been related to establishing a micropropagation system with emphasis on optimising the concentrations of plant growth regulators for multiplication and rooting of explants. Very little published information is available either on the effect of temperature and light environments on the growth of *Alstroemeria in vitro* or on the effect of different methods of subculture. The only published reports on plant growth regulators relate to the use of auxins for rooting and cytokinins for improving growth and shoot proliferation.

The aims of this study were to investigate the effects of (i) temperature and

light regimes on the growth of the rhizome of *Alstroemeria in vivo* and *in vitro* and (ii) plant growth regulators and subculturing methods on the growth of rhizome explants *in vitro*. The latter part of the study involved attempts to break apical dominance expressed within explants, in order to increase the rate of axillary bud outgrowth.

The following review of the literature includes a general description of the growth of *Alstroemeria* and a review of the work published on the plant *in vivo* and *in vitro*. An overview of the concept of apical dominance, and the mechanisms by which the plant growth regulators used in this study are thought to affect it, is also included.

1.2 *ALSTROEMERIA*

1.2.1 Taxonomic Description

The genus *Alstroemeria* belongs to the family Alstroemeriaceae in the order Alstroemeriales, which is included in the division Corolliferae of the monocotyledons (Hutchinson, 1959). There are four genera in this family, i.e. *Alstroemeria* (50 species), *Bomarea* (150 species), *Leontochir* (one species) and *Schickendantzia* (two species).

Cytological studies have shown that species in the genus *Alstroemeria* possess 16 chromosomes (2n=16) (Lakshmi, 1980;Tsuchiya and Hang, 1987). The majority of *Alstroemeria* cultivars are triploids (2n=3x=24) with a few being either diploid or tetraploid (2n=4x=32), with aneuploidy occurring occasionally amongst the triploids and tetraploids.

Alstroemeria is a native of South America with Chile as its centre of distribution. Many of the species are endemic to certain countries or regions within them (Healy and Wilkins, 1985; Uphof, 1952), though some are known to have fairly wide distributions (Figure 1.1). The estimate of 50 species in the genus (Bayer, 1987) may prove to be conservative, if a full and detailed survey of the area was undertaken. Their habitats range from the tropical

Amazon area to the snowline of the high Andean plateau, through the high forests and river valleys, to the western coastal deserts of Chile (Healy and Wilkins, 1985; Verboom, 1979). Overall, the genus ranges from sea level to 3700m and from Ecuador, which is on the equator, to Patagonia in southern Chile. The genus, therefore, tolerates many varied and often extreme environments.

Full descriptions of the genus *Alstroemeria* can be found in Hutchinson (1959), Uphof (1952) and Willis (1985). The genus consists of herbaceous perennials with a rhizomatous rootstock, from which aerial shoots and roots, which may carry tubers, arise. The leaves are positioned alternately on the stem and are twisted through 180° at the base, causing inversion of the lamina. The inflorescences are composed of a whorl of simple or compound cymes, each carrying one to five sympodially arranged flowers. The perianth segments are free and arranged in two series, with six stamens that dehisce at daily intervals (Figure 1.2). The ovary is inferior, tri-locular and has axile placentation. The style is filiform and tri-lobed, becoming receptive after the anthers have dehisced. The ovules are numerous within each loculus and the mature fruit is a dry capsule that splits explosively. Many seeds are produced, containing copious endosperm and a small embryo.

1.2.2 Morphology and Development of the Rhizome Rootstock

The roots of *Alstroemeria* are both thin and fibrous and thick and fleshy, and may be produced on virtually any part of the rhizome. Some species lack the fibrous roots and possess only fleshy roots with root hairs. In others, the fleshy roots develop into thick tubers, the size depending on the species (Figure 1.3A) (Buxbaum, 1951). It was also reported that new roots arise mainly from the newly formed rhizome apex and, to a lesser extent, from the roots and tubers. The tubers are known to be local sources of starch (Mabberley, 1987) in quantities similar to that found in potato (Cox and MacMasters, 1947).

Figure 1.1: The distribution of the genus *Alstroemeria* in South America, * denoting presence in a country. (From Buxbaum, 1951)

Figure 1.2: The inflorescence and a portion of the stem from three hybrid cultivars of *Alstroemeria*

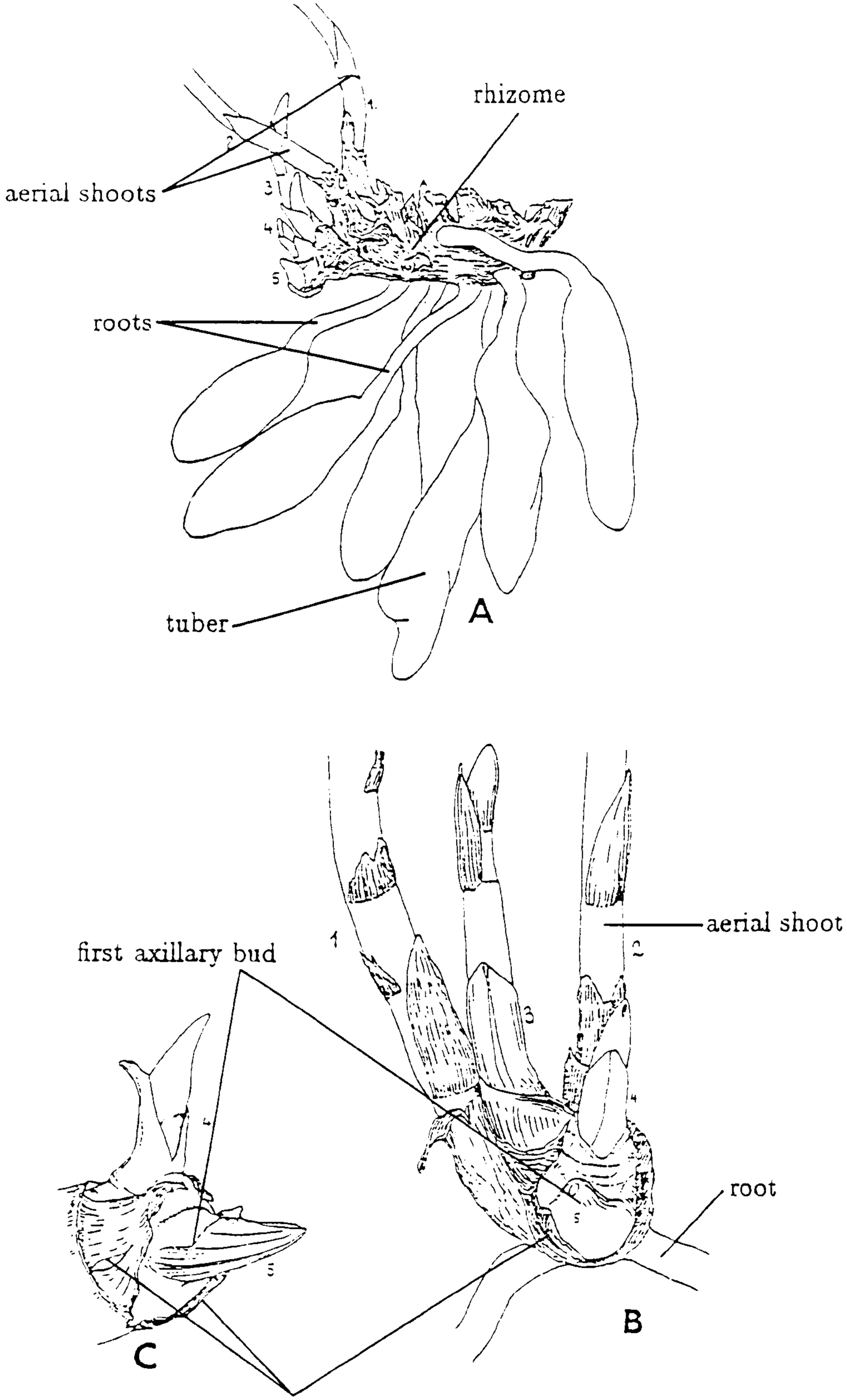


Figure 1.3: The morphology of the rhizome of *Alstroemeria*, showing the arrangement of the roots, shoots, scale leaves and axillary buds, viewed from **A** the side, **B** the front and **C** at the apex. The sequence of numbers 1-5, indicates the progression from the older to the youngest shoots, respectively. (From Buxbaum, 1951)

The aerial shoots arise from the rhizome and are positioned alternately in two ranks along its length (Figure 1.3A and B). The thickened apex develops a very large bud so that the rhizome appears to be monopodial (Figure 1.3C). However, this large bud is the axillary bud of the first scale leaf of the previous aerial shoot (Buxbaum, 1951). The first internode of the aerial shoot is greatly enlarged and the axillary bud is projected forward (Figure 1.4A and B). Occasionally this occurs to such an extent that further internodes are suppressed and the shoot may die. From this it can be seen that a chain of enlarged basal internodes is developed, imitating a long monopodial rootstock, whereas development of the rhizome is sympodial.

The second scale leaf of the aerial shoot (Figure 1.4A) also bears an axillary bud. This may produce new aerial shoots and consequently a lateral rhizome (Figure 1.5A and B), thereby beginning the ramification of the rhizome. The second axillary bud may often stay dormant for a long time, only developing when the base of the aerial shoot dies or is removed. No further axillary buds occur on the aerial shoot (Buxbaum, 1951; Heins and Wilkins, 1979; Priestly *et al.*, 1935).

1.2.3 Breeding

Alstroemeria breeding has been in progress for about 40 years, but only after the first ten years was it possible to release some new hybrids, as the species proved difficult to hybridize and only a small number of seeds were produced (Goemans, 1962). Early in this breeding programme it was observed that most two species hybrids were more or less fertile but as soon as a third species was used the resulting progeny were completely sterile.

A small number of *Alstroemeria* species have been used in the production of modern hybrids, with *Alstroemeria aurantiaca*, *Alstroemeria ligtu* and *Alstroemeria pelegrina* prominent amongst the probable progenitors, especially of the earlier hybrids (Broertjes and Verboom, 1974). It has been suggested that the cause of sterility in these hybrids was probably through interspe-

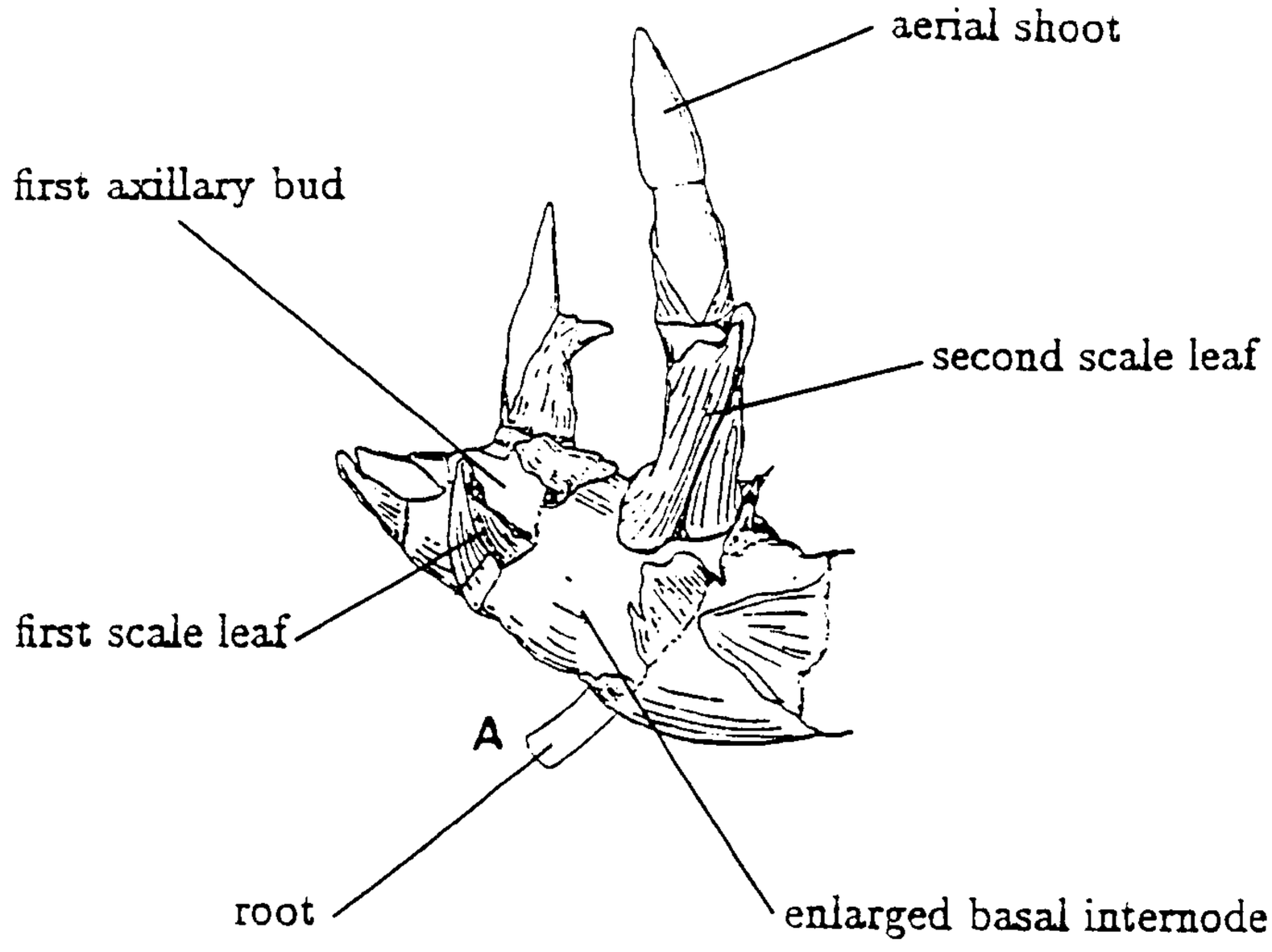


Figure 1.4: The morphology of the distal portion of the rhizome of *Alstroemeria*, showing the arrangement of shoots, roots, scale leaves and axillary buds from **A** the side and **B** the ventral surface. (From Buxbaum, 1951)

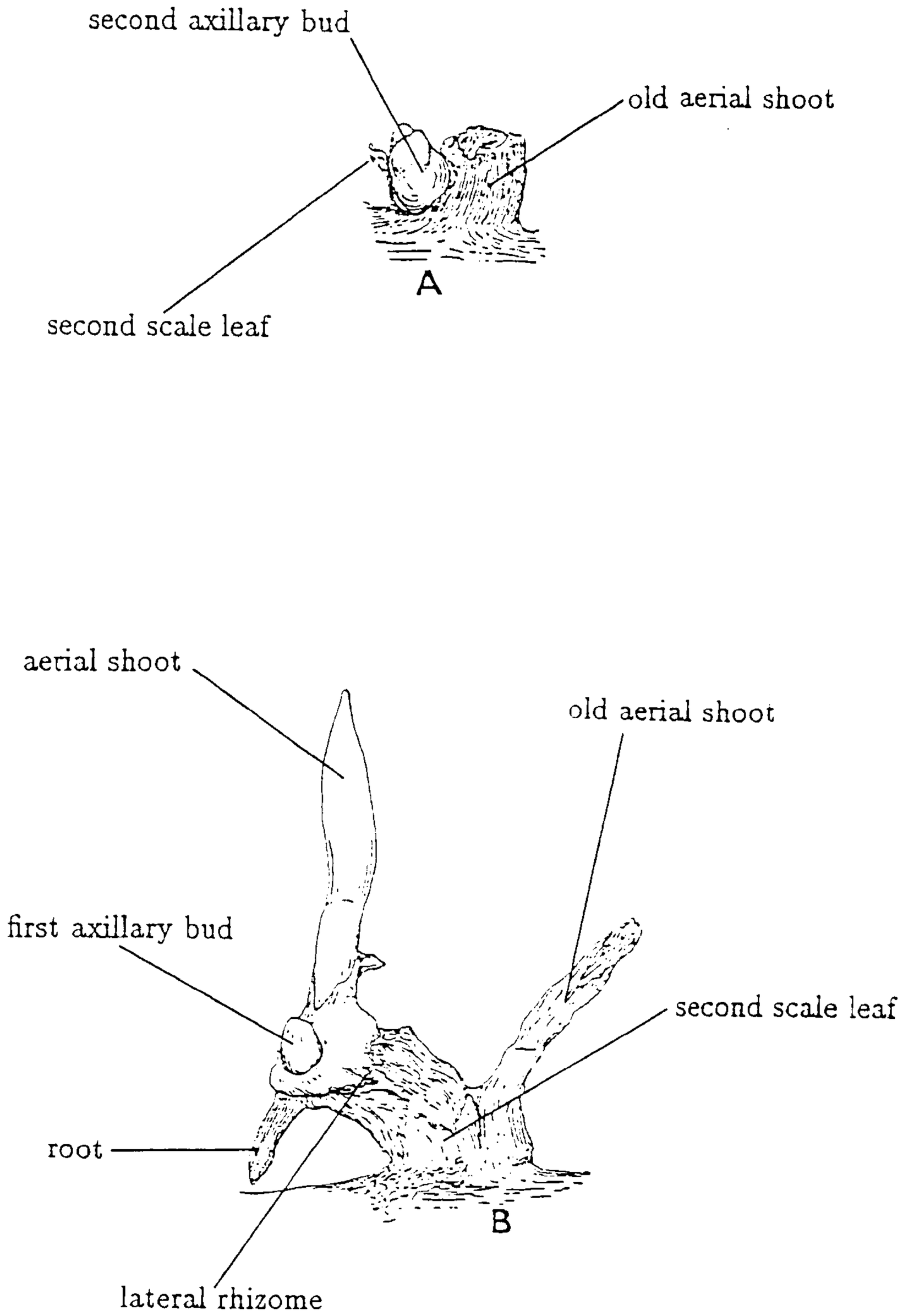


Figure 1.5: Stages in the development of a lateral rhizome from the second axillary bud of an aerial shoot. **A** shows an early stage with the bud enlarging and **B** a later stage with the lateral rhizome supporting its own root and shoot. (From Buxbaum, 1951)

cific hybrid sterility with the diploid chromosome number, because one of the parents was an unknown spontaneous tetraploid or one of the gametes had an unreduced chromosome number. The latter two mechanisms lead to sterile triploid plants (Broertjes and Verboom, 1974). Triploidy seems to be confirmed by differences in radiosensitivity between diploid and triploid categories (Broertjes and Verboom, 1974) and has been confirmed by cytological analysis for a large number of the modern hybrids (Hang and Tsuchiya, 1988; Tsuchiya *et al.*, 1987; Tsuchiya and Hang, 1987).

Due to the sterility of many hybrid cultivars, methods for inducing genetic variation and producing improvements in these cultivars are limited. Consequently, the majority of improvements to these hybrids have been through mutation breeding using X-rays (Broertjes and Verboom, 1974) and many commercially viable mutants of *Alstroemeria* have now been produced (Broertjes and van Harten, 1978, 1988). Actively growing rhizomes of very young plants are generally used and all of the mutants produced, so far, have been found to be 'solid' ones, i.e. not chimeral. It has been suggested that a sympodial growth pattern is responsible for this phenomenon (Broertjes and van Harten, 1988) rather than a unicellular origin of the meristem (Broertjes and Verboom, 1974).

1.2.4 Propagation

Due to the high incidence of sterility and the variability of the plants produced, seed propagation is uncommon in *Alstroemeria*. Consequently, they are propagated traditionally by splitting of the rhizomes. The plant clumps are lifted in summer and carefully divided into single rhizomes. From these rhizomes the apex and up to 10 cm of healthy rhizome is removed with roots and tubers, if present, and the aerial shoots are reduced to approximately 2 cm. These 'splits' are grown in small pots until they become established and then they are planted out into their flowering positions (Healy and Wilkins, 1981, 1986a; Molnar, 1975; Salinger, 1985; Verboom, 1979).

With the introduction of micropropagation techniques, much *Alstroemeria* propagation is now performed *in vitro*. This involves the manipulation of *Alstroemeria*, using standard aseptic techniques, with subsequent growth of cultures in controlled environment conditions of 15°C and a 12 hour daylength. The irradiance depends on the light source used, approximately 6 $W\,m^{-2}$ being used commercially (Parigo Horticultural Co., personal communication). Gabryszewska and Hempel (1984) suggest the use of higher temperatures (22-24°C) and a longer daylength of 16 hours.

The *in vitro* propagation system used commercially is the proliferation of axillary buds from rhizome explants with an actively growing apex (George and Sherrington, 1984; Hussey *et al.*, 1979). In this system the explants are grown on semi-solid medium based on that of Murashige and Skoog (MS) (1962). To this MS medium 6-benzylaminopurine (BAP) at 2-4 $mg\,l^{-1}$ is added for shoot proliferation (Hussey *et al.*, 1979; Parigo Horticultural Co., personal communication; Pierik *et al.*, 1988) and α-naphthaleneacetic acid (NAA), up to 1 $mg\,l^{-1}$, for rooting (Parigo Horticultural Co., personal communication; Pierik *et al.*, 1988). Rooted plants are moved to a glasshouse, transferred to compost in small pots and weaned, by passage through environments of decreasing humidity, until they are established (Parigo Horticultural Co., personal communication). At this point they are ready for planting out in their flowering positions.

Many monocotyledonous plants, including *Alstroemeria* and quite a large number of other flower crops, have low natural propagation rates due to restricted development of axillary meristems (Hussey *et al.*, 1980). As discussed earlier *Alstroemeria* has in fact only two axillary buds on each aerial shoot, one which continues the growth of the rhizome and a second which has the potential to grow into a lateral rhizome (Buxbaum, 1951; Heins and Wilkins, 1979; Priestly *et al.*, 1935). As outgrowth of these lateral rhizomes is generally slow then the throughput, or multiplication rate, of the rhizome *in vivo* is low. The adoption and application of *in vitro* propagation techniques to *Alstroemeria* does not increase the number of axillary buds present

but allows growth and production of lateral rhizomes throughout the year. These techniques may also be used to induce higher proportions of these axillary buds to grow out. Therefore, with these techniques, many more plants may be produced *in vitro* in one year than may be produced *in vivo*.

1.2.5 General Culture

In South America the species of *Alstroemeria* grow in a wide range of habitats and climates and consequently have very different environmental requirements. Due to the varied ancestry of the new commercial hybrids, these cultivars do not respond equally in the same greenhouse environments (Keil, 1986). Although many cultivars are now available, most of the cultural recommendations have resulted from studies on a few cultivars, principally 'Regina' and 'Orchid'. Consequently, it has been suggested that growers should experiment with *Alstroemeria* in their own setting, while carefully considering the available cultural information.

The general culture of *Alstroemeria* cultivars has been described by Anon (1980), Healy and Wilkins (1981, 1985, 1986), Molnar (1975), Salinger (1985), Verboom (1979), Wilkins *et al.* (1980), Wilkins and Heins (1976), and Wilson (1979). More specific details on the nutritional and spacing requirements of *Alstroemeria* can be found in Bik and Berg (1981) and Healy and Lang (1985) respectively. The following is a brief account of *Alstroemeria* culture.

Commercial culture of *Alstroemeria* is carried out under glass, as many of the new hybrid cultivars are not frost resistant. However, an increasing area is grown as a summer crop without the protection of glass, in both the U.K. and the Netherlands. The rhizome system is protected in the winter by a mulch of, for example, mushroom compost or straw (Parigo Horticultural Co., personal communication). The plants are usually grown in prepared ground beds and the plants are lifted and replaced every two or three years. Planting is carried out in the autumn, preferably at densities of five to six plants per square meter of bed. In some instances, planting may take place in

April or May, though in the U.K. autumn planting is still the more common. Great care should be taken at lifting as the fleshy roots are very brittle and damage easily, resulting in subsequent rotting (Robinson, 1963). The soil should be open, slightly acid and well drained, as waterlogging can cause poor growth or death. The plants make vigorous growth and high levels of nutrients are required (Parigo Horticultural Co., personal communication). Support for the stems is essential as they may reach a height of two meters or more.

Temperature is thought to be an important factor in the induction of flowering. With the rhizome as the site of perception, soil temperature is very important and should not exceed 18°C. Winter night temperature should be maintained at 10°C or above and raised to approximately 15°C in the spring. With many cultivars high temperatures and long days in summer prevent flower induction, such that flowering appears to be seasonal, occurring in spring and autumn. Shading may be used to avoid extremes of high temperature. In winter, low light and temperature levels tend to inhibit flowering. Supplementary lighting may be used to bring forward spring production, generally by increasing the daylength.

Thinning of weak vegetative stems helps prevent growth becoming too dense and enhances flower production. Cropping or removing any stems is achieved by separating them at the junction with the rhizome. *Alstroemeria* would seem to be relatively free of pests with occasional outbreaks of spider mites, whitefly, aphids and caterpillars. Botrytis can be a problem in dull weather and several viruses have now been isolated (Versluijs and Hakkaart, 1985).

1.2.6 Economic Importance

Alstroemeria has been grown in the United Kingdom as an herbaceous garden plant for at least 200 years, with the introduction of *A. pelegrina* to the Royal Botanical Gardens at Kew in 1753, and *A. aurantiaca* has been in cultivation since 1831 (Robinson, 1963). Over the last two decades, with

the introduction of the new hybrid cultivars from Europe, *Alstroemeria* has gained a great deal of popularity and consequently has become an important cut flower crop. The consumer appreciates the long vase life of the flowers of up to three weeks, which is manipulated to some extent by the grower (Healy and Lang, 1989), and the large range of flower colours available. The colour range, which includes varying shades and intensities of red, orange, pink, yellow and white, is a product of the diverse colour base found in the species. The inner whorl of tepals is generally marked with dark streaks and dots and it is often tinted with yellow or white. These elaborations of the inner whorl vary markedly between cultivars.

The attraction of *Alstroemeria* to the grower is the low temperature requirement for flower production, in comparison with other glasshouse cut flower crops (Healy and Wilkins, 1981), which can result in considerable energy savings. Also, with the introduction of new and improved cultivars, *Alstroemeria* is now moving into 'all year round' production with 'winter flowering types' (Eggington, 1986) and up to 200 stems can be produced from every square meter in production (Deen, 1986).

By 1986 the production area of *Alstroemeria* worldwide had risen to 275 hectares (Pierik *et al.*, 1988). Currently the Netherlands and Colombia are the world's two largest producers, with 94 per cent of Colombia's production being exported to the United States of America. In the period 1988-1989 over 166 million stems were produced in the Netherlands, an increase of 13 per cent on the previous year, and almost 78 million stems were produced in Colombia (Anon, 1989, 1990).

1.3 *IN VIVO* AND *IN VITRO* RESEARCH

1.3.1 *In Vivo*

Several papers have been published during the last 15 years, on the effects of light and temperature on the growth of *Alstroemeria in vivo*. However, only a few make any direct reference to the effects of these parameters on the

rhizome. Most of the papers deal with the time to flowering, the length of the flowering period and the percentage of shoots that are generative. Some refer to the total number of shoots produced, which is an index of the rhizome growth.

Powell and Bunt (1984, 1986), working on several cultivars, have shown that the date of propagation can have a marked effect on flower production. Plants propagated in January, March or May initiated flowers within two to four months, whereas those propagated in July, September or November remained vegetative until the following spring. Often, half of the plants propagated in May remained vegetative until the following January, at which time flower initiation occurred in both new and previously vegetative shoots. A period of shoot inhibition, or dormancy, was also reported to commence in May and last for between two to eight weeks, depending on the cultivar. Sims (1985) also considered the decline in shoot production seen in conditions of low temperature and short days to be a period of dormancy. This suggested that flower production patterns were affected less by propagation date than by season, the effect of the latter being controlled by complex interactions of temperature, daylength and possibly a vernalization requirement.

This vernalization factor had been demonstrated previously by Healy and Wilkins (1979), when they found that plants required a 5°C treatment for a minimum of four weeks at low irradiance levels to promote flowering. Further work (Healy and Wilkins, 1982a, 1982b) showed that this cold requirement can be fulfilled either at 5°C for a short period of time, i.e. six to eight weeks, or at 13°C over an extended period of up to 16 weeks. Total shoot production during the flowering period decreased as the duration of the 5°C treatment increased, but no difference in the total time to flowering was observed between plants under the two regimes.

Noordegraaf (1972, 1975) showed that flower initiation occurred earliest at 9°C, the lowest of the temperatures tested, and that there was virtually no flowering at 25°C, the highest temperature tested. However, subsequent

flower development was faster at the higher temperatures. The total number of shoots increased over the temperature range of 13°C to 25°C, however, the percentage of generative shoots declined. Similar results have been reported by Chepkairor and Waithaka (1988).

Heins and Wilkins (1979) showed that growing *Alstroemeria* at a soil temperature of 15°C for 40 days and then at 21°C for 21 days resulted in a higher proportion of vegetative shoots than at a constant 15°C. However, a higher percentage of the shoots flowered from plants grown at the constant 15°C soil temperature. A constant soil temperature of 16°C (Blom and Piott, 1990) produced a decrease in flower production of 15 per cent, irrespective of cultivar, during the spring and summer. In the autumn and winter, increases and decreases were observed which were cultivar dependent. Annual production was not affected but the ratio of flower production between the spring/summer and the autumn/winter decreased from 3.1 to 2.2 for uncontrolled and constant soil temperatures, respectively.

Keil-Gunderson *et al.* (1989) investigated the effect of different air and substrate temperatures and concluded that the greatest production of flowers occurred with a 20°C mean daytime air temperature, combined with a root temperature of 12 to 14°C which favoured year round production. Warmer day temperatures gave improved flower grade and stem length, whereas cooler air temperatures produced higher yields of generative shoots. When plants were grown in a constant air temperature of 13°C with the rhizome and roots at 5 to 25°C, the highest number of generative shoots, 33 per cent, was produced at a rhizome temperature of 10°C (Healy and Wilkins, 1986c). When the soil temperature was 13°C, no differences in the percentage of generative shoots were seen, regardless of the air temperature or the daylength. Treatments which resulted in a high percentage of generative shoots also produced high dry weights for roots and rhizomes.

The effect of soil cooling and supplementary lighting has also been considered by Lin (1984, 1985), who showed that an air temperature of 15 to 18°C and

a soil temperature of between 11 and 14°C resulted in an increased number of flowering shoots in spring/summer and also in autumn/winter. Supplementary high pressure sodium lighting provided for 16 hours a day in the spring and summer produced plants with fewer flowering shoots, whereas in the autumn and winter, this treatment increased the number and quality of flowering shoots. However, soil cooling also decreased total shoot production, with or without the use of supplementary lighting. Leliveld (1973) demonstrated that planting in October rather than in December or January can greatly increase yields and advance flowering by at least three weeks. Other workers (Healy *et al.*, 1982; Healy and Wilkins, 1986b; Krogt, 1985; Lin and Molnar, 1983) have reported similar increases in the number and quality of flowering shoots and the advancement of flowering due to supplementary lighting. This occurred when natural daylength was short and therefore at times of the year when the stimulus for shoot and flower initiation was absent.

Night interruption by incandescent light (Healy *et al.*, 1982; Healy and Wilkins, 1979; Heins and Wilkins, 1976, 1979) for up to five hours, which effectively produces a long day treatment, produced earlier flowering and increased the number of flowering stems. Similar results with long day/short night treatments have also been reported by Noordegraaf (1972, 1975) and Lin and Molnar (1983), from which a 16 hour daylength was suggested as the upper limit for maximum flower production. Extending this time caused flowering to occur even earlier but fewer shoots were produced. In these experiments incandescent lighting was used as an extension of eight hours of natural daylength, not as a night interruption.

As may be expected, under conditions of short days i.e. eight to ten hours, the flowering of *Alstroemeria* cultivars is severely inhibited, irrespective of soil or air temperatures (Healy *et al.*, 1982; Healy and Wilkins, 1979; Heins and Wilkins, 1979; Lin and Molnar, 1983; Noordegraaf, 1975). However, shoot production is not inhibited. Dambre (1988) used a short day treatment of nine hours in an attempt to prevent the onset of dormancy in *Alstroemeria* cultivars which, as already stated, often occurs in summer. In the first few

weeks of the short day treatment, large numbers of shoots were formed on the rhizome, i.e. up to five times the number formed under natural daylength conditions. However, 90 per cent of these shoots were vegetative, which was thought to be associated partly with the daylength and high temperatures encountered in this season which, as previously stated, are thought to suppress flower formation. Rhizome formation and growth were also suppressed by short day treatments. After the treatment was stopped the number of flowering plants increased and there was an increase in rhizome numbers and weight.

In an early publication, Noordegraaf (1975) stated that shoot development and flower initiation are not promoted by the same factors and that flower formation has an inhibitory effect on shoot development. These interactions remain unresolved. Keil-Gunderson *et al.* (1989) suggest that *Alstroemeria* flowering is influenced by two primary factors, rhizome temperature and photoperiod, while Healy and Wilkins (1986c) state that flower induction is not a single event on the rhizome but a continuous process for each shoot as the rhizome elongates.

The latest summary of this interaction (Blom and Piott, 1990) suggests that the control of flowering is biphasic. Plants require a cold induction treatment, or thermophase, as a prerequisite to flowering. The mechanism that triggers a flush once the thermophase has been fulfilled is still unclear. However, a photoperiod, or photophase, is strongly implicated. Cessation of flowering after a flush and dormancy of the rhizome is believed to be due to high soil temperatures, lack of plant growth substances and long photoperiods.

1.3.2 *In Vitro*

Although the commercial propagation of *Alstroemeria* is carried out *in vitro*, very few publications have been produced on the effect of light, temperature or methods of culture. It would appear that only Ziv *et al.* (1973) has tried a medium other than MS for the propagation of *Alstroemeria*. White's medium

(1963) was compared with MS medium but no differences were observed in the growth of cultures. Recently, Smith and Bridgen (1987) referred to tests on different media components, but few results are presented in the abstract published.

Different explant sources have also been assessed, for example flower pedicels, subapical segments of inflorescence or vegetative stems and rhizome segments (Hussey *et al.*, 1979; King and Bridgen, 1987; Lin and Monette, 1987; Ziv *et al.*, 1973). For good explant growth and ease of production, culturing from rhizome segments with an intact apex has been suggested (Hussey *et al.*, 1979; Lin and Monette, 1987). Cultures initiated from rhizome segments sliced longitudinally (Lin and Monette, 1987), or into halves or quarters, give a poorer response compared with those from the intact rhizome. Pierik *et al.* (1988) demonstrated that the larger the explant, in relation to the number of aerial shoots it bears, the lower the rate of axillary bud outgrowth, and suggested that an explant comprising a single shoot was the most efficient way of multiplying *Alstroemeria*.

Although Ziv *et al.* (1973) and Lin and Monette (1987) used different auxins and cytokinins, respectively, in their work, neither made any direct comparisons of effects within these groups of compounds. Gabryszewska and Hempel (1984, 1985) screened the effects of the cytokinins BAP and 6-(γ,γ-dimethylallylamino)-purine (2iP) and the auxins NAA and indole-3-butyric acid (IBA) on rhizome branching, aerial shoot production and rooting, of *Alstroemeria in vitro*. They concluded that the auxins stimulated rooting and aerial shoot growth but had no effect on rhizome branching, with NAA being the most effective. Cytokinins stimulated rhizome branching and aerial shoot production but inhibited rooting, with BAP being the most effective. Similar results were reported by Pierik *et al.* (1988) after screening the same growth regulators as above, with the addition of the auxin indole-3-acetic acid (IAA) and the cytokinins 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA), 6-(4-hydroxy-3-methyl-2-butenylamino)purine (zeatin) and 6-furfurylaminopurine (kinetin). Bridgen and Winski (1989), however, re-

ported few differences between the cytokinins BAP, kinetin and 2iP, on the growth of *Alstroemeria* in culture.

With the exceptions of Gabryszewska and Hempel (1984), who reported the need for a higher irradiance when rooting *Alstroemeria*, and Lin and Monette (1987), who suggested a temperature of 8°C for initiating *Alstroemeria* cultures with multiplication at 22°C, only two publications present data on the effects of temperature and light on the multiplication of *Alstroemeria* rhizome explants *in vitro*. Pierik *et al.* (1988) reported that irradiances between 1.5 and 9.7 $W\,m^{-2}$ had no effect on the multiplication of the rhizome, though shoot length increased at the lower irradiances. Bridgen and Winski (1989) concluded that 120 $\mu E\,m^{-2}s^{-1}$ was the optimum irradiance for rapid propagation. In addition, multiplication rate was not significantly affected by daylength, when comparison was made between explants cultured at daylengths of 8 or 16 hours, at an irradiance of 6 to 8 $W\,m^{-2}$ (Pierik *et al.*, 1988). When multiplication rate was compared at temperatures between 15 and 24°C, rising by 3°C intervals, no significant difference was seen between 15 and 21°C but there was a significant decrease in multiplication rate at 24°C (Pierik *et al.*, 1988). An optimum temperature of 15°C was established by Bridgen and Winski (1989) after comparing multiplication rates at 10, 15 and 20°C. These results show that *Alstroemeria* can be cultured at 15°C and a daylength of 8 hours with an irradiance of 6-8 $W\,m^{-2}$, without causing a decrease in the rhizome multiplication rate. Different cultivars would, although following a similar pattern, elicit a different range of responses.

The *in vitro* culture of *Alstroemeria* is also being studied to achieve organogenesis and embryogenesis for the production of somaclonal variants (Bridgen, 1986; Gonzalez Benito and Alderson, 1990). The *in vitro* rescue of hybrid embryos, that would not reach maturity in the seed, has also been reported (Winski and Bridgen, 1988). Likewise, meristem culture and virus elimination from *Alstroemeria* have also been studied (Hakkaart and Versluijs, 1985).

1.4 APICAL DOMINANCE

1.4.1 The Mechanism of Apical Dominance

Recent reviews of this topic can be found in Tamas (1987) and Roberts and Hooley (1988). The growing shoot apex is known to exert correlative influence over a range of developmental events including axillary bud growth, the orientation of laterals and the development of rhizomes and stolons. Not only do the apical tissues of a plant have a profound influence on the form which a plant adopts but they also have the ability to restrict the development of lateral bud meristems, maintaining them in a quiescent state until the apex loses its capacity to dominate or is removed from the plant.

Axillary buds in several plant species have been shown to originate directly from the growing shoot apex. A small group of cells in the axil of a leaf primordium becomes isolated from the apical meristem and develops into the apex of the axillary bud. After further development a visible bud is formed (Tamas, 1987). The ability of a plant to sustain a supply of replacement apices is not only of major ecological advantage but is also an excellent example of the way in which plant growth and development are integrated. This phenomenon has commercial value in the agricultural and horticultural industries, as axillary outgrowth can be used to increase yields and plant numbers, the latter via techniques of vegetative propagation.

Since removal of the shoot apex stimulates growth of lateral meristems, it is evident that the apex is the source of some correlative signal. However, in some grass species defoliation is also required before lateral bud growth is stimulated. This suggests that, in these species, hormones other than those produced by the apical bud may control lateral bud growth (Richards *et al.*, 1988). It has been postulated that this signal is provided by the auxin IAA, which has been shown to have a basipetal polarity of transport from the shoot apex in internodal segments of *Nicotiana tabacum* and coleoptiles of *Zea mays* (Hertel and Leopold, 1963; Sheldrake and Northcote, 1968). There is evidence that the majority of auxin transport takes place in cells associated

with the internal phloem and in cells close to the cambium. Jacobs and Gilbert (1983) have shown that in *Pisum sativum* stem tissue, the plasma membranes of the basal ends of parenchyma cells sheathing the vascular bundles is the location of the presumptive auxin transport carrier.

Auxin moving down the plant was thought to have a direct effect on the growth of axillary buds, with dominance often becoming less pronounced with increasing distance from the apex. This is considered as an effect of decreasing concentration or supply. Since the direct theory of auxin inhibition of lateral buds was proposed, more recent work, reviewed by Guern and Usciati (1972) and Hillman (1984, 1986), has shown the role of auxin in the maintenance and release of apical dominance to be more complex and probably less central and direct than previously envisaged.

The concept that auxin acts at the shoot apex by diverting molecules towards it, has been considered for many years and is now gaining a greater acceptance. This is leading to a greater emphasis on possible hormonal control of transport flows and distribution patterns of metabolites, ions and water. As these would include plant growth regulators (PGRs) and plant nutrients, directed movement to the apex would bias competition for these growth constituents, thereby limiting the growth of lateral buds (Leakey *et al.*, 1978; McIntyre, 1969, 1972, 1977; McIntyre and Damson, 1988). The stimulation of lateral bud outgrowth by such compounds as polar auxin inhibitors consequently has become more difficult to interpret, than when axillary bud activity was regarded as being closely controlled by auxin supply (Rubery, 1987b).

It has also been reported that calcium ion extrusion, normally stimulated by IAA (De Guzman and Fuente, 1984), can be prevented by inhibitors of polar auxin transport, and that the absence of calcium inhibited IAA transport. This suggests that the calcium status of the cell is directly linked to auxin transport and that a change in the direction and rate of IAA movement is likely to alter both the internal concentration and polar distribution of cal-

cium within the cell. These changes could then set off different developmental effects in the cell (Hepler and Wayne, 1985).

The basic mechanism of apical dominance, therefore, remains unresolved even though extensive information is available on specific hormonal effects. All the classes of major growth substances are known to have at least some effect on axillary bud growth but their interaction is still largely undefined. In general, a hormone regime that enhances vigour of the apical bud enhances apical dominance, whereas when the apical bud is less vigorous, lateral bud growth may continue under the influence of growth promotive hormones in the axillary bud (Tamas, 1987).

1.4.2 Growth Regulator Control of Apical Dominance

1.4.2.1 Triiodobenzoic acid

2,3,5-Triiodobenzoic acid (TIBA) is a synthetic plant growth regulator which increases lateral branch development in fruit trees and stimulates branching in plants such as soybean. Increases in soybean yields were also reported but are thought to be due mainly to a reduction in crop lodging, as TIBA is also known to decrease plant height (Roberts and Hooley, 1988). However, the effects of TIBA have been found to be unpredictable under different environmental conditions.

TIBA is classed as a member of a group of specific and highly potent inhibitors of polar auxin transport, known as phytotropins (Rubery, 1987a). Morris *et al.* (1973) showed that application of TIBA to intact pea seedlings (*Pisum sativum*) inhibited the basipetal transport of IAA, as it did in the coleoptile of *Zea mays* (Hertel and Leopold, 1963). In *Dracaena marginata* stem segments, a 60 per cent decrease in basipetal auxin transport occurred after the application of TIBA to the basal cut ends (Saltveit and Fonteno, 1983). Inhibition of the increase in diffusible auxin has also been reported after TIBA treatment at the first node of tulip flower stalks (*Tulipa gesneriana* cv. 'Paul Richter') (Okubo and Uemoto, 1985). TIBA also inhibits

the growth of hypocotyl sections of soybean induced by 2,4-dichlorophenoxy-acetic acid (2,4-D) by as much as 60 per cent (Widholm and Shaffer, 1970). Auxin uptake into tobacco stem segments is stimulated up to threefold by the action of TIBA, probably as a result of inhibition of a component of auxin efflux from the cells (Rubery, 1979). Similar results have also been recorded for *Pisum sativum* (Davies and Rubery, 1978).

Phytotropins inhibit polar auxin transport by blocking secretion of auxin from the basal ends of cells (Rubery, 1987a) and TIBA was the first chemical recognised in this group (Niedergang and Skoog, 1956). The mechanism of polar auxin transport is now thought to be an 'energetically downhill auxin anion efflux' (Goldsmith, 1977; Rubery, 1980), with inhibition acting at the level of the auxin efflux carrier within the plasma membrane (Davies and Rubery, 1978; Rubery, 1987b). Further work has shown TIBA to be a non-competitive inhibitor of the efflux carrier for polar auxin transport (Depta *et al.*, 1983). It therefore seems likely that it binds to a regulatory site different from the substrate site. This site could reside on a separate regulatory subunit, either permanently associated with the catalytic subunit of the efflux carrier or capable of interacting with it, if the proteins are mobile within the membrane. TIBA itself has also been found to be transported in a polar fashion (Thomson *et al.*, 1973).

Consequently, a two site model has been proposed to resolve this apparent contradiction of TIBA being a non-competitive inhibitor and also a substrate of the polar transport system. This suggests that for the auxin efflux carrier, auxins and TIBA have separate specific binding sites and that the occupation of one of the sites allows ligand translocations, whereas occupation of both sites prevents any transport (Depta and Rubery, 1984). It is therefore apparent that the biological properties of TIBA are consistent with a critical role for auxins in such developmental events as growth and apical dominance (Roberts and Hooley, 1988).

1.4.2.2 Cytokinins and thidiazuron

There is strong evidence that cytokinins are key factors in promoting bud growth (Tamas, 1987). Application of cytokinins to quiescent axillary buds from a range of species has been shown to stimulate their growth, for example in soybean (Ali and Fletcher, 1971) and in bulbous and cormous, monocotyledonous plants (Hussey, 1976). Increases in the rate of tillering have also been found from the application of BAP to ryegrass (*Lolium multiflorum*) *in vitro* (Dalton and Dale, 1981, 1985). BAP is known to inhibit polar auxin transport (Harrison, 1982), a mechanism, as stated previously, thought to play an important role in apical dominance.

Apical dominance may be maintained by regulating cytokinin distribution within the plant (Phillips, 1975). This assumes that shoot growth requires root-produced cytokinin, which accumulates preferentially in the shoot apex, thereby depriving the axillary buds of their supply of cytokinin. Woolley and Wareing (1972) have shown that the application of IAA to the apical stump of decapitated *Solanum* cuttings leads to a decrease in the amount of BAP, applied at the base, reaching the axillary buds. This suggests that cytokinin transport to, and accumulation in, the axillary buds may be under apical control. Therefore, while cytokinins from the roots can enhance axillary bud growth, if the apical bud is highly dominant then the root-derived cytokinins are probably transported mainly to the apical bud, thereby enhancing apical dominance. It has also been shown (Wang and Wareing, 1979) that while shoots are able to synthesize cytokinin, repressed axillary buds are not. Consequently it would seem that a redirection of cytokinin supply or a decline in auxin supply, may be necessary to either initiate axillary bud growth or to enable axillary buds to synthesize cytokinin, respectively.

Thidiazuron, N-phenyl-N'-(1,2,3-thiadiazol-5-yl) urea, was first developed as a defoliant for cotton *Gossipium hirsutum* (Arndt *et al.*, 1976). It is absorbed by the leaves and causes premature formation of the natural abscission layer between the stem and petiole (Anon, 1986) and it also provides superior

control of regrowth vegetation after the leaf abscission (Gianfanga, 1987). The biochemical principles underlining the mode of action of thidiazuron are not yet fully understood.

Cytokinin activity of thidiazuron has been detected in callus bioassays of *Phaseolus lunatus* cv. 'Kingston' where it was found to be more active than zeatin (Mok *et al.*, 1982; Mok and Mok, 1985), analogues being less active than the parent compound. The effects of thidiazuron on shoot formation and stimulation of axillary bud growth have been determined for woody species and trees (van Nieuwkerk *et al.*, 1986; Wang *et al.* , 1986), broccoli (*Brassica oleracea* 'Italica' group) (Mok *et al.*, 1987) and several other test systems. Increases in cytokinin induce an increase in the activity of cytokinin oxidase in callus tissues of *Phaseolus vulgaris* cv. 'Great Northern' and the specificity of this enzyme suggests that it plays an important role in the regulation of cytokinin levels in plant tissues (Chatfield and Armstrong, 1986). Thidiazuron was found to be as effective as any adenine derivative in inducing this response from the enzyme. This suggests that thidiazuron along with other cytokinins may serve as a substrate for cytokinin oxidase.

Thidiazuron is classified as a cytokinin-active phenylurea derivative (Mok *et al.*, 1987). The cytokinin activity of such derivatives is very variable, many being virtually or completely inactive, with a few having activities equal to or greater than that of BAP (Takahashi *et al.*, 1978). In all reports to date the biological activity of thidiazuron was higher than or comparable to that of the most active adenine-type cytokinins.

The biosynthesis of ethylene is promoted by the synergistic action of auxin and cytokinin (Lau and Yang, 1973) and it has been shown that thidiazuron can substitute effectively for adenine-type cytokinins in enhancing ethylene production (Yip and Yang, 1986). This activity agrees with earlier findings that application of thidiazuron resulted in an increase in ethylene production in mung bean (*Vigna radiata*) and cotton (*Gossipium hirsutum*) (Suttle, 1984a, 1984b, 1985). This is thought to play an important role in the abscis-

sion of cotton leaves (Anon, 1986) and may play a role in the inhibition of polar auxin transport, leading to a loss or decrease in apical dominance.

From these observations the biological effects of thidiazuron conform, in general, to the properties established for the adenine-type cytokinins (Mok *et al.*, 1987). An interesting phenomenon associated with the use of thidiazuron *in vitro* is the change in cytokinin requirement after exposure to it. Cytokinin-dependent callus of some *Phaseolus lunatus* genotypes, maintained on medium containing thidiazuron, displayed cytokinin autonomy in subsequent passages (Capelle *et al.*, 1983). This may be linked to an increase in endogenous cytokinin synthesis, known to be caused by thidiazuron (Thomas and Katterman, 1986).

1.4.2.3 Auxin-cytokinin synergism

The effects of cytokinins are often transitory *in vivo*, however bud outgrowth can be prolonged by combined treatment with auxin, a phenomenon also evident *in vitro* (Roberts and Hooley, 1988). The mechanism of this reaction still remains unclear. Sachs and Thimann (1967) demonstrated that individual buds treated with kinetin followed by auxin, resembled uninhibited buds in the lengths of their internodes. This was thought to be due to release from inhibition by the cytokinin and promotion of elongation by the auxin. When applied alone, neither PGR could achieve this result.

Auxin induces ethylene production in some plant species (Kang *et al.*, 1971; Sakai and Imaseki, 1971) and the addition of cytokinin has a synergistic effect on auxin-induced ethylene production (Lau and Yang, 1973; Yoshii and Imaseki, 1981), i.e. it enhances its production. Work on *Pisum sativum* cv. 'Alaska' (Burg and Burg, 1968) suggested that IAA stimulated ethylene production led to inhibition of bud growth. However, more recent work on *Phaseolus vulgaris* (Yeang and Hillman, 1981, 1982) has shown that increasing the ethylene level in the apical shoot can lead to development of the inhibited axillary buds and that inhibitors of ethylene production reverse this

process. Van Dijck *et al.* (1988) also concluded that the increase in ethylene production, stimulated by the synergistic effect of cytokinin and auxin added to the culture medium *in vitro*, is positively correlated with the outgrowth of lateral buds in some bromeliads. Pierik (1987) found similar results with a cultivar of the bromeliad genus *Vriesea*, where axillary shoot formation was enhanced by adding auxin and cytokinin in a ratio of 1:10 to the culture medium. In cultures of *Plantago ovata* (Barna and Wakhlu, 1988), though addition of auxin did not significantly affect the number of axillary shoots produced, it enhanced their quality and significantly increased their length.

It is known that ethylene inhibits polar-auxin transport (Morgan *et al.*, 1968) which, as stated previously, is thought to be a key factor in the maintenance of apical dominance. Therefore, the production of ethylene could be a means by which the synergistic interaction of auxin and cytokinin operates.

1.4.2.4 Gibberellins

There are few reports on the relationship between axillary bud development and endogenous gibberellin levels and the data that exists is often contradictory. Studies with applied gibberellins indicate a possible role in the regulation of bud development. Gibberellic acid (GA_3) enhances apical dominance in many plants, such as *Pisum sativum* and *Zingiber officinale* cv. 'Chinese' (Furutani and Nagao, 1986; Jacobs and Case, 1965; Torrey, 1973), probably as a result of the more efficient release of IAA by the dominant apex, as GA_3 has been found to stimulate the basipetal transport of IAA (Jacobs and Case, 1965; Palmer and Halsall, 1969; Pilet, 1965; Stowe and Yamaki, 1957). In contrast, the external application of GA_3 may also enhance the outgrowth of buds of some plants, such as *Nelumbo lutea*, *Hedera helix* cv. 'Baltica', *Hordeum* and *Avena* (Kane *et al.*, 1988; Galston and Davies, 1969; Lewnes and Moser, 1976; Scott *et al.*, 1967).

Kinetin strongly promotes the release of axillary buds from apical dominance when applied together with GA_3 (Catalano and Hill, 1969; Sachs and Thi-

mann, 1964). It has, however, been suggested that this may be the action of GA_3 on the buds after their release from inhibition by kinetin.

As stated previously, apical dominance becomes less pronounced the further from the apex the bud is situated and that this is thought to result from decreasing concentration or supply of auxin. Therefore, it can be postulated that the capacity of GA_3 to cause elongation of shoots could be a means by which this effect is increased. It has been established that GA_3 can increase cell proliferation (Liu and Loy, 1976) and elongation in newly formed cells (Raskin and Kende, 1984) and intercalary meristems (Kaufman, 1965, 1967).

Cellular elongation is a complex mechanism involving the physical process of the force of turgor and a change in cell wall metabolism (Metraux, 1987; Stuart and Jones, 1977). The latter affects the yielding of the cell wall and GA_3 has been shown to have little effect on elasticity but a profound effect on the irreversible, or plastic, component of extensibility (Adams *et al.*, 1975; Stoddart, 1987). There seems to be no information linking these two phenomena.

1.4.2.5 Paclobutrazol

Extensive field testing has shown paclobutrazol, [(2RS,3RS)-1-(chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol], to be a broad spectrum growth retardant producing reductions in vegetative growth, generally with no adverse effects to the plants (Lever *et al.*, 1982). The group of compounds to which paclobutrazol belongs, the triazoles, are the most highly active growth retardants demonstrated so far (Hedden and Graebe, 1985).

Studies with apple cultivars (Quinlan and Richardson, 1984; Stinchcombe *et al.*, 1984; Tromp, 1987) have shown that paclobutrazol can cause decreases in shoot length and leaf size and promote flowering, though the time of flowering was sometimes delayed. Enhancement of fruit set has been recorded but without any effect on yield. Similar results were reported for strawberry (McArthur and Eaton, 1987) where fewer numbers of larger berries were

produced and yield was unaffected. In studies with watermelon (Huang *et al.*, 1987) and cultivars of plum (*Prunus domestica*) (Webster and Quinlan, 1984), plant growth was inhibited and yields were often severely reduced. Changes in ear and grain number and modification of canopy structure, associated with changes in plant morphology, have been produced in graminaceous crops and turf grasses, after treatment with paclobutrazol (Hebblethwaite *et al.*, 1982; Lever *et al.* 1982; Shearing and Batch, 1982).

Paclobutrazol has also been used effectively to produce more compact ornamental plants, often with enhanced flowering. These include chrysanthemum and poinsettia (*Euphorbia pulcherrima*) (Menhenett, 1983), Easter lily (*Lilium longiflorum*) (Jiao *et al.*, 1986; Rees *et al.*, 1982), tulip (Hanks and Menhenett, 1983; Rees *et al.*, 1982) and many others (Galston and Shearing, 1985; Lever *et al.*, 1982; Shanks, 1980). By 1984, control of plant growth by paclobutrazol had been proven for over 60 species (Anon, 1984).

Paclobutrazol is readily taken up passively through roots, stem tissue and foliage and it is transported acropetally, almost exclusively in the xylem. Consequently paclobutrazol sprayed onto foliage accumulates in the leaves and little reaches the meristematic areas of cell division and cell elongation, the site of action of paclobutrazol. To reach these meristematic areas uptake of PP333 is required through the roots or exposed stems (Anon, 1984; Lever *et al.*, 1982).

The primary role of paclobutrazol is as a growth retardant, though it does also possess fungicidal properties (Lever *et al.*, 1982). It is also assumed that the compound interferes directly or indirectly with the regulation of endogenous levels of abscisic acid (ABA) and cytokinins. These are thought to be closely connected with a variety of secondary physiological responses including increased chlorophyll concentration, altered carbohydrate status, delayed senescence and increased stress tolerance (Gehlot *et al.*, 1989; Grossman *et al.*, 1987). The latter may result from a change in the water use of the plants, as has been recorded in apples following the application of pa-

clobutrazol (Atkinson, 1986; Atkinson and Chauhan, 1987). This may be caused by decreased levels of water uptake, associated with decreases in leaf area, stomatal conductivity and root size and density, that are often produced by the action of paclobutrazol on plants. These changes together with increased epicuticular wax, have been demonstrated in *in vitro* material of chrysanthemum. It has also been shown that these changes confer resistance to desiccation to the explants, which leads to better establishment *ex vitro* (Smith *et al.*, 1990).

Commercial paclobutrazol is a racemic mixture of two enantiomers, the 2S,3S form which is known to inhibit stem elongation and the 2R,3R form which has fungicidal activity but little impact on growth (Lenton *et al.*, 1987; Lurssen, 1987). These phenomena are caused by the selective action of the two enantiomers on the inhibition of gibberellin biosynthesis or interference with sterol metabolism, respectively (Dalziel and Lawrence, 1984; Hedden and Graebe, 1985; Lurssen, 1987). The inhibition of gibberellin biosynthesis is thought to be caused by the inhibition of *ent*-kaurene oxidase in the oxidation of the gibberellin precursor *ent*-kaurene to *ent*-kaurenoic acid (Dalziel and Lawrence, 1984; Hedden and Graebe, 1985; Graebe, 1987). Therefore, the action of paclobutrazol as an anti-gibberellin prevents cell division and elongation and other growth responses normally associated with the action of gibberellins. The action of paclobutrazol on sterol metabolism involves inhibition of sterol demethylation in fungi (Baldwin and Wiggins, 1984) which prevents the biosynthesis of ergosterol, a vital component of fungal cell membranes, thereby affecting some fundamental property of these membranes.

The biosynthetic pathways of sterol and gibberellin have features in common and paclobutrazol has been reported to reduce the sterol levels of plant tissues (Baldwin and Wiggins, 1984). However, the enantiomers are not specific inhibitors of the two pathways. Consequently, a third mode of action, namely changes in endogenous ABA levels, is implicated to explain the observed effect on water consumption and stomatal movement.

1.4.2.6 Ethylene and abscisic acid

Although these growth regulators have not been used in the present study, they have been implicated in the mechanisms discussed previously. Consequently, their interaction with apical dominance will be considered here briefly.

Ethylene

There is considerable evidence that ethylene may also play a role in apical dominance, though its gaseous nature makes it unlikely as a correlative signal (Roberts and Hooley, 1988). Ethylene pretreatment can severely inhibit polar auxin transport, with species differing in their individual sensitivity (Morgan *et al.*, 1968). Pretreatment of whole plants for several hours (Burg and Burg, 1967) or excised segments (Osborne and Mullins, 1968) appears to be necessary for polar auxin transport to be inhibited, with recovery occurring after the removal of the ethylene (Beyer, 1973). This suggests that ethylene may reduce the level of auxin carriers in the plasma membrane, possibly by regulating gene expression or intra-cellular protein movement.

Abscisic acid

Some workers have reported that axillary bud growth inhibition is correlated with the ABA content of the buds. If the shoot is decapitated, or otherwise treated to release the buds from inhibition, the ABA content of the buds is seen to decrease rapidly (Tamas, 1987). In non-branching mutants of tomato, the ABA content of the axillary buds is much higher than in normal plants (Tucker, 1976). ABA treatment of axillary buds inhibits their growth, whereas the application of ABA to the shoot apex releases axillary buds from inhibition (Tucker, 1977). This response is thought to be caused by the inhibition of apical bud growth, with the consequent loss of its dominance effect.

There are no indications that ABA functions as the correlative signal released by shoot apices. The available evidence suggests that ABA acts within the

axillary buds as a secondary factor, whose level can be regulated by the dominant apex. Decapitation lowered ABA levels in axillary buds of *Phaseolus* plants but this was prevented by the application of IAA to the cut stump (Tucker, 1978). It therefore appears, that the polar transport of IAA can maintain a high level of ABA in the axillary buds. It is also thought, that the effect of ABA may be modified by other plant growth substances (Tamas, 1987).

Chapter 2: MATERIALS AND GENERAL METHODS

2.1 SOURCE OF PLANT MATERIAL

Plants of four hybrid cultivars of *Alstroemeria*, 'Valiant', 'Parade', 'Eleanor', and a 'Butterfly type', were supplied by Parigo Horticultural Company (Spalding, U.K.). In this thesis the 'Butterfly type' will be referred to as 'Butterfly'. *Alstroemeria* cultivars are often divided into groups on the basis of their growth characteristics. The four cultivars chosen originate from four different groups and, therefore, together they exhibit a range of growth characteristics for *Alstroemeria* cultivars *in vivo* and *in vitro*.

2.2 GROUP BACKGROUNDS AND GENERAL CHARACTERISTICS

In this thesis, aerial shoots are referred to as shoots.

To protect the rights of the breeder the species used in the following breeding programmes will be referred to as α, β, γ and δ. This information was supplied by Parigo Horticultural Co..

- Species α, a native of cool plains and mountain foothills, including pine and *Nothofagus* forest where the climate is generally frost-free.
- Species β, a native of frost-free areas of the Pacific coast, where a cool spring usually gives way to warm summers and it is often dry.
- Species γ, a native of the northern most regions of South America and a shade loving plant.

- Species δ, a native of the Pacific coastal regions.

All of these parent species were collected from Chile, though they are not all restricted to this country.

Group A (sterile triploids)

Species α ♀ x Species β ♂

↓

Hybrid 1 ♀ x Species α ♂

↓

cv. 'Eleanor'

This group performs well *in vitro* but shows poor establishment *in vivo*. All cultivars with hybrid 1 as the female parent grow well *in vitro*. However, when established the rhizome is large and the shoots are tall and strong. Generally no flowers are produced in the winter.

Group B (sterile triploids)

Species β ♀ x Species γ ♂

↓

Hybrid 2 ♀ x Species α ♂

↓

cv. 'Carmen'

Radiation | Mutation

↓

cv. 'Valiant'

This group multiplies poorly *in vitro* but possesses good growth *in vivo*. However, the rhizome is small and weak and the shoots, though reasonably tall, are also weak. There is some flower production through the winter.

Group C (sterile triploids)

These are products of the same programme as that for group B. The cultivar chosen from this group was 'Parade'. Growth *in vitro* is relatively poor, whereas *in vivo* it is good. However, the rhizome, though slow to extend, is not as small and weak as that in group B and shoot growth is tall and strong. The stems remain vegetative in winter.

Group D (fertile diploids)

Species δ ♀ x Species γ ♂

↓

cv. 'Butterfly types'

The growth of these cultivars *in vitro* is generally very poor, whereas *in vivo* they are easy to establish and grow vigorously. Morphologically this group is quite dissimilar to the others as the stems are shorter, the leaves have a different texture and the close positioning of the shoots on the rhizome makes it shorter and more compact. Flowering is generally confined to late summer and autumn.

The number and distribution of the roots and tubers on the rhizome varies markedly between the groups. Figure 2.1 shows the rhizome rootstocks of the four cultivars chosen. It can be seen, therefore, that *in vivo* the cultivar 'Eleanor' shows poor establishment, whereas the cultivar 'Butterfly' shows good establishment, the converse being true *in vitro*. The cultivars 'Valiant' and 'Parade' are intermediates between the two cultivars 'Eleanor' and 'Butterfly'.

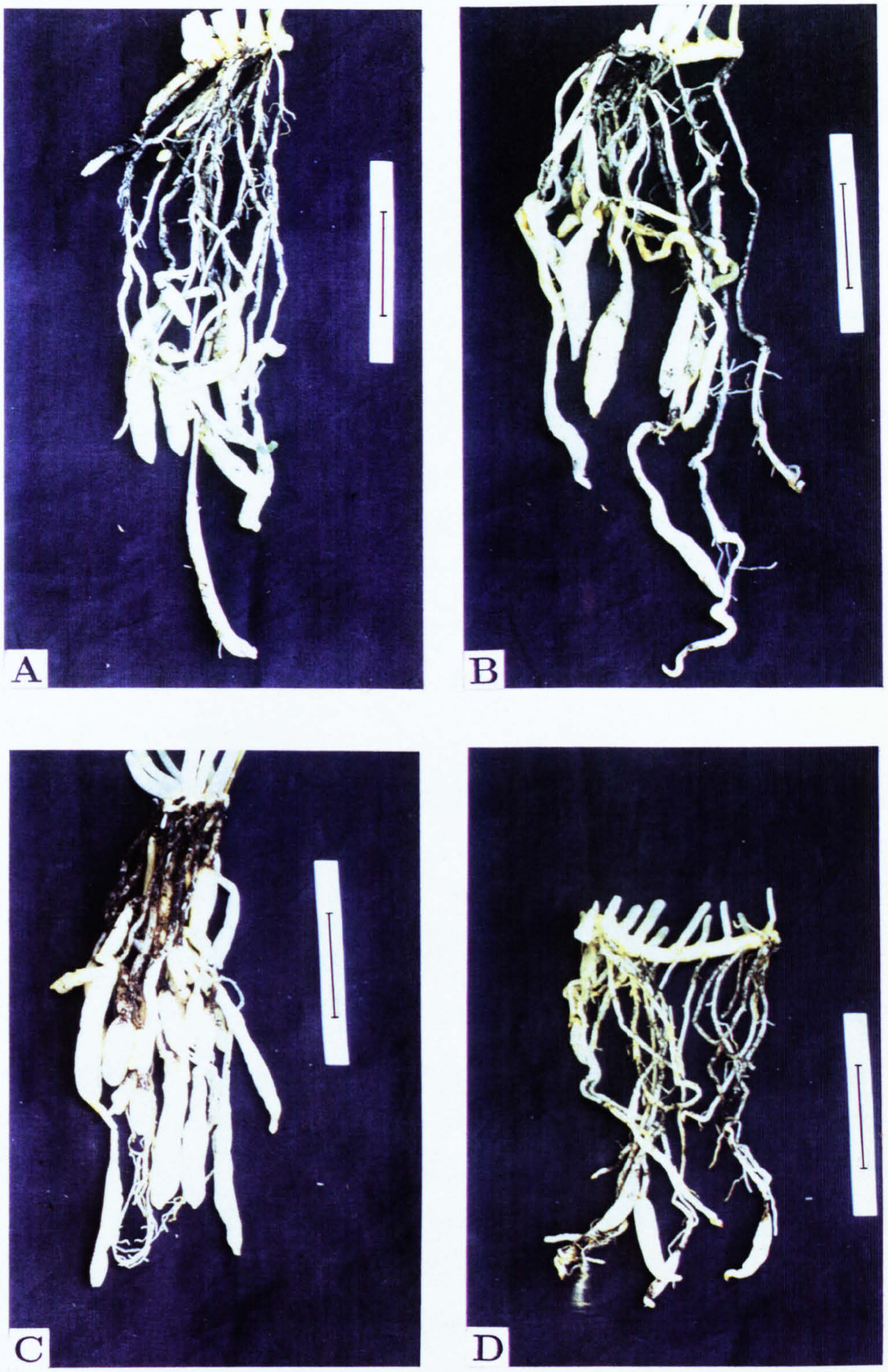


Figure 2.1: The rhizome rootstocks of the four cultivars chosen. Each rhizome segment consists of ten enlarged basal internodes. **A** = 'Valiant', **B** = 'Parade', **C** = 'Butterfly' and **D** = 'Eleanor'. Bar = 5 cm

2.3 GENERAL CULTURAL CONDITIONS

2.3.1 *In Vivo*

At the beginning of each experiment plant material was supplied as freshly lifted plant clumps for splitting by hand, or as small plants in 9 cm pots that had been produced from micropropagation. These 'splits', consisting of approximately 10 enlarged basal internodes, and small plants were transferred to three litre pots containing Fisons Levington M2 compost. This compost consists of a medium-coarse grade of peat and has a medium nutrient level, with a pH range of 5.3 to 6.0 (Anon, 1987). As the plants grew fairly tall and the stems were fragile, they were supported with split canes and string. The pots were transferred to their experimental positions and after approximately 12 weeks the plants were given a liquid feed of Maxicrop Triple (1:1000). This fertilizer is produced from a seaweed extract with small quantities of added nitrogen, phosphorus and potassium (Maxicrop International Limited, personal communication).

These were the standard cultural conditions for all *in vivo* experiments, conditions specific to each experiment are described in the relevant sections.

2.3.2 *In Vitro*

Rhizome cultures of the four cultivars, supplied by Parigo Horticultural Co., were maintained as a multiplying stock, from which material was removed for use in experiments. A stock of material was necessary as the meristems used in the micropropagation of *Alstroemeria* are all situated underground on the plant, which makes them difficult to disinfect when initiating. The stock cultures were subcultured every four or five weeks and maintained at 15°C, a 12/12 hour (day/night) daylength cycle (Parigo Horticultural Co., personal communication) and an irradiance of approximately 20 $W\,m^{-2}$ (120 $\mu E\,m^{-2}s^{-1}$), provided by Philips Color 29, 65-80 W, warm white fluorescent tubes. Any changes to these conditions are described in the relevant sections. All cultures, stock and experimental, were grown on *Alstroemeria*

production medium (Parigo Horticultural Co., personal communication). In some experiments, however, different combinations of PGRs were added to this medium.

Unless otherwise stated, *in vitro* material was subcultured every four weeks. This included removing all of the roots and the shoots above the first node, separating lateral rhizomes for subsequent culturing , removing some of the older part of the rhizome explant and transferring them to fresh medium. For experimental work, a 'standard explant' consisted of three enlarged basal internodes, with the roots removed and the shoots excised above the first node. At subculturing the explants were returned to this initial condition before continuing through the next four weeks of the experiment, after which subculturing would again occur. All of the *in vitro* experiments were carried out for 12 weeks only, i.e. three subculture cycles, as it had been reported (Parigo Horticultural Co., personal communication) that if no change had occurred within this time, then it was unlikely to do so.

2.4 MEDIA PREPARATION

The *Alstroemeria* production medium was based on MS revised medium (Appendix A), with elevated concentrations of vitamins and sucrose and reduced agar (Appendix B). It also contained 4 mg l^{-1} BAP, unless stated otherwise.

Stock solutions of the medium constituents (Appendices A and B) were stored in the dark at 5°C, with the exception of the vitamins and amino acids which were stored frozen. To prepare culture media, the appropriate volumes of stock solutions were mixed and sucrose and growth regulators (Appendix C) were added. Distilled water was added to obtain the required volume and the medium was then adjusted to a pH of 5.8 using solutions of potassium hydroxide and hydrochloric acid. In this study *Alstroemeria* production medium was always used semi-solid, agar (Sigma) at 8 g l^{-1} being dissolved by autoclaving at 104°C (5 p.s.i.) for ten minutes. The medium was dispensed in 25 ml aliquots into 140 ml screw top glass jars and sterilized by

autoclaving at 121°C (15 p.s.i.) for 15 minutes. This jar type and medium volume were used in all of the *in vitro* experiments.

Due to the thermolabile nature of GA_3, it had to be added after the medium had been sterilized. Consequently, pH adjusted solutions of GA_3 were filter sterilized with Sartorius Minisart NML 0.2 μm pore size disposable filters, and added to the cooling medium. This was then dispensed aseptically into previously sterilized culture jars.

2.5 DATA RECORDING AND ANALYSIS

All experimental designs were randomised blocks, with the details of the designs being given in each section. The *in vivo* experiments were assessed at intervals of either four or eight weeks by recording (i) the numbers of shoots, lateral rhizomes and tubers and (ii) the dry weights of the shoots, whole root system and the complete rhizome with laterals. These experiments were repeated to produce replication through time. The repetitions were performed over the same period one year later, to prevent any seasonal effects occurring. The two data sets were then combined to give a mean value for each parameter per plant. In the *in vitro* experiments cultures were assessed at subculturing on the fourth, eighth and twelfth weeks from the start of the experiments. The numbers of shoots, lateral rhizomes and roots were recorded. However, the first set of data for each experiment was not used, as it was considered that the explants were becoming accustomed to the new media or environments during the initial four weeks. The second and third sets were combined as one data set, as they were considered to be repeats of the experiment, producing a mean value for each parameter per culture jar. The means of the numbers produced for each treatment were calculated using all non-infected material. Data presented in figures and tables are the means per explant.

When the data consisted of scores or counts, it was considered to be discontinuous and was analysed by fitting a linear model with a binomial or

poisson distribution of the errors. These are both considered as analyses of deviance. From these analyses, values for chi-square (χ^2) were produced, from which the statistical probability (p) could be worked out. Continuous data, for example weights of samples, were subjected to an analysis of variance, from which values for the standard error of the difference of the means (sed) and the statistical probability (p) were produced. All analyses were performed using the Genstat 5 statistical computing package (Lawes Agricultural Trust, 1988, Rothamsted Experimental Station). Differences were considered significant for p=0.05 or less.

As this study was principally concerned with the effects of the treatments on the cultivars and substantial differences between the cultivars have already been demonstrated (Section 2.2), no analyses of the variation in growth response between cultivars in experiments will be made. An exception to this is the experiment designed to quantify the differences apparent *in vitro* (Section 5.3).

Chapter 3: THE EFFECT OF TEMPERATURE, IRRADIANCE AND DAYLENGTH ON RHIZOME GROWTH *IN VIVO*

3.1 INTRODUCTION

Of the limited number of papers published on the effects of changes in environmental factors on the growth of *Alstroemeria in vivo*, only a few make specific reference to how these factors influence the growth of the rhizome. Consequently, experiments were carried out to study the growth response of rhizomes across ranges of temperature, irradiance and daylength, *in vivo*.

In each experiment, the environmental factors were constant with the exception of the single variable being studied. All of the experiments were carried out in the period between autumn to spring in order to avoid extremes of temperature in the glasshouses. Suitable plant material was also more available in the autumn.

3.2 THE EFFECT OF TEMPERATURE

3.2.1 Materials and Methods

Potted 'split' plant material (Section 2.3.1) of the cultivars 'Valiant', 'Parade' and 'Butterfly' were grown in three controlled environment Saxcil cabinets, set at constant temperatures of 8, 13 and 18°C, for 24 weeks. The cabinets were set on a 12/12 hour (day/night) daylength cycle, lighting being supplied by Philips Color 29 warm white fluorescent tubes 65-80 W, that gave approximately

28 W m^{-2} (180 μE $m^{-2}s^{-1}$) of light, from behind a white translucent perspex screen. Growth analysis was carried out at intervals of eight weeks, over the 24 week period, at which times four plants of each cultivar from each treatment were removed and assessed (Section 2.5). This experiment was subsequently repeated using new plant material and the data analysed (Section 2.5), with an experimental design of 3 temperatures x 8 replicates, with 4 assessments for each cultivar.

3.2.2 Results

Temperature had a greater effect on the dry weight of plants than on the numbers of lateral rhizomes, roots and shoots produced (Tables 3.1 and 3.2). These differences were generally apparent by week 24. At 8°C all of the cultivars performed poorly, producing generally fewer parts and lower dry weights than at the other two temperatures. Differences between growth at 13 and 18°C tended to be small. There was often a decline in growth at 18°C to levels lower than that at 13°C, especially in the cultivar 'Parade' and to a lesser extent 'Valiant' (Figure 3.1).

This trend supports the observation made during this experiment that increasing the temperature results in more plants failing to establish viable new growth from 'splits' and subsequently dying. This was particularly significant for the cultivar 'Valiant' (Figure 3.2). Often, some shoots emerged initially but within a few weeks they had collapsed and died. The shoots generally did not wilt but suddenly collapsed due to deterioration at their base. Closer examination revealed that the rhizome segments of these plants had not produced any new roots and had completely degenerated. Some of the tubers on these dead plants were still intact and were unchanged from when they were planted.

Time to flowering, although not quantified, was retarded for all cultivars at 8°C, with a similar time to flowering for the plants in the 13 and 18°C temperature regimes. Some material of all the cultivars at all the temperatures

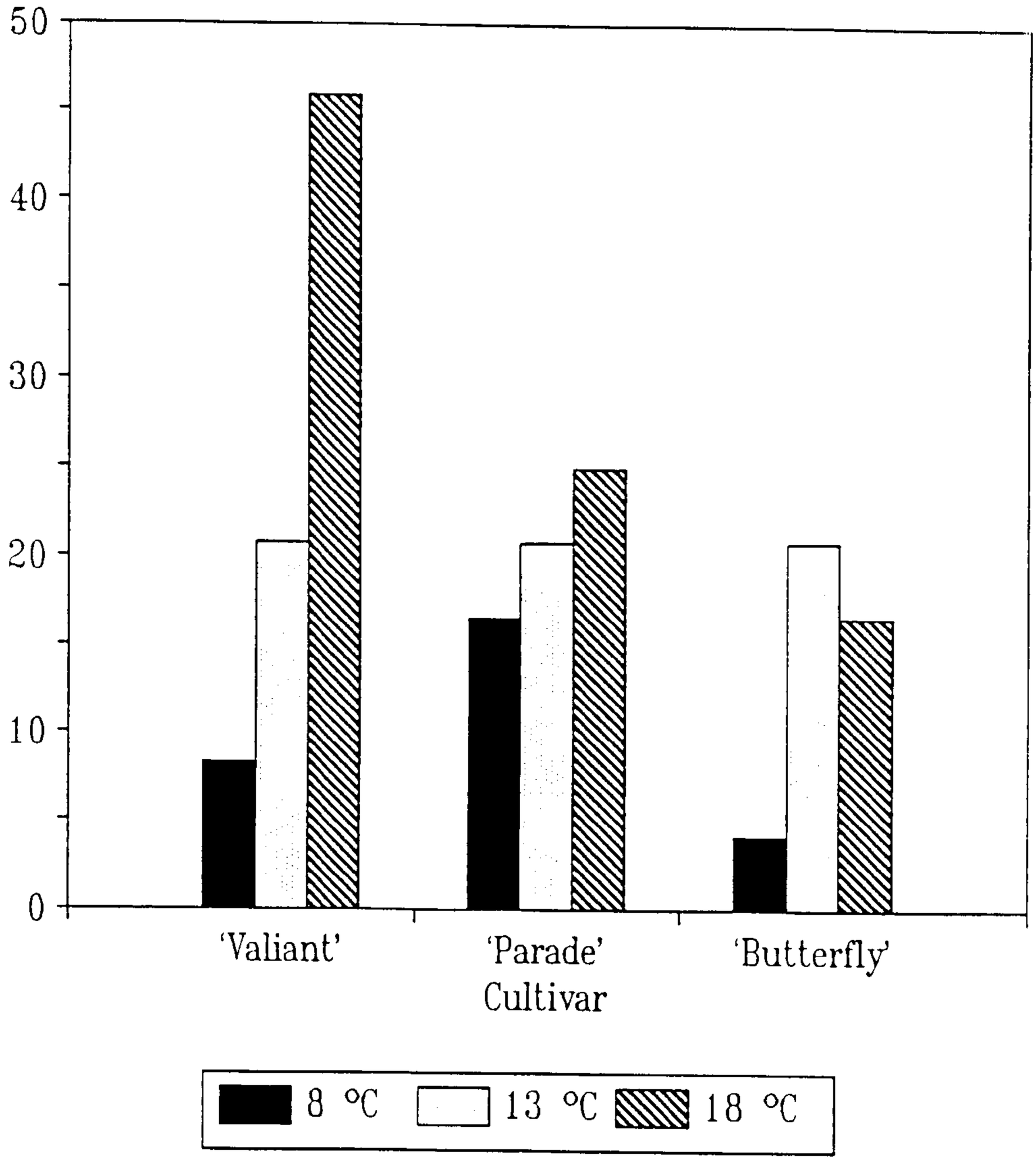
Table 3.1: The effect of temperature on the numbers of lateral rhizomes, tubers and shoots produced by the cultivars 'Valiant', 'Parade' and 'Butterfly'

Cultivar	Temperature (°C)	Period of growth (weeks) 0	8	16	24
		Number of lateral rhizomes			
	8	0.0	0.0	0.4	1.0
'Valiant'	13	0.0	0.3	1.3	3.1
	18	0.0	0.0	4.0	5.0
		not significant			
	8	0.0	0.6	0.8	1.6
'Parade'	13	0.0	0.2	1.5	5.9
	18	0.0	0.0	2.3	2.0
		not significant			
	8	0.0	2.1	2.6	4.8
'Butterfly'	13	0.0	2.0	4.1	7.3
	18	0.0	3.4	5.0	6.2
		not significant			
		Number of tubers			
	8	4.4	4.8	4.9	8.1
'Valiant'	13	4.5	5.6	8.9	31.0
	18	4.1	4.3	8.5	33.0
		not significant			
	8	0.8	0.4	2.9	4.3
'Parade'	13	1.5	2.7	6.0	21.0
	18	0.7	2.7	3.8	13.1
		not significant			
	8	3.3	2.9	5.3	7.8
'Butterfly'	13	3.7	4.6	10.0	38.0
	18	3.7	2.8	19.1	40.0
		p=0.05			
		Number of shoots			
	8	0.0	2.0	2.8	5.9
'Valiant'	13	0.0	2.6	5.0	16.3
	18	0.0	2.3	9.8	19.0
		not significant			
	8	0.0	2.1	3.1	7.3
'Parade'	13	0.0	2.5	4.1	15.0
	18	0.0	2.8	6.2	9.5
		not significant			
	8	0.0	3.6	7.8	15.9
'Butterfly'	13	0.0	6.5	13.8	29.5
	18	0.0	8.3	19.5	28.1
		not significant			

Table 3.2: The effect of temperature on the dry weights of rhizome, roots and shoots produced by the cultivars 'Valiant', 'Parade' and 'Butterfly'

Cultivar	Temperature (°C)	Period of growth (weeks) 0	8	16	24
		Rhizome dry weight (g)			
	8	1.04	0.29	0.44	0.62
'Valiant'	13	1.04	0.34	0.59	1.11
	18	1.04	0.31	0.91	1.33
		p=0.05, sed=0.12, d.f.=63			
	8	0.92	0.56	0.70	1.34
'Parade'	13	0.92	0.42	1.14	2.20
	18	0.92	0.90	1.06	1.30
		not significant			
	8	0.33	0.38	0.56	0.99
'Butterfly'	13	0.33	0.42	1.01	2.21
	18	0.33	0.40	1.13	3.38
		p=0.001, sed=0.15, d.f.=63			
		Root dry weight (g)			
	8	1.76	2.05	3.24	4.82
'Valiant'	13	1.76	3.15	4.85	16.91
	18	1.76	2.28	6.07	11.32
		not significant			
	8	2.61	1.47	4.14	6.33
'Parade'	13	2.61	1.75	8.07	24.99
	18	2.61	4.31	4.50	15.26
		p=0.01, sed=2.17, d.f.=63			
	8	2.45	1.68	3.02	4.15
'Butterfly'	13	2.45	3.08	9.23	30.77
	18	2.45	2.19	8.32	38.33
		p=0.001, sed=2.76, d.f.=63			
		Shoot dry weight (g)			
	8	0.00	0.07	0.19	0.80
'Valiant'	13	0.00	0.10	1.89	7.03
	18	0.00	0.07	4.74	20.23
		p=0.001, sed=1.60, d.f.=63			
	8	0.00	0.13	0.75	5.38
'Parade'	13	0.00	0.24	4.85	40.27
	18	0.00	0.56	11.30	18.33
		p=0.01, sed=3.65, d.f.=63			
	8	0.00	0.25	1.66	5.34
'Butterfly'	13	0.00	1.36	4.60	20.89
	18	0.00	2.27	13.80	19.89
		p=0.001, sed=2.46, d.f.=63			

Figure 3.1: Growth of the cultivars 'Butterfly' (**A**) and 'Valiant' (**B**) after 24 weeks at three different temperatures. Left to right: 8, 13 and 18°C. Scale = 1m



'Valiant'	p=0.01
'Parade'	not significant
'Butterfly'	p=0.05

Figure 3.2: The effect of temperature on plant deaths in the cultivars 'Valiant', 'Parade' and 'Butterfly'

was in flower or had buds developing by week 16. The time to flowering of the cultivar 'Butterfly' seemed to be least affected by temperature.

3.3 THE EFFECT OF IRRADIANCE

3.3.1 Materials and Methods

Small plants (Section 2.3.1) of the cultivars 'Valiant' and 'Eleanor' were grown for 20 weeks in a glasshouse, set at a mean daily temperature of 15°C and a 12/12 hour (day/night) daylength cycle. The daylength extension was produced by high pressure sodium lamps (G.E.C. RC HPS/U 2 Solarcolour, 400 W), that gave approximately 28 W m^{-2} (88 μE $m^{-2}s^{-1}$) of light. Environments of 60 and 50 per cent irradiance were produced by covering the plants with graded shade netting, 40 and 50 per cent shade respectively (Rokolene protection netting, Roko Containers, S. and E. Strauss Ltd.), supported on a metal frame.

Two plants of each cultivar from each treatment, were removed for growth analysis (Section 2.5) at intervals of four weeks, over the 20 week period. Temperature was monitored in the different irradiance regimes to ensure that no significant change in the temperature occurred in the different treatments. Irradiance was also monitored to ensure that no changes occurred due to alterations in the properties of the netting during the experimental period. The experiment was subsequently repeated using new plant material and the data analysed (Section 2.5), with an experimental design of 3 irradiances x 4 replicates, with 6 assessments for each cultivar.

3.3.2 Results

There were no losses of plants following transfer to the three litre containers. Overall, irradiance had a greater effect on dry weight than on the number of lateral rhizomes, tubers and shoots produced (Tables 3.3 and 3.4). Some trends of decrease in number and dry weight with decreasing irradiance were evident but they were generally only apparent by weeks 16 or 20. Growth was

always best at 100 per cent irradiance, regardless of the cultivar. However, differences perceived between the 60 and 50 per cent irradiance levels were not consistent, depending on the cultivar and the growth parameter being studied. Of the cultivars studied, 'Eleanor' seemed to be the more sensitive to changes in irradiance received. At the end of the experiment there was little visible difference between the treatments within a cultivar (Figure 3.3).

Time to flowering, although not quantified, appeared to be similar for both of the cultivars across the range of irradiances studied. Buds and some flowers were evident at all irradiances for the cultivar 'Valiant' by week 8, whereas for the cultivar 'Eleanor', only buds were developing at this time, but more were present in the 100 per cent irradiance treatment. Both cultivars were in flower in all the irradiance treatments by week 12.

3.4 THE EFFECT OF DAYLENGTH

3.4.1 Materials and Methods

Small plants (Section 2.3.1) of the cultivars 'Valiant' and 'Eleanor' were grown for 20 weeks in a glasshouse maintained at a mean daily temperature of approximately 15°C. Daylengths of 8, 12 and 16 hours were achieved with automated blackout curtains, set for a maximum daylength of 8 hours, with supplementary lighting for daylength extension to 12 and 16 hours. The supplementary lighting of approximately 7.5 $W\,m^{-2}$ (5 $\mu E\,m^{-2}s^{-1}$) was provided by 100 W tungsten bulbs.

Two plants of each cultivar from each treatment were removed for growth analysis (Section 2.5) at intervals of four weeks, over the 20 week period. Temperature readings were taken to ensure that the tungsten lights were not producing any significant heating effects in the extended daylength environments. This experiment was subsequently repeated using new plant material and the data analysed (Section 2.5), with an experimental design of 3 daylengths x 4 replicates, with 6 assessments for each cultivar.

Table 3.3: The effect of irradiance on the numbers of lateral rhizomes, tubers and shoots produced by the cultivars 'Valiant' and 'Eleanor'

Cultivar	Irradiance (%)	Period of growth (weeks) 0	4	8	12	16	20
		Number of lateral rhizomes					
'Valiant'	100	0.0	0.0	4.8	5.0	5.3	5.0
	60	0.0	0.3	2.3	1.8	3.5	4.5
	50	0.0	0.8	0.5	2.3	1.8	2.3
		p=0.001					
'Eleanor'	100	0.1	1.3	1.5	2.0	2.0	6.3
	60	0.1	0.0	0.0	0.3	0.8	1.3
	50	0.1	0.5	0.0	0.5	1.0	1.8
		not significant					
		Number of tubers					
'Valiant'	100	15.7	14.0	15.0	19.5	49.5	67.3
	60	15.7	15.5	18.0	25.8	28.3	39.8
	50	15.7	18.3	14.0	26.3	23.5	33.8
		not significant					
'Eleanor'	100	5.5	8.7	8.8	12.3	15.8	27.0
	60	5.5	8.5	10.5	11.5	10.3	21.3
	50	5.5	6.8	8.5	9.0	12.0	18.5
		not significant					
		Number of shoots					
'Valiant'	100	3.3	3.8	8.8	13.0	18.8	29.0
	60	3.3	3.5	6.5	10.5	12.0	18.0
	50	3.3	5.0	9.0	9.5	13.8	12.5
		not significant					
'Eleanor'	100	3.5	5.3	8.5	10.8	11.8	14.0
	60	3.5	5.5	6.0	10.5	10.3	11.8
	50	3.5	6.3	7.5	8.5	11.8	11.5
		not significant					

Table 3.4: The effect of irradiance on the dry weights of lateral rhizomes, roots and shoots produced by the cultivars 'Valiant' and 'Eleanor'

Cultivar	Irradiance (%)	Period of growth (weeks) 0	4	8	12	16	20
		Rhizome dry weight (g)					
'Valiant'	100	0.66	0.57	1.05	1.38	2.25	3.69
	60	0.66	0.76	0.81	1.37	1.49	2.29
	50	0.66	0.67	1.01	1.02	1.18	1.82
		p=0.001, sed=0.16, d.f.=45					
'Eleanor'	100	0.27	0.45	0.59	1.14	1.29	1.91
	60	0.27	0.36	0.46	0.80	1.36	2.04
	50	0.27	0.36	0.41	0.45	0.91	1.01
		p=0.001, sed=0.15, d.f.=45					
		Root dry weight (g)					
'Valiant'	100	3.98	4.82	4.45	7.99	19.65	25.26
	60	3.98	5.02	4.54	9.31	12.31	15.90
	50	3.98	4.05	6.33	8.00	8.84	17.51
		not significant					
'Eleanor'	100	1.53	2.90	3.21	6.71	10.57	15.67
	60	1.53	2.50	2.92	4.29	11.96	15.19
	50	1.53	2.51	2.62	2.88	5.06	7.66
		p=0.05, sed=1.43, d.f.=45					
		Shoot dry weight (g)					
'Valiant'	100	0.86	2.64	5.80	22.54	37.65	54.95
	60	0.86	1.79	5.82	17.44	32.70	53.18
	50	0.86	2.02	6.45	15.30	25.97	43.96
		not significant					
'Eleanor'	100	1.09	2.96	10.11	40.00	62.94	88.72
	60	1.09	2.76	6.72	23.29	47.89	61.82
	50	1.09	3.56	6.37	17.61	44.04	56.75
		p=0.001, sed=2.69, d.f.=45					

Figure 3.3: Growth of the cultivars 'Valiant' (**A**) and 'Eleanor' (**B**) after 20 weeks at three different irradiances. Left to right: 50, 60 and 100 %. Scale = 1m

3.4.2 Results

The daylength treatments produced only a few significant differences for the two cultivars, across the range of growth parameters studied (Table 3.5 and 3.6). Increasing daylength caused a significant decrease in the number of lateral rhizomes produced by the cultivar 'Valiant' and also a significant increase in the number of tubers produced and the dry weight of the root system for the cultivar 'Eleanor'. Some other differences were possibly becoming evident by weeks 16 or 20.

Time to flowering in the cultivar 'Valiant' was not markedly affected by daylength. All plants in all of the treatments possessed buds by week 4. For the cultivar 'Eleanor', flowering was delayed by four to eight weeks in the daylength of 12 hours and by eight to twelve weeks in the eight hour daylength. By week 16 there were buds or flowers in all of the treatments. In the eight hours daylength regime, shoots of the cultivar 'Eleanor' were noticeably thicker with smaller leaves and they were approximately half the height of shoots in the other two treatments (Figure 3.4).

It was not possible to produce photographs of the rhizome rootstocks to complement those of the whole plants at the end of the experiments (Figures 3.1, 3.3 and 3.4), due to the congested nature of the roots and rhizome and the damage that they consequently suffered when being removed and cleaned of compost.

3.5 DISCUSSION

As expected, plants of the three cultivars grown at 8°C exhibited the slowest growth rates. It is well established that the metabolism and growth rate of plants are positively correlated with temperature. It has also been considered that the decline in shoot production at low temperatures is a change towards a period of dormancy for *Alstroemeria*, (Healy and Wilkins, 1985; Sims, 1985). Commercially, it is known that the growth of *Alstroemeria* is inhibited if temperatures are allowed to fall too low and many of the new hybrid

Table 3.5: The effect of daylength on the numbers of lateral rhizomes, tubers and shoots produced by the cultivars 'Valiant' and 'Eleanor'

Cultivar	Daylength (hours)	Period of growth (weeks) 0	4	8	12	16	20
		Number of lateral rhizomes					
'Valiant'	8	0.0	0.0	2.0	3.8	4.0	5.30
	12	0.0	0.0	1.0	1.0	2.0	2.50
	16	0.0	0.8	1.0	1.3	1.0	0.60
		p=0.05					
'Eleanor'	8	0.1	0.0	0.0	0.8	0.8	1.3
	12	0.1	0.3	0.5	1.5	1.8	1.5
	16	0.1	0.0	1.3	0.8	0.8	3.5
		not significant					
		Number of tubers					
'Valiant'	8	10.7	15.0	17.0	21.3	23.0	31.0
	12	10.7	14.8	17.5	16.3	20.5	29.3
	16	10.7	17.8	19.3	24.0	28.5	27.0
		not significant					
'Eleanor'	8	5.5	7.0	6.5	10.3	10.0	13.8
	12	5.5	9.3	9.5	10.8	13.5	13.8
	16	5.5	10.5	11.3	20.5	18.8	36.3
		p=0.05					
		Number of shoots					
'Valiant'	8	3.3	4.8	6.0	11.3	13.5	19.3
	12	3.3	3.5	5.0	7.0	9.5	12.3
	16	3.3	5.0	5.5	7.0	9.8	10.5
		not significant					
'Eleanor'	8	3.5	4.3	5.3	8.3	10.5	14.5
	12	3.5	5.5	6.3	9.5	11.3	11.3
	16	3.5	5.0	5.3	6.3	8.0	12.8
		not significant					

Table 3.6: The effect of daylength on the dry weights of lateral rhizomes, roots and shoots produced by the cultivars 'Valiant' and 'Eleanor'

Cultivar	Daylength (hours)	Period of growth (weeks) 0	4	8	12	16	20
		Rhizome dry weight (g)					
'Valiant'	8	0.66	0.63	0.86	0.97	1.39	2.03
	12	0.66	0.56	0.74	0.79	0.91	1.12
	16	0.66	0.67	0.95	0.95	0.93	1.28
		not significant					
'Eleanor'	8	0.27	0.31	0.34	0.58	1.28	1.22
	12	0.27	0.33	0.34	0.41	0.71	0.83
	16	0.27	0.33	0.39	0.63	0.75	1.36
		not significant					
		Root dry weight (g)					
'Valiant'	8	3.98	3.48	4.81	5.01	7.57	9.06
	12	3.98	3.87	4.57	5.13	6.51	8.18
	16	3.98	4.46	4.34	8.58	9.37	10.31
		not significant					
'Eleanor'	8	1.53	1.80	1.96	3.05	5.03	5.94
	12	1.53	2.15	2.64	3.14	4.58	5.47
	16	1.53	2.12	2.77	5.68	9.31	19.86
		p=0.05, sed=1.60, d.f.=45					
		Shoot dry weight (g)					
'Valiant'	8	0.86	1.46	3.98	10.55	17.64	34.52
	12	0.86	1.47	3.97	7.69	17.02	23.77
	16	0.86	1.25	3.18	4.64	14.23	23.44
		not significant					
'Eleanor'	8	1.09	1.99	2.48	10.47	19.67	40.01
	12	1.09	2.55	5.06	11.34	19.48	34.30
	16	1.09	2.96	5.83	10.17	16.23	24.16
		not significant					

Figure 3.4: Growth of the cultivars 'Valiant' (**A**) and 'Eleanor' (**B**) after 20 weeks at three different daylengths. Left to right: 8, 12 and 16 hours. Scale = 1m

cultivars are known to be sensitive to frost (Parigo Horticultural Co., personal communication).

Dormancy can also occur in *Alstroemeria* when temperatures are too high (Dambre, 1987; Powell and Bunt, 1984, 1986), for example between 18 and 20°C in northern temperate zones of the world (Parigo Horticultural Co., personal communication). The data from the temperature experiment indicates that the increase in growth rate slows down as the temperature is raised from 13 to 18°C, especially for the cultivars 'Valiant' and 'Parade'. Similar temperatures, i.e. 17 to 21°C, are also considered to be too high for good flowering of *Nerine flexuosa* (Fortanier *et al.*, 1979), and temperatures above 20°C are known to keep *Iris* bulbs in a vegetative state (Rees, 1972). The growth rate of *Asparagus* spears is also known to decline at temperatures exceeding 30°C (Kim *et al.*, 1989). For two of the three cultivars, production of lateral rhizomes was lower at 18 °C than at 13°C. High temperature has also been demonstrated to produce a decrease in the rate of tillering in ryegrass (*Lolium spp.*) (Mitchell, 1953a, 1953b). As *Alstroemeria* is generally propagated vegetatively (Section 1.2.4), a temperature regime that does not maximize lateral rhizome production will lead to a lower level of new plant production, which is an important commercial consideration. An optimum temperature for the growth of *Alstroemeria* would appear to be between 13 and 18°C.

Many of the results obtained may be linked with survival mechanisms inherent in *Alstroemeria*, which help the plant to overcome periods of either high or low temperature stress. The increase in tuber number in two of the cultivars supports this theory. Dambre (1987) has reported that in *Alstroemeria*, storage organs are produced as temperatures rise, i.e. before the above ground parts die down and the plant becomes dormant, in order to survive hot dry summers.

A possible reason for the rapid decline in plant growth at 18°C may have been the use of constant temperature regimes, consequently the plants were

not subjected to cooler night temperatures as they would be in their native habitats. Therefore, if summer dormancy is controlled by day degrees at or above a critical temperature, this could be achieved much faster under a constant temperature regime. This type of effect has been indicated in the early growth of ginger (*Zingiber officinale*) where best growth at a constant temperature was produced at 27.5°C, whereas fluctuating temperatures produced an optimum of 29°C (Evenson *et al.*, 1978). The perception of depth and the production of contractile roots in *Gladiolus* is also related to temperature fluctuation. Halevy (1986) demonstrated that when grown at constant temperatures, *Gladiolus* corms produced few contractile roots, yet, if the temperature was allowed to fluctuate significant increases in the number of these roots were produced.

Rees (1972) states that for many types of bulbs, the pattern of growth i.e. summer dormancy and a cold requirement for subsequent growth, are related to extremes of environment and a short growing season in the plant's native habitat. With *Alstroemeria*, although mechanisms for surviving periods of stress seem to be present, these are not equivalent to the distinct phases of growth seen in bulbs. This has been shown by the year round pattern of flowering exhibited by some of the new *Alstroemeria* cultivars (Eggington, 1986) and the work on temperature manipulation and constant flowering of *Alstroemeria* cultivars (Blom and Piott, 1990; Keil-Gunderson *et al.*, 1989; Lin, 1984, 1985).

The failure to establish some of the 'splits' was shown to be related to temperature. It would seem that the 'splits' can initially produce shoots from reserves in the rhizome, but if new roots are not produced fast enough, the rhizome is exhausted and the whole plant dies. This effect is accelerated by higher temperature, as it causes the shoots to grow faster, thereby demanding greater resources from the rhizome and causing it to dry out more quickly. It is not clear why the roots and tubers on the 'split', present when planted, fail to support it. In replanted *Alstroemeria*, new roots are formed mainly at the newly formed apex of the rhizome and also from the roots and tubers

that already exist (Stinson, 1952). However, the plants are sensitive to being moved (Robinson, 1963). In some way the normal function of these organs may have been disrupted by the treatment received during lifting from the glasshouse bed. This may have prevented new growth on the pre-existing root system. Frequently, many of the roots and tubers on lifted plant clumps are damaged or broken off. It is also possible that the tubers did not receive the stimuli required for the mobilization and translocation of their reserves, due to the inappropriate environmental conditions at the time of potting the 'splits'.

Overall, the results obtained from this experiment do seem to confirm the 15°C temperature considered as the maximum for efficient plant and flower production by commercial growers of *Alstroemeria.*

Reducing irradiance produced an overall decrease in each of the parameters studied for both the cultivars 'Valiant' and 'Eleanor', however, the degree to which the decrease occurred was cultivar dependent. These effects probably resulted from reductions in the rate of photosynthesis caused by the decreases in irradiance, which would lead consequently to poorer growth. Similar results of reduction in overall plant growth and quality were found with Easter Lily (Miller and Langhans, 1989), *Elymus repens* and *Agrostis gigantea* (Skuterud, 1984) and ryegrass (Mitchell, 1953a, 1953b).

The cultivar 'Eleanor' appeared to be more sensitive to changes in irradiance, and this is supported by the fact that in winter this cultivar produces no flowers, whereas the cultivar 'Valiant' continues to produce flowers throughout this period (Parigo Horticultural Co., personal communication). Light levels are generally low in the winter and may cause flower bud abortion in the cultivar 'Eleanor'.

Higher irradiance would seem to favour *Alstroemeria* production, however, this was not tested in the present experiment. The interaction of temperature, daylength and irradiance can cause buds to dry up and plants to become dormant in the summer (Section 1.3.1.) (Parigo Horticultural Co.,

personal communication), consequently shading is often provided. Puntieri and Gomez (1988) have shown that *Alstroemeria aurantiaca* favours a forest environment rather than an open site for growth. In forests it is often the dominant understorey plant, whereas in low scrubland it co-exists with several species of shrubs and herbs.

The levels of supplementary lighting used in the daylength experiment were not high enough to allow the plants under the 12 and 16 hour daylengths to produce greater amounts of photosynthates than those at the 8 hour daylength. As a consequence, plant production was approximately equal. As daylength is usually considered as a stimulus for change in the developmental stage of a plant, the supplementary lighting was only intended to initiate such changes and not to influence photosynthesis.

The increase in shoot number with decrease in daylength, though not significant, supports the observation of Dambre (1987) who found increased numbers of shoots were produced at a short day of nine hours. However, the present study does not corroborate the other conclusion made by Dambre, that short days suppressed rhizome formation and growth. In ginger (*Zingiber officinale*) increases in daylength from 10 to 16 hours was seen to decrease rhizome swelling and enhance vegetative growth (Adaniya *et al.*, 1989). The increase in tuber number of the cultivar 'Eleanor' and the increase in root dry weight, probably resulting from the increase in the tuber number, may again be part of the plant's natural strategy for overcoming periods of environmental stress.

Increases in daylength are often accompanied by increases in temperature, hence an increase in photoperiod may be a signal or stimulus for storage organ production prior to a period of summer dormancy. This confirms the statement by Dambre (1987) that at the end of the spring flowering period, a period of generally long days and rising temperatures, storage organs are formed and plants form few new shoots and few, if any, flowers. The inhibition of flowering by shorter daylengths further supports the findings of

other workers on this aspect of the control of *Alstroemeria* flowering (Healy *et al.*, 1982; Healy and Wilkins, 1979; Heins and Wilkins, 1979; Lin and Molnar, 1983; Noordegraaf, 1975). Varying the daylength has produced similar effects on plant height of Easter Lily (*Lilium longiflorum* Thunb. var. 'Croft'), but no significant change in the time to flowering was found (Smith and Langhans, 1962). However, increasing daylength has been shown to cause a decrease in the cold treatment required for flowering of *Lilium* cultivars (Weiler and Langhans, 1972; Weiler, 1973). Growth and flowering of *Nerine flexuosa alba* was found to be unaffected by daylength (Fortanier *et al.*, 1979).

The data means and the results of the statistical analyses for the experiments in this chapter should be interpreted with caution because the number of replicates used was low. Means with high standard deviations may then result, especially if one or two extreme pieces of data are recorded. Consequently, differences between the means in the treatments may be exaggerated.

In the temperature experiment there were eight replicates for each cultivar in each treatment, however, due to the death of plants caused by the treatments, there were usually less than this number. In the other two experiments, there were four replicates for each cultivar in each treatment. The low replication could explain why fairly strong trends seem to have been established, which were not significant when analysed.

3.6 CONCLUSION

For maximum rhizome production a temperature of between 13 and 18°C, a high irradiance and a short daylength are required. However, as *Alstroemeria* is a cut flower crop and flowering is inhibited by high irradiance and short daylengths, which do not normally occur together, a compromise position of some shading in the summer and a daylength of at least 12 hours are employed for efficient flower production.

The experiments performed lasted for periods of either 20 or 24 weeks, after which the size of the pot would have restricted the growth of the plants. Many of the trends only began to show after the plants had been growing for at least 16 weeks. Any repeat experiments should therefore use larger pots and longer periods of time to enable the trends to develop further. This could provide a better understanding of the effects of the environmental parameters studied.

Chapter 4: FACTORS AFFECTING THE ESTABLISHMENT OF 'SPLIT' PLANT MATERIAL OF *ALSTROEMERIA*

4.1 INTRODUCTION

High temperatures were found to be detrimental to the establishment of plants from 'splits' (Section 3.2.2). In contrast, when small potted plants (Section 2.3.1) were used in experiments in Chapter 3, none of the plants died. It was further observed that 'splits' often produced new shoots well in advance of new roots and those that did were more likely to die. Also, those cultivars that normally produce fewer tubers, were generally seen to have poorer rates of 'split' establishment.

As a consequence of these observations, three experiments were set up to investigate factors influencing the establishment of 'splits'. The first examined the effect of the range of temperatures used in the earlier experiment (Section 3.2.1) with different durations at these temperatures. The second investigated whether the 'splits' relied on the roots and tubers present at planting for their establishment, or whether they could establish using new roots that they might produce. The last experiment used an *in vitro* system to determine whether the establishment of 'splits' could be enhanced by extracts from the tubers of *Alstroemeria*.

4.2 THE EFFECT OF TEMPERATURE

4.2.1 Materials and Methods

Three controlled environment growth rooms set at constant temperatures of 8, 13 and 18°C were used, each with a daylength cycle of 12/12 hours (day/night) and irradiance of approximately 10 W m^{-2} (60 μE $m^{-2}s^{-1}$) provided by Philips Color 29, 65-80 W, warm white fluorescent tubes. Pot grown plants of the cultivars 'Valiant', 'Butterfly' and 'Eleanor' were used. The plants of the cultivars 'Valiant' and 'Eleanor' were derived from 'splits' that had been potted into three litre pots, approximately five months earlier. The plants of the cultivar 'Butterfly', however, had been raised from seed, germinated at least five months before the experiment began. All of the plants were removed from their pots and then treated in a manner similar to that of plants lifted from ground beds, i.e. split for replanting (Section 1.2.4). Great care was taken to prevent any root or tuber damage. The 'splits' obtained were replanted in three litre pots containing Fisons Levington M2 compost. Sixty pots of each cultivar were transferred to each of the three temperatures. At intervals of one week, five plants from each cultivar were transferred to a glasshouse and grown on for a further twelve weeks. From previous observations, it was concluded that plants which did not establish would be dead within 12 weeks. The glasshouse had no supplementary lighting and was maintained at a mean daily temperature of 15°C. After twelve weeks in the glasshouse the establishment of the plants was assessed, by recording the numbers of dead and established plants, with an experimental design of 3 temperatures x 5 replicates, with 12 durations for each cultivar.

4.2.2 Results

No significant differences in plant establishment were found for any of the cultivars in any of the temperature treatments. The plant deaths that occurred did not exceed five per cent for any cultivar in any treatment (Table 4.1).

Table 4.1: The effect of establishment temperature on plant deaths in the cultivars 'Valiant', 'Eleanor' and 'Butterfly'

Cultivar	Temperature (°C)	Plant deaths (%)
	8	0.0
'Valiant'	13	0.0
	18	0.0
	8	1.7
'Eleanor'	13	1.7
	18	0.0
	8	4.2
'Butterfly'	13	4.2
	18	2.1

No differences significant

4.3 THE EFFECT OF REMOVAL OF THE ROOTSYSTEM

4.3.1 Materials and Methods

'Splits' produced from the division of two year old plant clumps were planted into three litre pots containing Fisons Levington M2 compost. Prior to planting all of the roots and tubers were removed as close to the rhizome as possible. The pots were placed in a glasshouse without supplementary lighting and set at a mean daily temperature of 15°C. Ten pots of each of the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor' were used. A similar set of plants were produced with their root systems intact as a control.

During a 10 week period the cultivars were assessed by recording the pots that initially produced shoots, whether or not they subsequently died and by scoring pots at the end of the 10 weeks, according to whether they contained dead or established plants. This experiment was repeated at a similar period one year later, when more plant material was available. This increased the

total replicate number for each cultivar to 20 plants in each treatment.

4.3.2 Results

Between 60 and 80 per cent of the 'splits' without roots of the cultivars 'Valiant', 'Parade' and 'Butterfly' and approximately 40 per cent of the cultivar 'Eleanor' produced shoots initially. None of the splits without a root system contained any living material by the end of the 10 week period. All of the rhizome segments had dried up and it was apparent that very few new roots had been produced.

When the root system was intact, between 70 and 90 per cent of the splits of the cultivars 'Valiant', 'Parade' and 'Butterfly' and approximately 55 per cent of the cultivar 'Eleanor' produced shoots initially. By the end of the 10 week period between 55 and 75 per cent of the pots of the cultivars 'Valiant', 'Parade' and 'Butterfly' and approximately 25 per cent of the cultivar 'Eleanor' contained viable plant material. The plants that failed to establish had dried up, as happened to the 'splits' without roots.

4.4 THE EFFECT OF TUBER EXTRACTS *IN VITRO*

4.4.1 Materials and Methods

Total tuber extracts were prepared from fresh tubers removed from pot grown plants of the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor' (Appendix D). These extracts were filter sterilized and then added to *Alstroemeria* production medium, without BAP, in the way described for GA_3 (Section 2.4). The extracts were added to the medium to provide a concentration of 10 per cent. Rhizome explants of the cultivars 'Valiant' 'Parade' and 'Eleanor' were transferred onto media containing extract from the same cultivar and onto media containing extract from the tubers of the cultivar 'Butterfly'. The latter was performed to see, if any effect was produced, whether it was a specific interaction between a cultivar and the compounds within its tubers or whether a similar action was occurring within all cultivars and tubers.

Some explants were also cultured on unsupplemented *Alstroemeria* production medium as a control.

Five 'standard explants' were placed in each jar (Section 5.2) with 10 jars for each treatment. The cultures were incubated at 15°C and a 12/12 hour (day/night) daylength cycle, with light provided by Thorn Pluslux 3500, 70 W, fluorescent tubes giving approximately 5 $W\,m^{-2}$ (28 $\mu E\,m^{-2}s^{-1}$) of light. Cultures were assessed (Section 2.5) at intervals of four weeks, over a 12 week period and the data analysed (Section 2.5) with an experimental design of 3 treatments x 20 replicates for each cultivar.

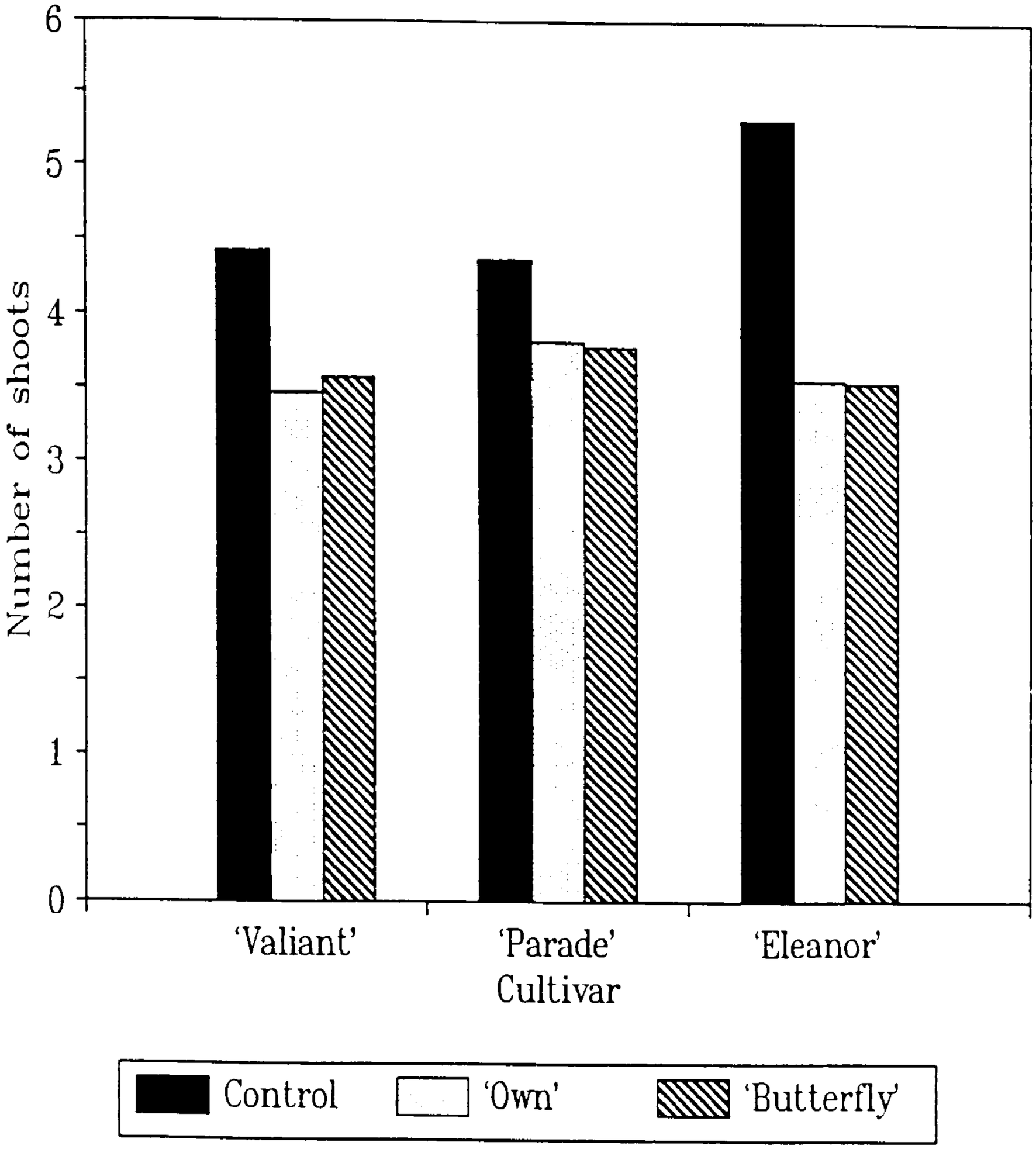
4.4.2 Results

The tuber extracts severely inhibited the growth of the rhizome and roots of all cultivars (Figures 4.1 and 4.2 and Table 4.2). Little difference was seen between the response of the explants on the medium containing extract from the same cultivar or medium containing extract from the cultivar 'Butterfly'.

A marked difference was seen in the length of the shoots produced. Many of the shoots produced by explants growing on media containing extracts were between two and four times longer than those on the control medium, with a few being up to six times as long. This effect of the extracts was not quantified.

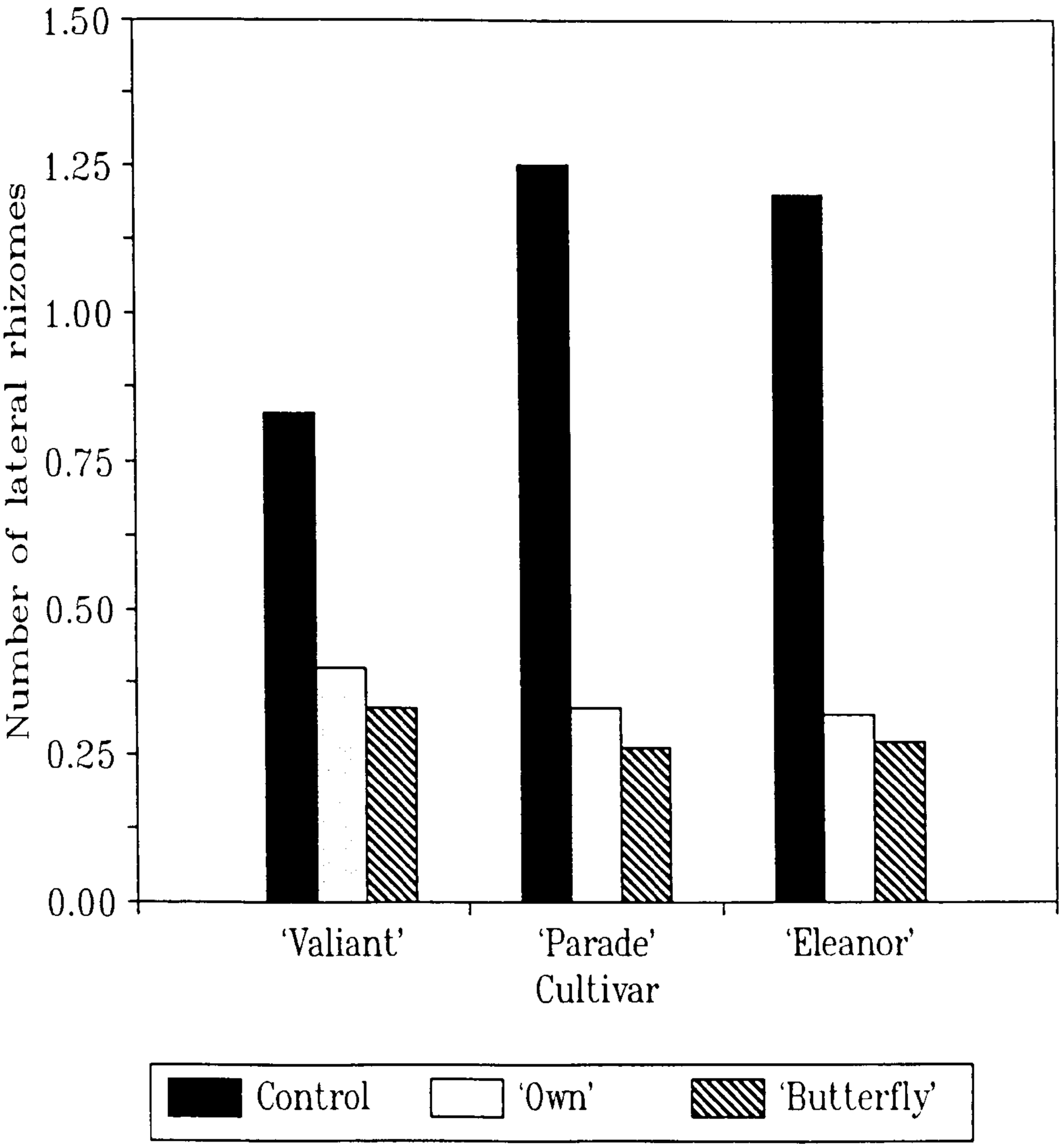
4.5 DISCUSSION

The results from the temperature experiment did not confirm the earlier observations that an increase in temperature caused a significant decrease in 'split' establishment. This second experiment indicated that, within the range studied, temperature had no effect on the establishment of *Alstroemeria* 'splits'. Since the methods and the cultural conditions employed were virtually identical in the two experiments, the discrepancy in the results would strongly suggest that there were differences in the 'splits' used. Two main differences were recognised. The first was the age of the plants from which



'Valiant'	p=0.05
'Parade'	p=0.01
'Eleanor'	p=0.001

Figure 4.1: The effect of extracts from their own tubers and those of the cultivar 'Butterfly', on shoot production by rhizome cultures of the cultivars 'Valiant', 'Parade' and 'Eleanor'



'Valiant'	not significant
'Parade'	p=0.001
'Eleanor'	p=0.01

Figure 4.2: The effect of extracts from their own tubers and those of the cultivar 'Butterfly', on lateral rhizome production by rhizome cultures of the cultivars 'Valiant', 'Parade' and 'Eleanor'

Table 4.2: The effect of extracts from their own tubers and those of the cultivar 'Butterfly', on root production by rhizome cultures of the cultivars 'Valiant', 'Parade' and 'Eleanor'

	Cultivar		
Tuber extract	'Valiant'	'Parade'	'Eleanor'
Control	0.85	0.89	0.62
'Own'	0.64	0.66	0.38
'Butterfly'	0.60	0.54	0.45

No differences significant

the 'splits' had been derived. 'Splits' of the cultivars 'Valiant' and 'Eleanor' were produced from pot grown 'splits', which had been established for approximately five months. Those of the cultivar 'Butterfly' were produced from seed raised plants, approximately five months old. Therefore, none of these plants had been growing in production beds for at least two years or had been cropped for flowers. Consequently, this plant material could be considered as physiologically younger than that used in the earlier experiment and had not experienced the reduction of resources in the rhizome rootstock associated with flower production and cropping. Hence, the 'splits' used in the second experiment were probably more vigorous than those used in the earlier experiment.

The second difference, and probably the more important of the two, was the treatment received by the plants during lifting and removal of the soil prior to preparing 'splits' from the rhizomes. In commercial practice, clumps of *Alstroemeria* are lifted mechanically from the glasshouse soil, with the soil being removed fairly roughly. In the second experiment, the compost was removed carefully from the rhizome and roots of pot grown plants, thus minimising damage to the root system. The reduced incidence of plant deaths in the second experiment may, therefore, have resulted from the 'splits' being

less damaged and, as a consequence, in a better physiological state to sustain the growth of the 'split' and subsequent plant establishment.

The slightly higher incidence of plant deaths for the cultivar 'Butterfly', in comparison to the other two cultivars, may be attributed to the small size of the plants, i.e. they were only about five months old from germination of the seed. Consequently, the 'splits' of the cultivar 'Butterfly' were sometimes smaller than those normally used commercially.

The removal of the roots on splits confirmed the importance of the original root system to the propagule for subsequent establishment. Rootless 'splits' failed to establish. The results also indicated that there were sufficient reserves within the rhizome to sustain initial shoot production, but not sufficient to sustain plant growth or to prevent the reserves of the rhizome from being exhausted before the plant becomes established. The major problem at this stage appears to be that the rate of new root production, at least initially, lags considerably behind that of the production of shoots. Consequently, uptake of water and nutrients into the rhizome does not match the requirements for the growth of the shoots already formed.

Comparisons can be made with observations made in Chapter 3. If the roots on a 'split' are damaged, and hence not functional, then such a propagule will act in a similar way to one that is rootless. It may, therefore, produce shoots and suffer the same problems of exhaustion of reserves followed by death. Failure to establish could, therefore, be related to the number of roots that are damaged and not functioning properly. The different capacities to establish, exhibited by the cultivars studied, may be associated with factors such as the morphology of their root systems and the numbers of roots and tubers they produce. These factors have been shown to vary greatly between cultivars (Figure 2.1).

The reliance on a pre-existing, undamaged root system and the observation that shoot production is generally in advance of root production, may be a function of the shoots being a greater sink for nutrients than the root system.

Such a mechanism could be purely a passive movement of compounds to these greater sinks, or possibly a directed movement to the shoots that is also controlled by the shoots, at least in respect to the younger parts of the rhizome. The latter could be analogous to that of the directed movement of nutrients, thought to be involved in the maintenance of apical dominance in plants (Chapter 7). It is also possible that shoot and root production are events genetically programmed to occur at different times on the rhizome. More detailed studies would be necessary to clarify these points.

The profound changes in explant growth on media supplemented with tuber extracts, may have been mediated by either an active inhibition of growth of the rhizome, preferential transport of resources to the shoots or a specific enhancement of shoot growth. The presence of a growth inhibitor would leave more resources for shoot growth, whereas preferential transport of resources or the action of a specific growth promoter could inhibit rhizome growth by increasing competition for resources in favour of the shoots.

The timing and conditions for tuber production and the observation that intact tubers can be present on dead rhizomes (Chapter 3), together with the present observation that shoot growth was promoted at the expense of rhizome growth in the presence of tuber extract, indicates that the tuber in *Alstroemeria* may be more important than previously thought. It would appear that the tubers contain compounds which encourage rapid growth of shoots, possibly for use after periods of environmental stress. In their natural habitats such stress may have caused the plants to lose their aboveground growth. There may, therefore, be a requirement for specific conditions before the contents of the tubers are mobilized for subsequent utilization elsewhere in the plant.

The distribution of tubers on the root systems varies between cultivars (Figure 2.1). For example, the cultivar 'Eleanor' has very few tubers compared with the other three cultivars. If the rhizome of 'Eleanor' is shortened further than shown, it would possess few or no tubers. However, the experiment on

the effect of temperature on 'split' establishment showed that there was no difference in the levels of establishment between the cultivar 'Eleanor' and the other cultivars. This suggests that the tubers are not a prerequisite for successful establishment of 'splits'.

More detailed studies at a biochemical level are required to determine if the change in growth caused by the tuber extracts was produced by a plant growth regulator, a carbohydrate or some other organic or inorganic compound.

4.6 CONCLUSION

It would appear to be essential to keep damage of the root system of *Alstroemeria* 'splits' to the absolute minimum in order to achieve successful plant establishment. The tubers probably have only a small role in 'split' establishment. However, they may be of importance for overcoming periods of stress and for maximizing initial shoot growth, when conditions more favourable for plant growth return. More work is needed to elucidate the nature of the active component(s) in tuber extracts before any conclusions on the mechanism of the tuber interaction can be made. It should also be remembered that the tuber extract experiment was performed *in vitro* and although parallels may be drawn, the other experiments reported so far have been concerned with plant responses *in vivo*. Consequently, caution must be exercised in drawing conclusions from this *in vitro* experiment.

Chapter 5: PRELIMINARY INVESTIGATIONS *IN VITRO*

5.1 INTRODUCTION

The competition between plants in a crop situation is well documented, with information also available in this area for *Alstroemeria* (Healy and Lang, 1985). However, there are few published reports on the effect of explant density on the competition between explants in culture vessels. This limited evidence suggests that the availability of a nutrient factor is important, as reducing the number of *Hemerocallis* explants in culture vessels significantly increased multiplication rates (Lumsden *et al.*, 1990). Morphogenesis has also been shown to be affected by changes in medium-explant volume ratio and also by the shape of the container (Cousson *et al.*, 1989; Tran Thanh Van, 1981). An experiment was, therefore, designed to determine the optimum number of explants that could be incubated in a specified culture vessel, before growth of explants was significantly decreased. This information, it was hoped, would ensure that rhizome production and overall explant growth in subsequent experiments would be optimised, by preventing any interactive effects between the explants.

A second experiment was designed to determine whether the size difference seen *in vitro* was correlated with the multiplication rates of different cultivars. It had been suggested that the smaller the explant, the lower its propagation rate.

5.2 EFFECT OF EXPLANT DENSITY ON GROWTH *IN VITRO*

5.2.1 Materials and Methods

The cultivar 'Eleanor' was selected for this experiment because it possessed the largest rhizome of the four cultivars. It was considered that any interactions would be greatest between the explants of this cultivar. One, two, three, four, five or six 'standard explants' were cultured on 25 ml of *Alstroemeria* production medium, using ten jars for each of the explant densities. The cultures were incubated at 15°C and a 12/12 hour (day/night) daylength cycle, with an irradiance of approximately 20 $W\,m^{-2}$ (120 $\mu E\,m^{-2}s^{-1}$), provided by Philips Color 29, 65-80 W, warm white fluorescent tubes, for 12 weeks. The cultures were assessed (Section 2.5) at intervals of four weeks, when only the shoot number was recorded for each treatment, to provide an overall measure of the explant size. Any lateral rhizomes or roots produced, would not be large enough after a maximum of only four weeks' growth, to significantly affect the size of the explants. The data were analysed (Section 2.5), giving an experimental design of 6 treatments x 20 replicates.

5.2.2 Results

There was little difference in shoot production between the explant densities of one to five in a culture jar (Table 5.1), although a small decrease in shoot production was apparent with increasing explant number. The explant density of six per jar did, however, result in a significant decrease in shoot production.

5.2.3 Discussion

The interaction between the explants at a density of six per culture jar, supports the results of the studies mentioned earlier (Section 5.1). This inhibition of shoot production may have been due to by-products of the metabolism of the explants and/or to a competitive effect for nutrients within

Table 5.1: The effect of explant density *in vitro* on shoot production in the cultivar 'Eleanor'

Explant density	Number of shoots
1	4.6
2	4.5
3	4.2
4	4.2
5	4.1
6	3.4

p=0.01

the medium.

On the basis of this result five 'standard explants' per culture jar was selected as the explant density for subsequent experiments in this study. Any variation in this number is detailed at the beginning of the appropriate sections.

5.3 COMPARISON OF THE GROWTH *IN VITRO* OF THE FOUR *ALSTROEMERIA* CULTIVARS

5.3.1 Materials and Methods

The cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor' were cultured on *Alstroemeria* production medium using five 'standard explants' per jar. Ten jars of each cultivar were incubated at 15°C and a 12/12 hour (day/night) daylength cycle, with an irradiance of approximately 20 $W\,m^{-2}$ (120 $\mu E\,m^{-2}\,s^{-1}$), provided by Philips Color 29, 65-80 W, warm white fluorescent tubes, for 12 weeks. The cultures were assessed at intervals of four weeks and the data analysed (Section 2.5), giving an experimental design of 4 cultivars x 20 replicates.

5.3.2 Results

There were no significant differences in lateral rhizome and root production between the four cultivars (Table 5.2). However, there were significant differences in shoot production, with the cultivar 'Eleanor' producing the highest numbers and the cultivar 'Butterfly' the lowest numbers. The morphology of the cultivars 'Valiant' and 'Parade' appeared to be more like that of the cultivar 'Eleanor' (Figure 5.1), with the levels of shoot production tending to be closer to that of the cultivar 'Butterfly' (Table 5.2). In addition to the differences between the cultivars, there was also a considerable amount of variability within the cultivars (Figure 5.1).

Table 5.2: Comparison of the growth *in vitro*, of the *Alstroemeria* cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'. Mean numbers of lateral rhizomes, shoots and roots

Cultivar	Lateral branches	Shoots	Roots
'Valiant'	0.5	4.2	0.06
'Parade'	0.6	4.4	0.09
'Butterfly'	0.3	4.0	0.00
'Eleanor'	0.5	4.9	0.00
	not significant	p=0.01	not significant

5.3.3 Discussion

The results from this experiment confirm the information on the four cultivars supplied by Parigo Horticultural Co. Ltd., i.e. the cultivar 'Eleanor' shows the best growth *in vitro* with the cultivar 'Butterfly' showing the poorest growth, the cultivars 'Valiant' and 'Parade' being intermediate (Section 2.2). However, these differences in growth were only expressed in the number of shoots produced and not in root or lateral rhizome production, the latter being of primary importance in the micropropagation of *Alstroemeria* hybrid cultivars.

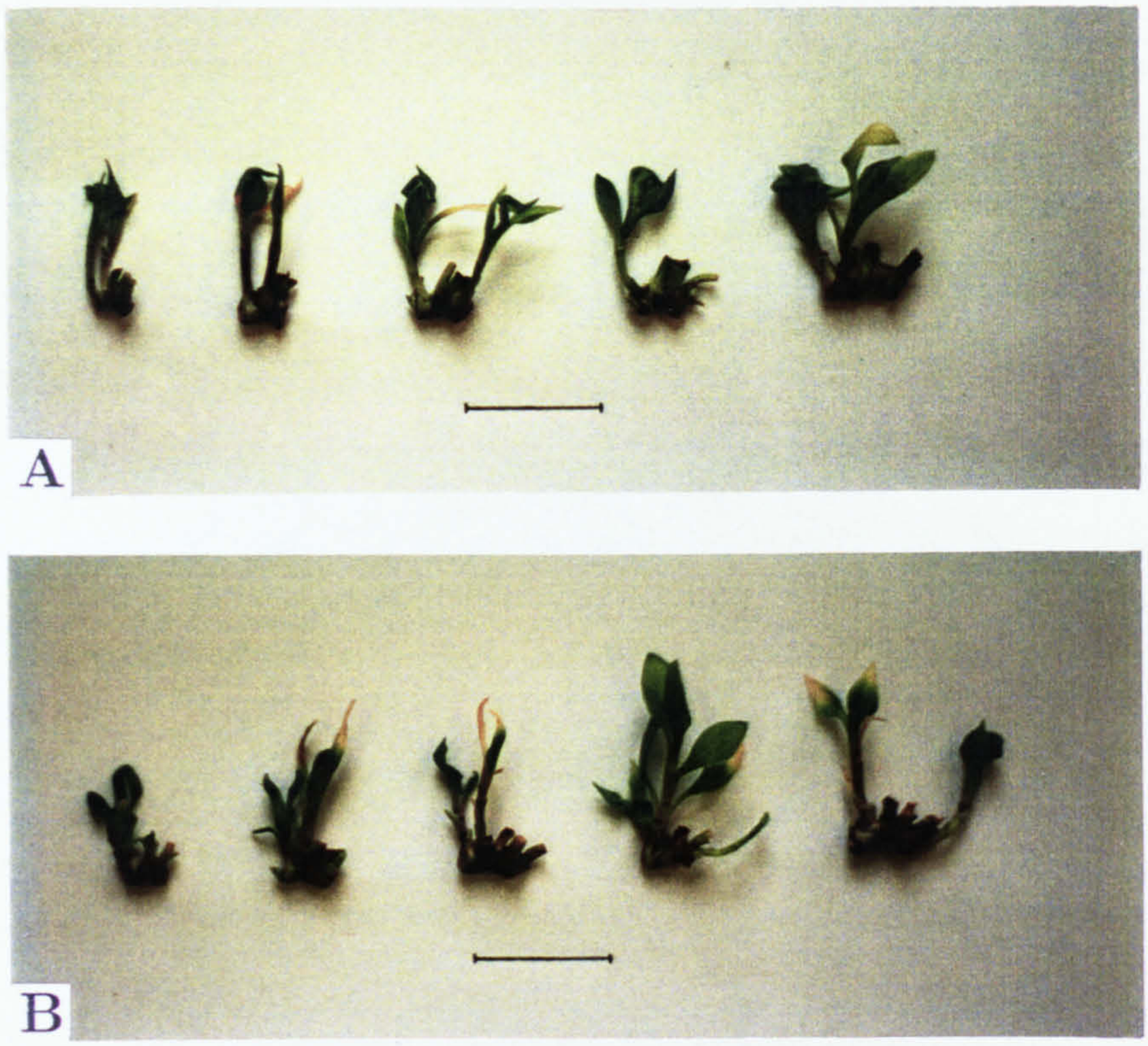


Figure 5.1: Cultures of the cultivars 'Valiant' (**A**), 'Parade' (**B**), 'Butterfly' (**C**) and 'Eleanor' (**D**), after four weeks growth *in vitro*. Bar = 2 cm

The suggestion that variation in multiplication rate existed between the cultivars, may have arisen due to differences in growth rates leading to explants of the more vigorous cultivars, being maintained *in vitro* at sizes larger than that of less vigorous cultivars. This could lead to a higher rate of lateral rhizomes being produced from the former, as more of the second axillary buds (Section 1.2.2) would be present per explant.

Confirmation of differences in the *in vitro* growth of the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor', justifies the selection of these four cultivars for this study. The variability seen within the cultivars may, however, make future experimental assessment within this study somewhat problematic, especially when replicate number is low.

Chapter 6: THE EFFECT OF TEMPERATURE, IRRADIANCE AND DAYLENGTH ON RHIZOME GROWTH *IN VITRO*

6.1 INTRODUCTION

Of the relatively few published reports on the effects of the culture environment on the growth of *Alstroemeria in vitro*, only four of these mention changes in temperature and light, with only Bridgen and Winski (1989) and Pierik *et al.* (1988) considering these physical factors of the culture environment in any detail.

Experiments were designed to assess the effect of temperature, irradiance and daylength on the growth of rhizome explants *in vitro*. In each experiment, the environmental factors were identical with the exception of the factor being studied. The broad ranges of irradiance and daylength were used in order to establish upper and lower limits of these factors for *Alstroemeria in vitro*. Bridgen and Winski (1989) and Pierik *et al.* (1988) reported that the upper temperature limit for *Alstroemeria* was approximately 20°C with an optimum of 15°C. Therefore, the range of temperatures chosen for the present study was below that limit in order to provide more information on the lower temperature tolerance of *Alstroemeria in vitro*.

6.2 THE EFFECT OF TEMPERATURE

6.2.1 Materials and Methods

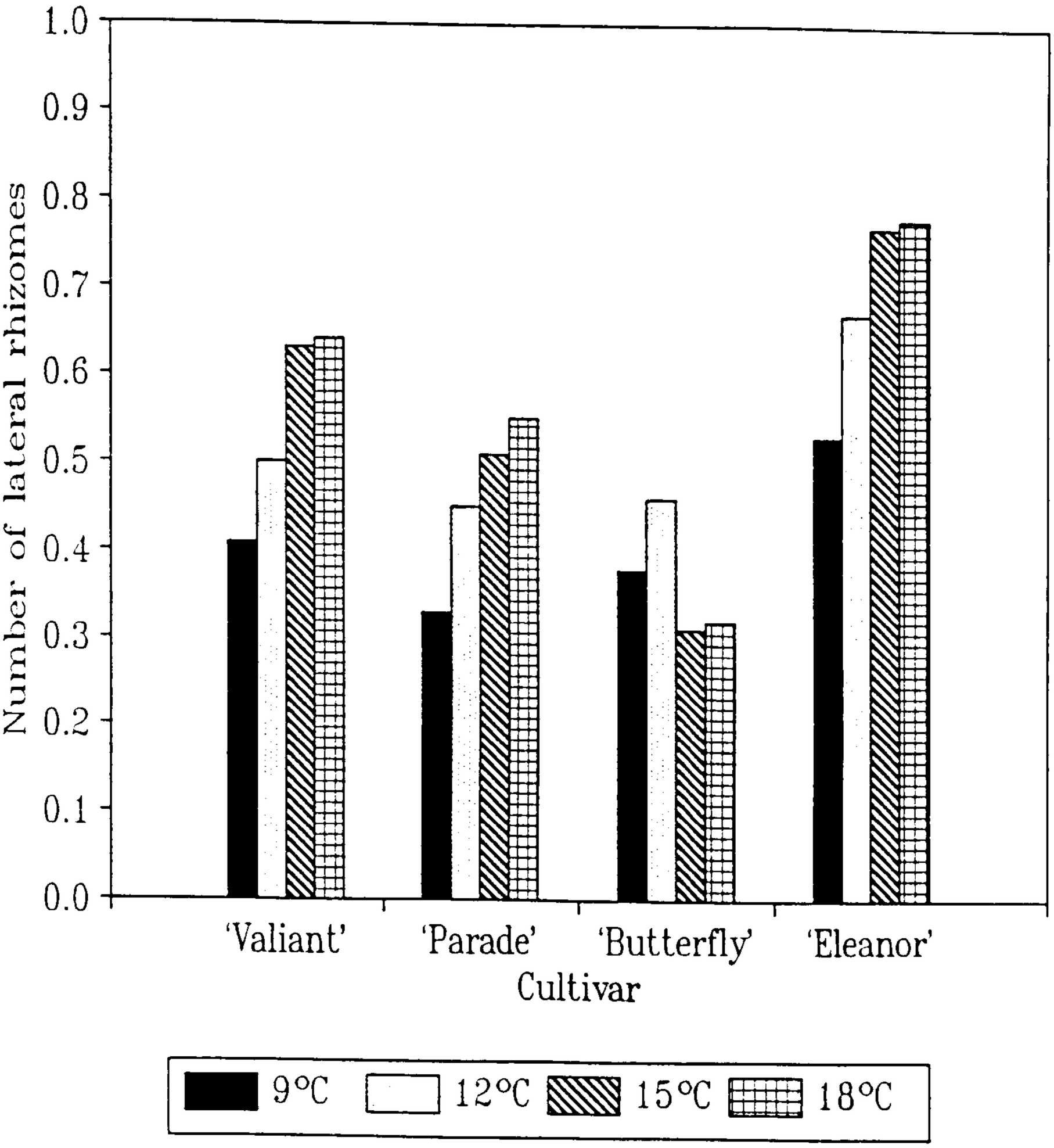
Five 'standard explants' of the cultivars 'Valiant', 'Parade' and 'Eleanor' and three of the cultivar 'Butterfly' were placed into culture jars containing *Alstroemeria* production medium. Ten jars of each cultivar were incubated at 9, 12, 15 and 18°C, with a 12/12 hour (day/night) daylength cycle and an irradiance of approximately 6 $W\,m^{-2}$ (26 $\mu E\,m^{-2}s^{-1}$), provided by Philips Color 32, 15 W, Delux warm white fluorescent tubes. The cultures were assessed (Section 2.5) at intervals of four weeks, over a 12 week period and visual observations of the quality of the explants were also recorded. The experiment was subsequently repeated and the data analysed (Section 2.5) with an experimental design of 3 temperatures x 40 replicates for each cultivar.

6.2.2 Results

Temperature affected significantly the growth of the rhizome cultures. The greatest effect was on the number of lateral rhizomes (Figure 6.1). The cultivars 'Valiant', 'Parade' and 'Eleanor' all exhibited an increase in the number of lateral rhizomes with increase in temperature. However, the increase produced between 15 and 18°C was only small. In contrast, the cultivar 'Butterfly' showed a decline in lateral rhizome production between 12 and 15°C and little difference between 15 and 18°C.

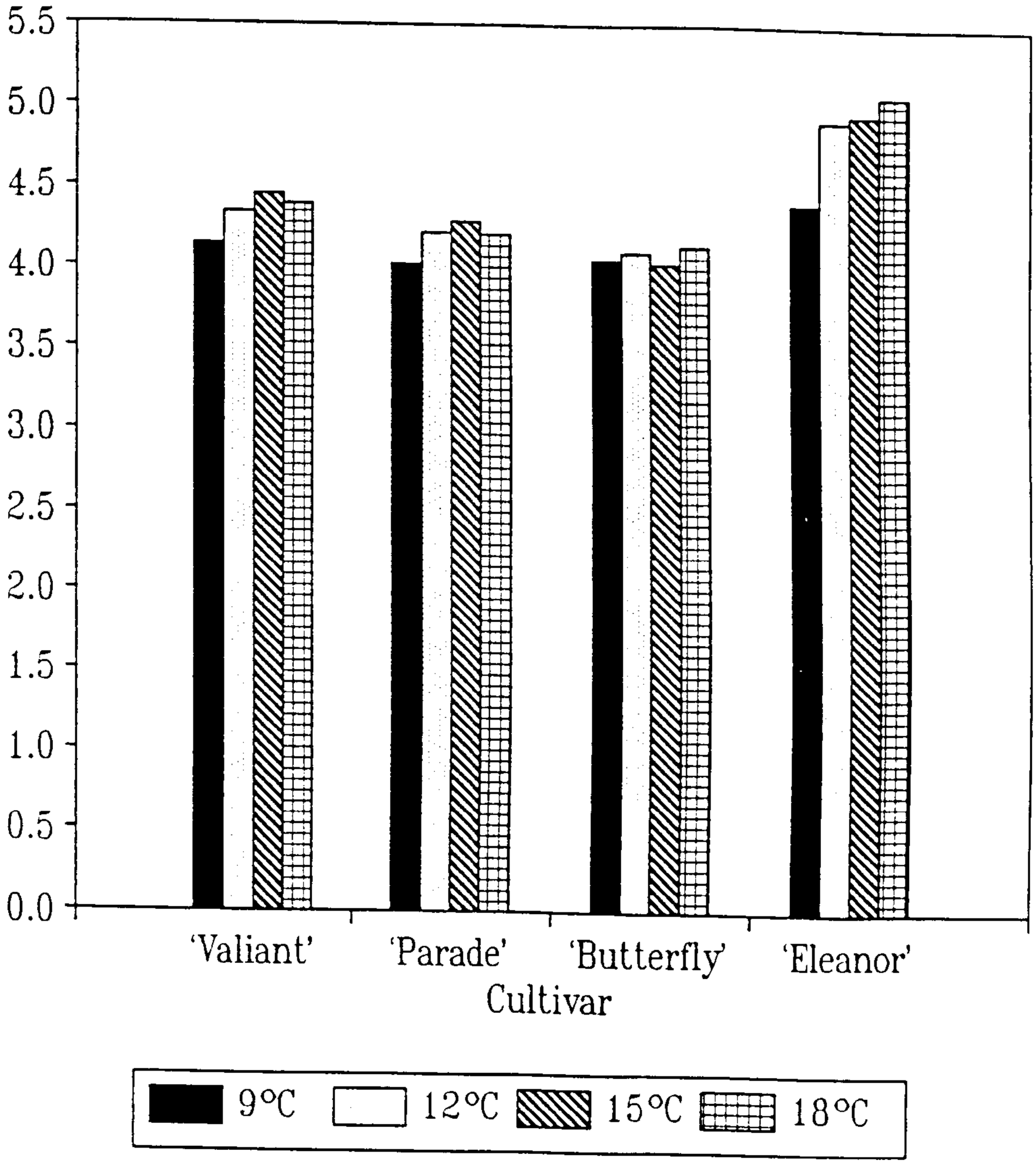
All of the cultivars showed a trend of increasing shoot number with increase in temperature. However, only the increases for the cultivars 'Valiant' and 'Eleanor' were significant (Figure 6.2). A slight decline in shoot number in the cultivars 'Valiant' and 'Parade' was observed between 15 and 18°C. Shoot number for the cultivar 'Butterfly' remained fairly constant above 12°C. 'Eleanor' was the only cultivar which exhibited a continuous increase in shoot number with increase in temperature.

Very few roots were produced by the four cultivars and any differences were not significant (Table 6.1). A slight increase in shoot height was observed



'Valiant'	p=0.001
'Parade'	p=0.01
'Butterfly'	not significant
'Eleanor'	p=0.001

Figure 6.1: The effect of temperature on the number of lateral rhizomes produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'



'Valiant'	p=0.05
'Parade'	not significant
'Butterfly'	not significant
'Eleanor'	p=0.001

Figure 6.2: The effect of temperature on the number of shoots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Table 6.1: The effect of temperature on the number of roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Temperature (°C)	Cultivar 'Valiant'	'Parade'	'Butterfly'	'Eleanor'
9	0.15	0.06	0.00	0.04
12	0.15	0.06	0.00	0.04
15	0.05	0.06	0.00	0.00
18	0.07	0.05	0.00	0.00

No differences significant

with increasing temperature. This increase was cultivar dependent, with the more vigorous cultivars *in vitro* showing the greatest increases in shoot length.

6.3 THE EFFECT OF IRRADIANCE

6.3.1 Materials and Methods

Five 'standard explants' of each of the four cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor' were placed into culture jars containing *Alstroemeria* production medium. Ten jars of each cultivar were incubated at three irradiances of approximately 20, 10 and 5 $W\,m^{-2}$ (120, 60 and 30 $\mu E\,m^{-2}s^{-1}$, respectively), provided by Philips Color 29, 65-80 W, warm white fluorescent tubes with a 12/12 hour (day/night) daylength cycle. The temperature was maintained at 15°C (Section 6.5).

The two lower irradiances were achieved by placing the culture jars under neutral density filters which reduced light transmission by 50 and 75 per cent. This filter type is designed to reduce the levels of all the wavelengths of light equally (Rosco Cinegel, Colour Correction Filters, 3402, Roscosun N3). To produce the light reduction of 50 per cent, one layer of the filter was used

and for the reduction of 75 per cent, two layers of the filter were used. Light readings were taken under one and two layers of the filter.

The cultures were assessed (Section 2.5) at intervals of four weeks, over a period of 12 weeks and visual observations of the quality of the explants were also recorded. The experiment was subsequently repeated with new explant material and the data analysed (Section 2.5), with an experimental design of 3 irradiances x 40 replicates for each cultivar.

6.3.2 Results

The range of irradiances used had no significant effect on the growth of the rhizome cultures (Table 6.2). For the cultivars 'Valiant', 'Parade' and 'Butterfly', there was a slight trend of decreasing numbers of lateral rhizomes with decrease in irradiance. A slight increase in shoot length was also evident as irradiance decreased. Again, this was cultivar dependent, with the more vigorous cultivars *in vitro* exhibiting the greatest differences.

6.4 THE EFFECT OF DAYLENGTH

6.4.1 Materials and Methods

Five 'standard explants' of each of the three cultivars 'Valiant', 'Parade' and 'Eleanor' and three 'standard explants' of the cultivar 'Butterfly' were placed into culture jars containing *Alstroemeria* production medium. Ten jars of each cultivar were incubated in each of four daylengths, i.e. 8, 12, 16 and 20 hours of light per 24 hour period. The temperature was maintained at 15°C (Section 6.5) and an irradiance of approximately 6 $W\,m^{-2}$ (26 $\mu E\,m^{-2}$-s^{-1}) was provided by Philips Color 32, Delux warm white fluorescent tubes. The cultures were assessed (Section 2.5) at intervals of four weeks, over a 12 week period and visual observations on the quality of the explants were also recorded. The experiment was subsequently repeated with new explant material and the data analysed (Section 2.5), with an experimental design of 3 daylengths x 40 replicates for each cultivar.

Table 6.2: The effect of irradiance on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	Irradiance (%)	Lateral rhizomes	Shoots	Roots
'Valiant'	100	0.61	4.37	0.07
	50	0.55	4.42	0.06
	25	0.53	4.35	0.07
'Parade'	100	0.39	4.26	0.03
	50	0.30	4.18	0.03
	25	0.31	4.20	0.02
'Butterfly'	100	0.34	4.07	0.00
	50	0.31	4.08	0.00
	25	0.31	4.06	0.00
'Eleanor'	100	0.64	5.20	0.00
	50	0.67	5.31	0.00
	25	0.63	5.24	0.00

No differences significant

6.4.2 Results

Daylength produced no significant changes in growth of the rhizome cultures. A slight trend of increasing lateral rhizome number with increasing daylength, occurred in the cultivars 'Valiant', 'Butterfly' and 'Eleanor', whereas the cultivar 'Parade' showed no response at all (Table 6.3). The number of shoots and roots produced were unaffected by the range of daylengths used. An exception to this was the number of roots produced by the cultivar 'Valiant', which decreased as daylength increased.

A decrease in shoot length was observed with increase in daylength, however, as with the effects of irradiance (Section 6.4.2) the decrease was slight and cultivar dependent.

6.5 DISCUSSION

All plants have their own temperature tolerances *in vivo* and *in vitro*. For *Alstroemeria in vitro* it would appear that there is a tendency for rhizome production and multiplication rate to reach a level between 12 and 15°C, which can not be significantly improved upon by further increases in temperature. This may be a consequence of maximal uptake of nutrients from the medium and/or the optimal metabolism of the explant at these higher temperatures.

These results agree with the findings of Pierik *et al.* (1988) and Bridgen and Winski (1989). Pierik *et al.* (1988) noted that no significant differences were found between 15 and 21°C, and Bridgen and Winski (1989) established that 15°C was the optimum for the growth of *Alstroemeria in vitro*, after comparing multiplication rates at 10, 15 and 20°C. Based on the result from the present experiment, the experiments assessing the effects of irradiance and daylength were conducted at 15°C.

Irradiance and daylength both influence the total light energy received by plant cultures. In the present study these two factors had no significant ef-

Table 6.3: The effect of daylength on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	Daylength (hours)	Lateral rhizomes	Shoots	Roots
'Valiant'	8	0.58	4.39	0.22
	12	0.61	4.37	0.18
	16	0.61	4.39	0.11
	20	0.66	4.33	0.13
'Parade'	8	0.42	4.20	0.03
	12	0.39	4.26	0.03
	16	0.39	4.18	0.05
	20	0.44	4.19	0.06
'Butterfly'	8	0.29	4.02	0.00
	12	0.34	4.07	0.00
	16	0.44	4.06	0.00
	20	0.37	4.10	0.00
'Eleanor'	8	0.59	5.20	0.00
	12	0.64	5.20	0.03
	16	0.71	5.21	0.06
	20	0.73	5.23	0.02

No differences significant

fects on any of the growth parameters studied. At irradiances of 2.5 $W\,m^{-2}$ or less, rhizome multiplication was unaffected, however, the shoots began to etiolate at these light levels (Parigo Horticultural Co., personal communication). Pierik *et al.* (1988) also found that varying the irradiance from 1.5 to 9.7 $W\,m^{-2}$ and using daylengths of 8 and 16 hours, for the culturing of *Alstroemeria*, had no effect on rhizome multiplication. However, Bridgen and Winski (1989) in an abstract quote an optimum irradiance of 120 $\mu E\,m^{-2}\,s^{-1}$ for the rapid propagation of *Alstroemeria in vitro*. Unfortunately, no experimental details were provided.

The lack of response to the light treatments tested could have been due to an absence of photosynthetic ability within the explants. A lack of autotrophic growth may be attributed to low chlorophyll content and low photosynthetic activity of the tissues, thought to be caused by deficiencies in the culture conditions for good development of chloroplasts and active photosynthesis. Chloroplasts can develop and maintain some photosynthetic activity in cultured tissues, yet although light plays an important role in their growth, most chlorophyllous tissues can not grow without an exogenous supply of sugar (Yamada *et al.*, 1978). Studies on cauliflower (*Brassica oleracea* var. *botrytis*) (Grout and Aston, 1978) and further investigations on strawberry (*Fragaria* x *ananassa*) (Grout and Millam, 1985) cultured *in vitro*, have demonstrated that the explants showed no net carbon dioxide uptake and that they also possessed very low levels of photosynthesis. Their growth was seen to be dependent upon exogenous supplies of sugar added to the medium. Furthermore, it was noted that the leaves which developed *in vitro*, failed to develop significant photosynthetic ability and degenerated when transferred to an *in vivo* environment. However, the leaves which developed subsequent to transplanting *in vivo*, gained full photosynthetic competence. Grout and Millam (1985), following from the work of Wardle *et al.* (1979, 1981, 1983a, 1983b), also proposed that the major function of the leaves produced *in vitro* is as a transitory nutrient source, upon which the newly transplanted plantlet is dependent for its carbon and mineral sources, until it has produced new

leaves *in vivo*.

6.6 CONCLUSION

The present results indicate that 15°C is the lowest temperature at which rhizome explants of *Alstroemeria* should be cultured *in vitro*. At temperatures lower than this, significant decreases in explant growth and in particular the rhizome multiplication rate, will begin to occur. With respect to irradiance, it is suggested that in order to prevent etiolation of the explants, the light levels should be kept at an irradiance of at least 5 $W\,m^{-2}$. For daylength, 8 hours of light in a 24 hour day/night cycle seems to be sufficient for good growth of the explants. Shorter daylengths may be feasible but as yet have not been assessed. However, if a shorter period of light is used, then a higher irradiance may be required, if it is the total light integral and not the length of the light period that is most important for the growth and development of the explants.

Chapter 7: THE INFLUENCE OF APICAL DOMINANCE ON THE MULTIPLICATION RATE OF THE RHIZOME *IN VITRO*

7.1 INTRODUCTION

The shoot apex has a very important role in the phenomenon of apical dominance (Section 1.4.1). It has been suggested that chemical signals from the apex of a plant produce interactions at other locations within the plant, by which the suppression of lateral bud growth and the dominance of the apex are established. It has also been shown that there are several different compounds and combinations of compounds which interfere with the mechanisms of apical dominance, thereby releasing axillary buds from their inhibited state of growth. This chapter describes experiments set up to determine to what extent apical dominance is preventing the outgrowth of axillary rhizomes and thereby keeping down the multiplication rate of rhizomes of *Alstroemeria in vitro*.

Removal of the shoot apex may affect the outgrowth of lateral rhizomes (Richards *et al.*, 1988; Pierik, 1987). Subdivision of the rhizomes into single expanded basal internodal segments should remove any influence of adjacent shoots, as has been demonstrated for *Agropyron repens* (Chancellor, 1974), where the smaller the fragment of rhizome used, the greater the number of axillary buds released from inhibition. In these ways it may be possible to remove the sites which produce the signal for maintaining the dominance of the apex. An experiment was set up to examine the effect of the physical manipulation of rhizome explants on apical dominance.

It is well documented that certain PGRs play a role in the control of apical dominance (Section 1.4.2). The following PGRs were tested.

i TIBA was selected for its action as an anti-auxin. The naturally produced auxin IAA, is considered to be important in the establishment and maintenance of apical dominance.

ii Thidiazuron was selected for its very active cytokinin-like mode of action. It is more active at lower concentrations than BAP and the latter is known to decrease apical dominance. Thidiazuron may therefore exhibit a greater effect on apical dominance.

iii NAA/BAP combinations were selected because of the known synergistic effect of auxins on some of the actions of cytokinins, the latter decreasing apical dominance, with auxin concentrations lower than that of the cytokinin.

iv GA_3 was selected for its capacity to increase the elongation of shoots. If the shoots and rhizome of *Alstroemeria* could be made to elongate, then the increased spatial separation of the axillary buds from the apex may reduce their inhibition by the apex.

v Paclobutrazol was selected for its anti-gibberellin effects. By causing a reduction in shoot growth, it may be possible to achieve a relocation of any unused resources into the growth of the axillary buds.

BAP was also added to the media containing TIBA, GA_3 and paclobutrazol, because it was considered that if any of these PGRs produced a positive response, then the addition of BAP may produce an additive or even synergistic effect.

7.2 MECHANICAL CONTROL OF APICAL DOMINANCE

7.2.1 Materials and Methods

Rhizome cultures of the cultivars 'Valiant', 'Parade' and 'Eleanor' were used to provide the following six types of explant for subculturing.

- **S1** Three enlarged basal internodes + rhizome apex + shoots.
- **S2** Three enlarged basal internodes + rhizome apex – shoots.
- **S3** Three enlarged basal internodes – rhizome apex + shoots.
- **S4** Three enlarged basal internodes – rhizome apex – shoots.
- **S5** Three single enlarged basal internodes + shoots.
- **S6** Three single enlarged basal internodes – shoots.

Five rhizome explants of a single type were placed in each culture jar containing *Alstroemeria* production medium. Five jars of each of the six explant types for each cultivar were incubated at a temperature of 15°C and a 12/12 hour (day/night) daylength cycle, with an irradiance of approximately 20 $W\,m^{-2}$ (120 $\mu E\,m^{-2}s^{-1}$), provided by Philips Color 29, 65-80 W, warm white fluorescent tubes.

After four weeks, the number of lateral rhizomes that had been produced was recorded. For the analysis of the data, the explant type S1 was considered as the control treatment, as it had not been physically manipulated in any way. The data were converted to a percentage of the total potential number of lateral rhizomes which could have been produced. The experiment was subsequently repeated and the data analysed (Section 2.5), with an experimental design of 6 treatments x 10 replicates for each cultivar.

The rhizome apices removed in some of the treatments were recultured on *Alstroemeria* production medium and returned to the stock cultures. The

cultivar 'Butterfly' was excluded from this experiment, because the small size of its rhizome explants made it impractical to carry out the physical manipulations necessary for the preparation of the range of explant types. It would have been impossible to ensure that no damage had occurred to the second axillary bud, when dividing the enlarged basal internodes or when removing the rhizome apex.

7.2.2 Results

The three cultivars exhibited very similar trends with respect to the six explant treatments used. Treatments S2 and S3 resulted in low lateral rhizome production, as in the control. By contrast, treatments S4, S5 and S6 produced significantly more lateral rhizomes (Figure 7.1, 7.2 and 7.3).

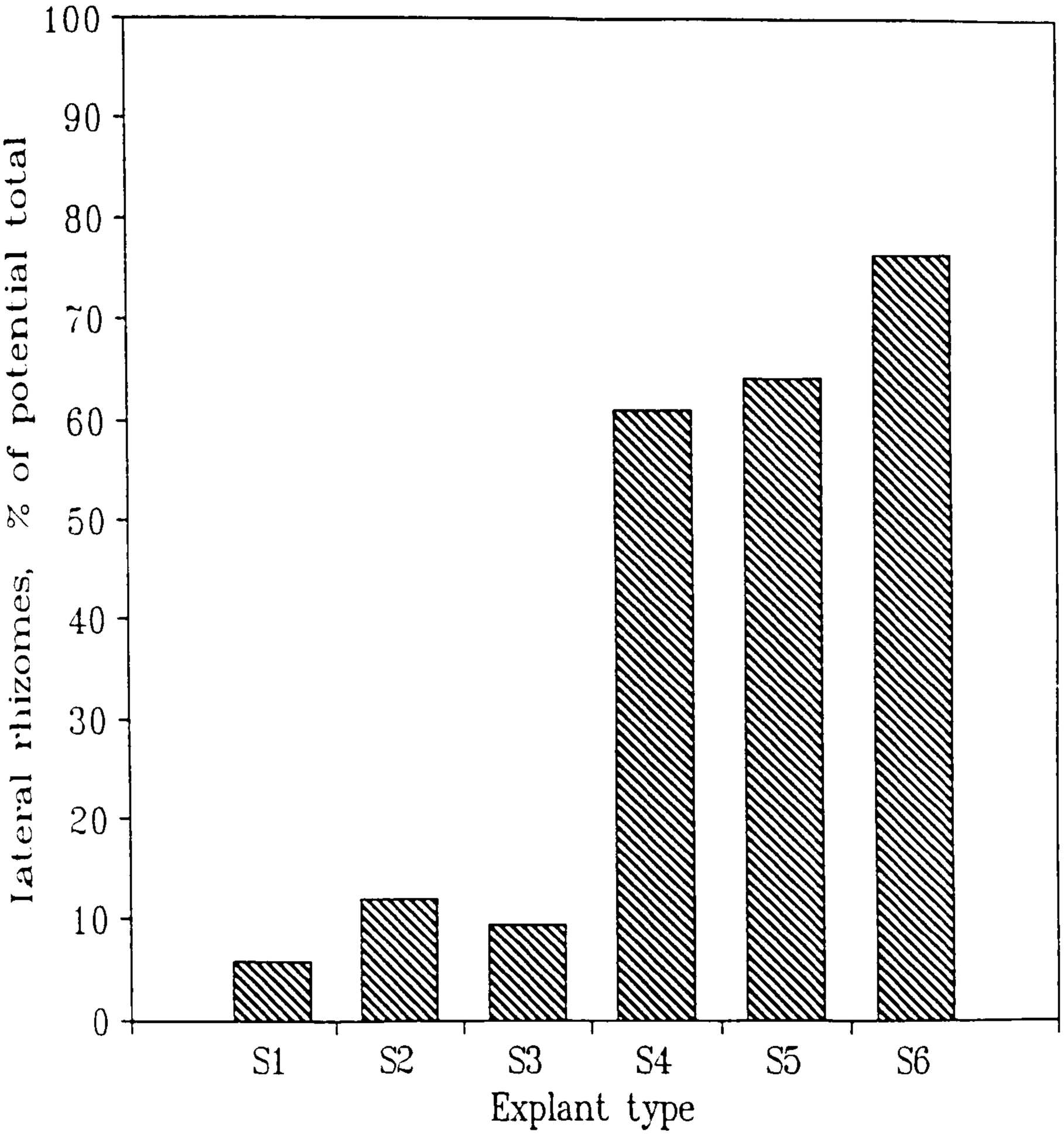
Removal of only the shoot or rhizome apices did not produce a significant difference in lateral rhizome production. However, removal of both types of apex produced an effect greater than the additive effect of the removal of individual apices. Subdivision of the rhizome further increased these effects, with single enlarged basal internodes that had had their shoots removed, producing the highest significant differences. The level to which these different manipulations affected lateral rhizome production was cultivar dependent.

7.3 CHEMICAL CONTROL OF APICAL DOMINANCE

7.3.1 Materials and Methods

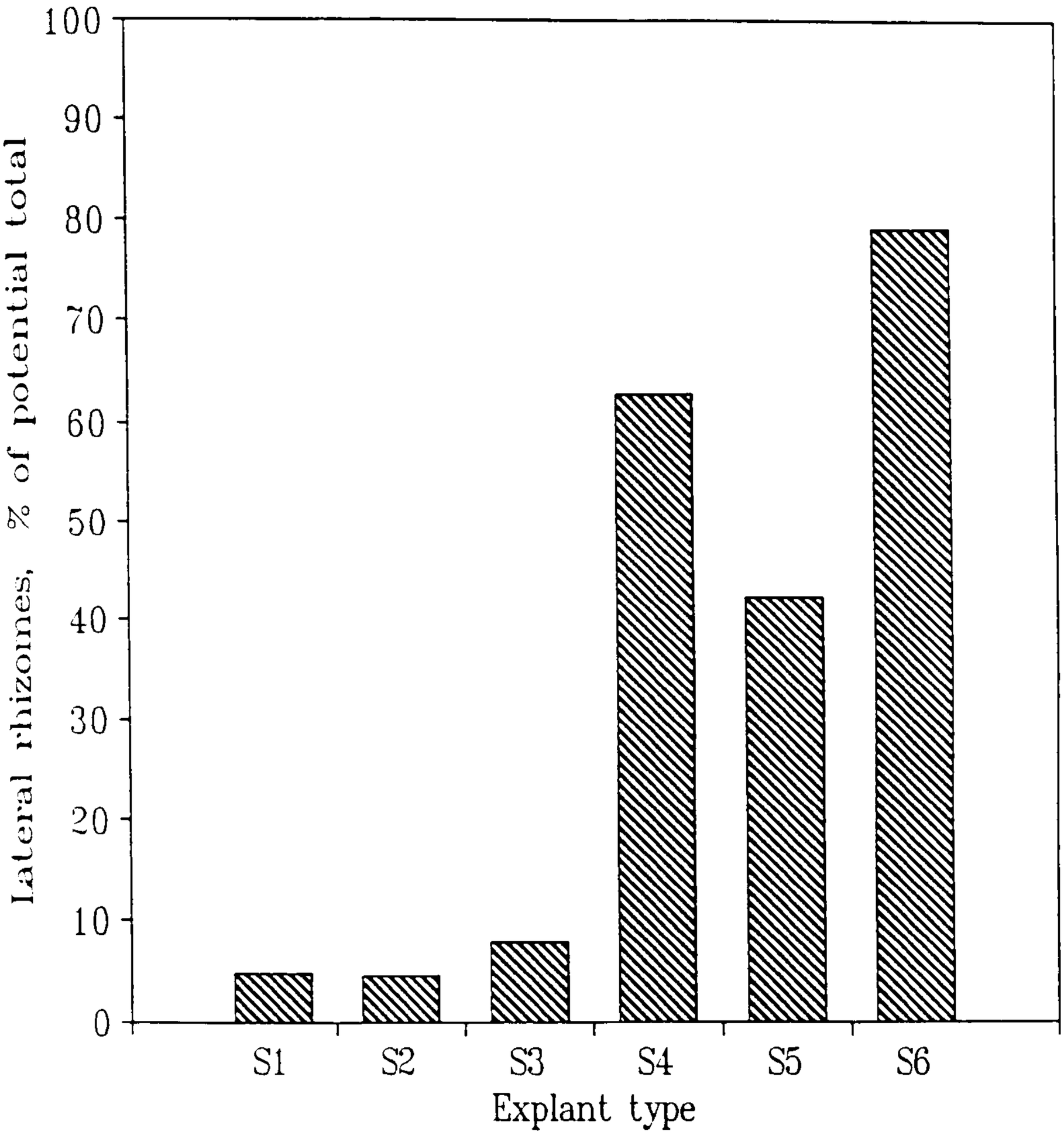
Five 'standard explants' of each of the cultivars 'Valiant', 'Parade' and 'Eleanor' and three for the cultivar 'Butterfly' were placed into culture jars containing *Alstroemeria* propagation medium, supplemented with the following combinations of PGRs.

i 0, 0.01, 0.10, 1.00 or 10.00 mg l^{-1} TIBA + 0 or 4 mg l^{-1} BAP.



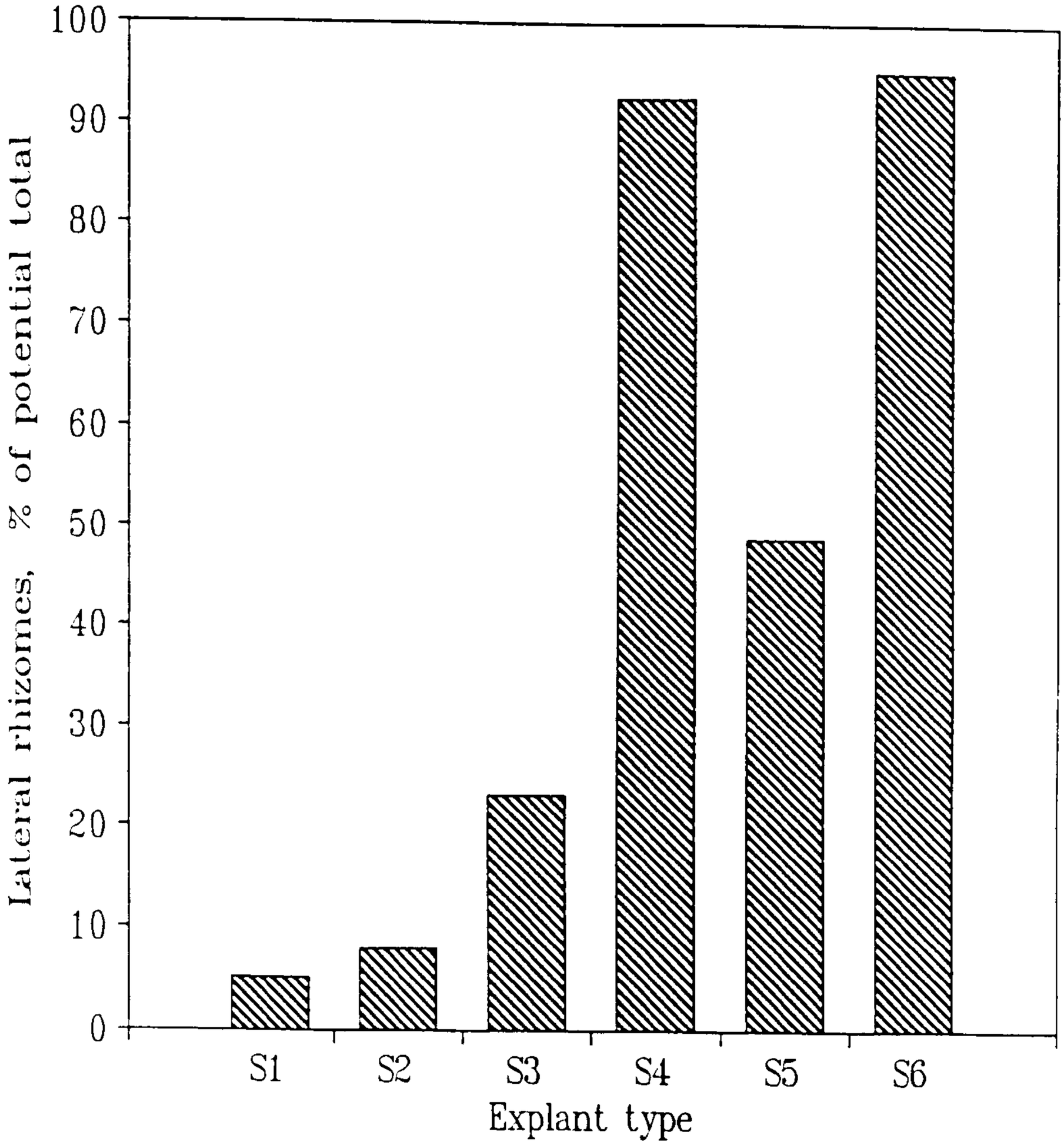
S1 3 enlarged basal internodes + rhizome apex + shoots (control)
S2 3 enlarged basal internodes + rhizome apex – shoots (not significant)
S3 3 enlarged basal internodes – rhizome apex + shoots (not significant)
S4 3 enlarged basal internodes – rhizome apex – shoots (p=0.01)
S5 3 single enlarged basal internodes + shoots (p=0.001)
S6 3 single enlarged basal internodes – shoots (p=0.001)

Figure 7.1: The effect of six explant types on the multiplication rate of the cultivar 'Valiant'



S1 3 enlarged basal internodes + rhizome apex + shoots (control)
S2 3 enlarged basal internodes + rhizome apex – shoots (not significant)
S3 3 enlarged basal internodes – rhizome apex + shoots (not significant)
S4 3 enlarged basal internodes – rhizome apex – shoots (p=0.001)
S5 3 single enlarged basal internodes + shoots (p=0.01)
S6 3 single enlarged basal internodes – shoots (p=0.001)

Figure 7.2: The effect of six explant types on the multiplication rate of the cultivar 'Parade'



S1 3 enlarged basal internodes + rhizome apex + shoots (control)
S2 3 enlarged basal internodes + rhizome apex – shoots (not significant)
S3 3 enlarged basal internodes – rhizome apex + shoots (not significant)
S4 3 enlarged basal internodes – rhizome apex – shoots (p=0.001)
S5 3 single enlarged basal internodes + shoots (p=0.05)
S6 3 single enlarged basal internodes – shoots (p=0.001)

Figure 7.3: The effect of six explant types on the multiplication rate of the cultivar 'Eleanor'

ii 0, 0.011, 0.022, 0.110, 0.220 or 0.440 mg l^{-1} thidiazuron and 0 mg l^{-1} thidiazuron + 4 mg l^{-1} BAP.

iii 0, 0.04, 0.10, 0.20, 0.40 or 1.00 mg l^{-1} NAA + 4 mg l^{-1} BAP.

iv 0, 0.10, 1.00, 10.00 or 100.00 mg l^{-1} GA_3 + 0 or 4 mg l^{-1} BAP.

v 0, 0.15, 0.30, 1.50 or 3.00 mg l^{-1} paclobutrazol + 0 or 4 mg l^{-1} BAP.

Three jars of each PGR combination were used for each cultivar. They were incubated at 15°C and a 12/12 hour (day/night) daylength cycle with an irradiance of approximately 20 W m^{-2} (120 μE $m^{-2}s^{-1}$), provided by Philips Color 29, 65-85 W, warm white fluorescent tubes. The cultures were assessed (Section 2.5) and visual observations of the quality of the explants were also recorded at intervals of four weeks, over a 12 week period. The data was analysed (Section 2.5), with an experimental design, depending on the PGRs, of either 6, 7 or 10 treatments x 6 or 12 replicates for each cultivar. Although these experiments were essentially looking for changes in the number of lateral rhizomes produced, the standard methods of assessment were continued because it was considered that the PGRs could manifest additional changes in the explants.

7.3.2 Results

7.3.2.1 General

None of the PGRs produced any significant differences in the number of lateral rhizomes, shoots or roots produced. In all instances, growth and explant quality were consistently better when BAP was present in the media. Root production was severely or completely inhibited by the presence of BAP. The degree to which these effects were exhibited was cultivar dependent.

7.3.2.2 TIBA

The presence of TIBA in the *Alstroemeria* production medium, with or without BAP, resulted in only a slight increase in the production of lateral rhi-

zomes and shoots. This response was, however, inconsistent. It did not appear to be dependent on the presence of BAP and there was much variation between the cultivars. The only consistent trend observed was that, regardless of how many roots were produced in the control treatment, there was always complete inhibition of root production at the highest concentration of TIBA. Root production was inhibited in virtually all treatments containing both TIBA and BAP (Table 7.1).

Rhizome and shoot quality appeared to deteriorate with increasing concentrations of TIBA. They became thinner and weaker in growth and some vitrification occurred at the highest concentrations. This was especially noticeable in the weaker growing cultivars 'Valiant' and 'Butterfly'. Due to the lack of response to the treatments and the deterioration in the size and quality of the explants, this experiment was not repeated.

7.3.2.3 Thidiazuron

The presence of thidiazuron in *Alstroemeria* propagation medium without BAP did not produce any significant changes in the number of lateral rhizomes and shoots. However, as with TIBA, the number of roots produced always declined with increasing concentrations of thidiazuron. At the highest concentrations of thidiazuron, root production was frequently completely inhibited. Thidiazuron appeared to inhibit root production less than BAP but, conversely, it stimulated axillary bud growth at the higher concentrations used, and for the cultivar 'Eleanor' at all concentrations tested, to a greater extent than BAP. Fewer shoots were generally produced in the presence of thidiazuron but these differences were not statistically significant (Table 7.2).

The incidence of vitrification increased with increasing concentrations of thidiazuron. The lowest concentration inducing vitrification appeared to be cultivar dependent. All of the cultivars exhibited vitrification in the presence of 0.1 $mg\,l^{-1}$ thidiazuron and all were severely vitrified at the highest

Table 7.1: The effect of TIBA and BAP on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	BAP ($mg\,l^{-1}$)	TIBA ($mg\,l^{-1}$)	Lateral rhizomes	Shoots	Roots
		0.00	0.2	4.1	0.2
		0.01	0.1	4.1	0.4
'Valiant'	0.0	0.10	0.3	4.1	0.4
		1.00	0.5	4.1	0.0
		10.00	0.4	4.3	0.0
		0.00	0.6	4.3	0.0
		0.01	0.6	4.6	0.0
'Valiant'	4.0	0.10	0.7	4.4	0.1
		1.00	0.8	4.6	0.0
		10.00	0.9	4.9	0.0
		0.00	0.1	3.8	0.2
		0.01	0.3	4.0	0.3
'Parade'	0.0	0.10	0.2	4.1	0.3
		1.00	0.1	3.9	0.1
		10.00	0.1	4.3	0.0
		0.00	0.6	4.7	0.0
		0.01	0.7	4.5	0.0
'Parade'	4.0	0.10	0.9	4.5	0.0
		1.00	0.9	4.4	0.0
		10.00	1.0	4.5	0.0
		0.00	0.0	4.2	0.1
		0.01	0.1	4.4	0.1
'Butterfly'	0.0	0.10	0.1	4.0	0.0
		1.00	0.1	4.3	0.0
		10.00	0.2	4.3	0.0
		0.00	0.3	4.5	0.0
		0.01	0.2	4.4	0.0
'Butterfly'	4.0	0.10	0.1	4.0	0.1
		1.00	0.1	4.3	0.0
		10.00	0.2	4.3	0.0
		0.00	0.2	4.4	0.8
		0.01	0.4	4.3	0.5
'Eleanor'	0.0	0.10	0.6	4.4	0.6
		1.00	0.4	4.1	0.2
		10.00	0.5	4.4	0.0
		0.00	0.2	5.1	0.0
		0.01	0.5	4.9	0.0
'Eleanor'	4.0	0.10	0.6	4.7	0.0
		1.00	0.4	5.1	0.0
		10.00	0.7	5.3	0.0

No differences significant

Table 7.2: The effect of thidiazuron on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	BAP ($mg\,l^{-1}$)	Thidiazuron ($mg\,l^{-1}$)	Lateral rhizomes	Shoots	Roots
'Valiant'	0.0	0.000	0.6	4.3	0.4
		0.011	0.4	4.0	0.2
		0.022	0.5	4.0	0.3
		0.110	0.6	4.5	0.1
		0.220	0.9	4.6	0.1
		0.440	0.8	4.3	0.0
	4.0	0.000	0.6	4.5	0.1
'Parade'	0.0	0.000	0.6	4.3	0.5
		0.011	0.6	4.1	0.3
		0.022	0.7	4.3	0.3
		0.110	0.7	4.1	0.6
		0.220	0.7	4.3	0.2
		0.440	0.9	4.2	0.1
	4.0	0.000	0.6	4.4	0.1
'Butterfly'	0.0	0.000	0.3	4.4	0.6
		0.011	0.3	4.4	0.3
		0.022	0.2	4.0	0.1
		0.110	0.3	4.1	0.1
		0.220	0.3	4.0	0.0
		0.440	0.2	4.4	0.0
	4.0	0.000	0.4	4.3	0.0
'Eleanor'	0.0	0.000	0.9	4.7	0.9
		0.011	0.9	4.6	0.7
		0.022	1.1	4.0	0.2
		0.110	1.2	4.3	0.0
		0.220	1.1	4.5	0.0
		0.440	0.9	4.7	0.0
	4.0	0.000	0.7	5.1	0.0

No differences significant

concentration. Again, due to the poor response to the treatments and the deterioration in the quality of the explants, this experiment was not repeated.

7.3.2.4 NAA/BAP

The addition of NAA to *Alstroemeria* production medium containing BAP did not affect lateral rhizome or shoot production. However, there appeared to be more roots at the highest concentration of NAA in the cultivars 'Valiant' and 'Parade' (Table 7.3). There were no differences in the appearance of the explants, for any of the cultivars in any of the treatments. This experiment was not repeated.

7.3.2.5 GA_3

The presence of GA_3 in *Alstroemeria* production medium, with or without BAP, often appeared to reduce slightly the numbers of lateral rhizomes, shoots and roots produced in all of the cultivars (Table 7.4). None of these results were, however, significant. As the concentration of GA_3 was increased, there was a tendency for shoots to become longer and thinner and for the rhizome to elongate. Vitrification occurred in the presence of 0.1 $mg\,l^{-1}$ GA_3 and above, with some of the explants appearing malformed at the highest concentrations. These effects were cultivar dependent but independent of the concentration of BAP in the medium.

As the concentration of GA_3 was increased, there was a tendency for shoots to become longer and thinner and for the rhizome to elongate. However, at the highest concentration tested, the shoots were shorter than those at most of the lower concentrations and the rhizome also appeared to be less extended (Figure 7.4). Once again, due to the deterioration in the quality of the explants and the apparent lack of response to the treatments, this experiment was not repeated.

Table 7.3: The effect of NAA and BAP on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	BAP ($mg\,l^{-1}$)	NAA ($mg\,l^{-1}$)	Lateral rhizomes	Shoots	Roots
'Valiant'	4.0	0.00	1.1	4.7	0.0
		0.04	0.7	4.3	0.0
		0.10	0.6	4.5	0.1
		0.20	0.7	4.3	0.1
		0.40	0.9	4.3	0.1
		1.00	0.6	4.3	0.3
'Parade'	4.0	0.00	0.5	4.3	0.0
		0.04	0.9	4.5	0.1
		0.10	0.7	4.3	0.0
		0.20	0.6	4.4	0.2
		0.40	0.6	4.4	0.1
		1.00	0.6	4.2	0.2
'Butterfly'	4.0	0.00	0.7	4.3	0.0
		0.04	0.5	4.0	0.0
		0.10	0.5	4.2	0.0
		0.20	0.7	4.2	0.0
		0.40	0.7	3.9	0.0
		1.00	0.3	4.1	0.0
'Eleanor'	4.0	0.00	0.4	4.8	0.0
		0.04	0.5	5.2	0.0
		0.10	0.8	4.8	0.0
		0.20	0.4	4.9	0.0
		0.40	0.7	4.9	0.0
		1.00	0.6	4.9	0.0

No differences significant

Table 7.4: The effect of GA_3 and BAP on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	BAP ($mg\,l^{-1}$)	GA_3 ($mg\,l^{-1}$)	Lateral rhizomes	Shoots	Roots
'Valiant'	0.0	0.0	0.4	4.3	0.6
		0.1	0.2	4.2	0.5
		1.0	0.3	4.1	0.2
		10.0	0.5	4.1	0.1
		100.0	0.3	4.1	0.1
'Valiant'	4.0	0.0	0.5	4.6	0.2
		0.1	0.5	4.4	0.1
		1.0	0.4	4.4	0.1
		10.0	0.4	4.5	0.0
		100.0	0.5	4.5	0.0
'Parade'	0.0	0.0	0.2	4.1	0.6
		0.1	0.1	4.0	0.3
		1.0	0.1	4.1	0.1
		10.0	0.1	4.1	0.1
		100.0	0.1	4.3	0.1
'Parade'	4.0	0.0	0.6	4.4	0.2
		0.1	0.3	4.4	0.0
		1.0	0.5	4.2	0.0
		10.0	0.2	4.1	0.0
		100.0	0.4	4.4	0.0
'Butterfly'	0.0	0.0	0.3	4.1	0.1
		0.1	0.0	3.9	0.0
		1.0	0.1	3.6	0.0
		10.0	0.2	3.8	0.0
		100.0	0.1	3.5	0.0
'Butterfly'	4.0	0.0	0.6	4.2	0.0
		0.1	0.4	4.1	0.0
		1.0	0.3	4.0	0.0
		10.0	0.3	4.0	0.0
		100.0	0.1	3.7	0.0
'Eleanor'	0.0	0.0	0.6	4.3	0.9
		0.1	0.1	4.2	0.7
		1.0	0.5	4.3	0.0
		10.0	0.7	4.2	0.1
		100.0	0.4	4.3	0.0
'Eleanor'	4.0	0.0	0.7	5.3	0.1
		0.1	0.8	5.2	0.0
		1.0	0.8	5.1	0.0
		10.0	0.9	5.1	0.0
		100.0	0.9	5.0	0.0

No differences significant

Figure 7.4: The effect of GA_3 on the morphology of rhizome explants of the cultivar 'Eleanor'. **A** – **C** = 0.0 mg l^{-1} BAP + 0.0, 1.0 and 100 mg l^{-1} GA_3, **D** – **F** = 4.0 mg l^{-1} BAP + 0.0, 1.0 and 100 mg l^{-1} GA_3, respectively. Bar = 1 cm

7.3.2.6 Paclobutrazol

Although treatments were not significant, some slight trends were apparent. The number of shoots produced increased as the concentration of paclobutrazol was increased for the cultivars 'Valiant' and 'Parade', with and without BAP, and the number of lateral rhizomes produced in the cultivar 'Valiant' also showed similar trends. The number of roots produced was always less in the presence of BAP. The cultivars 'Valiant' and 'Parade' also showed slight decreases in root number with increase in paclobutrazol concentration in the absence of BAP (Table 7.5).

Paclobutrazol had a very marked effect on explant morphology, with or without BAP in the medium (Figure 7.5). Shoot length and leaf size decreased with increasing concentrations of paclobutrazol. At the highest concentration, the leaves and shoots showed very little expansion or elongation, compared with those produced on the control media. The reduction in shoot length was accompanied by an increase in its diameter. This gave the rhizome the appearance of being shorter, however, the paclobutrazol treatments had caused no difference in rhizome length. Paclobutrazol in the media also affected the morphology of the roots. There was considerable swelling of the roots and a decrease in root length as the concentration was increased. Although paclobutrazol did not produce any significant results as regards the number of lateral rhizomes, shoots and roots produced, due to the very marked changes in the morphology of the explants, this experiment was repeated.

7.4 DISCUSSION

7.4.1 Mechanical Control of Apical Dominance

Pierik (1987) states that it has been known for many years that removal of the apical meristem releases axillary buds from the effects of apical dominance. However, in the case of *Alstroemeria* there are only two axillary buds on an

Table 7.5: The effect of paclobutrazol and BAP on the numbers of lateral rhizomes, shoots and roots produced *in vitro* by the cultivars 'Valiant', 'Parade', 'Butterfly' and 'Eleanor'

Cultivar	BAP ($mg\,l^{-1}$)	Paclobutrazol ($mg\,l^{-1}$)	Lateral rhizomes	Shoots	Roots
'Valiant'	0.0	0.00	0.2	4.1	1.1
		0.15	0.2	4.5	1.3
		0.30	0.2	4.3	1.5
		1.50	0.4	4.7	0.9
		3.00	0.4	4.6	0.8
'Valiant'	4.0	0.00	0.4	4.6	0.2
		0.15	0.5	4.8	0.5
		0.30	0.6	4.9	0.7
		1.50	0.8	4.9	0.5
		3.00	0.7	5.0	0.5
'Parade'	0.0	0.00	0.1	4.3	0.8
		0.15	0.1	4.3	0.7
		0.30	0.1	4.4	0.4
		1.50	0.1	4.4	0.5
		3.00	0.1	4.5	0.5
'Parade'	4.0	0.00	0.5	4.6	0.1
		0.15	0.5	4.7	0.5
		0.30	0.4	4.7	0.4
		1.50	0.8	5.2	0.4
		3.00	0.4	5.1	0.4
'Butterfly'	0.0	0.00	0.2	4.2	0.3
		0.15	0.1	4.4	0.4
		0.30	0.1	4.3	0.5
		1.50	0.1	4.4	0.1
		3.00	0.2	4.2	0.3
'Butterfly'	4.0	0.00	0.1	4.1	0.0
		0.15	0.6	4.4	0.0
		0.30	0.6	4.4	0.0
		1.50	0.4	4.5	0.0
		3.00	0.3	4.5	0.1
'Eleanor'	0.0	0.00	0.3	4.4	1.0
		0.15	0.2	4.2	1.2
		0.30	0.1	4.2	1.2
		1.50	0.2	4.1	1.2
		3.00	0.2	4.2	1.1
'Eleanor'	4.0	0.00	0.4	4.1	0.0
		0.15	0.3	4.2	0.1
		0.30	0.2	4.2	0.1
		1.50	0.4	4.1	0.1
		3.00	0.3	4.0	0.2

No differences significant

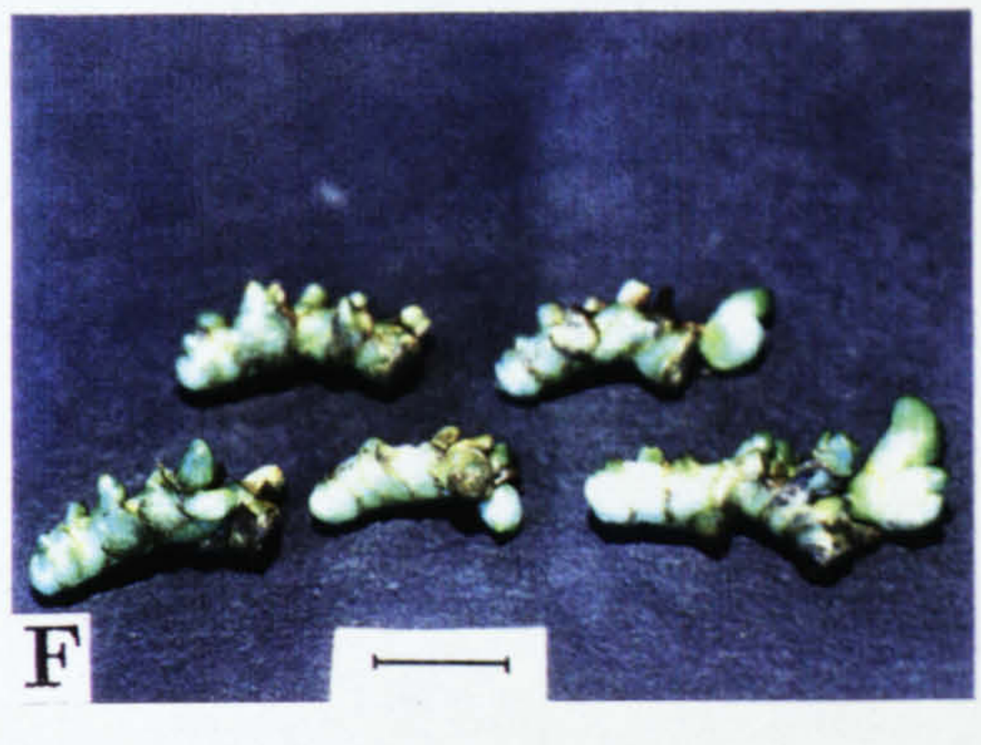


Figure 7.5: The effect of paclobutrazol on the morphology of rhizome explants of the cultivar 'Eleanor'. **A** - **C** = 0.0 mg l^{-1} BAP + 0.0, 0.3 and 3.0 mg l^{-1} paclobutrazol, **D** - **F** = 4.0 mg l^{-1} BAP + 0.0, 0.3 and 3.0 mg l^{-1} paclobutrazol, respectively. Bar = 1 cm

aerial shoot, one that continues the growth of the main rhizome and another which has the potential to grow out and produce branching of the rhizome system. There are also only two types of apices present, those at the tips of the aerial shoots and those at the distal ends of rhizomes (Section 1.2.2). Therefore, in common with most monocotyledonous plants (Hussey *et al.*, 1980), *Alstroemeria* has a low number of axillary buds and consequently the potential for axillary bud proliferation is limited.

From the results presented it would appear that these axillary buds are influenced by both the rhizome and shoot apices. Removal of either of these apices produced a low increase in axillary bud outgrowth. However, removal of both apices significantly increased lateral rhizome production, and the effect was far greater than the additive effects of the removal of the apices separately. This suggests that if one apex source is removed, the other may be able to compensate for this loss, by increasing the level of its own dominance effect. The shoot apex has been shown to maintain correlative inhibition over axillary bud growth (Roberts and Hooley, 1988; Tamas, 1987) and a similar mechanism has been proposed in rhizomes of *Agropyron repens* (Leakey *et al.*, 1975). However, an increase in a chemical signal is probably the least likely of the changes in mechanism which may be occurring. Apices are also considered to effect apical dominance by directing the movement of metabolites away from the axillary buds towards themselves (McIntyre, 1969, 1972, 1977; McIntyre and Damson, 1988).

Therefore, for *Alstroemeria in vitro* there would appear to be a two part system composed of shoot and rhizome apices, both competing for the nutrients, of which most are supplied by the media. Hence, when one is removed, the other may be able to direct more of the resources towards itself, with only a proportion of what could have been made available being directed into axillary bud growth. This would account for the small increases in lateral rhizome production observed when only one type of apex was removed.

The further increases reported after subdivision of the rhizome explants into

single enlarged basal internodes, suggests another complexity in the mechanism of inhibition of axillary bud growth in *Alstroemeria*. This may be considered to be a competitive or inhibitory effect, produced between the expanded basal internodes themselves. It has already been established, that each shoot will be competing for nutrients and thereby inhibiting axillary bud growth, yet even when the shoots have been removed, the level of lateral rhizome production is still not as high as when the shoots are removed and the expanded basal internodes are separated. Therefore, any effect of the shoot apices can essentially be disregarded. It is possible that,as the axillary buds are released from inhibition of growth, they begin to interact with one another, as each tries to establish dominance over the others. Consequently, explants that consist of several enlarged basal internodes, are seen to produce fewer lateral rhizomes compared with single enlarged basal internodes.

Chancellor (1974), working on the rhizome of *Agropyron repens*, reported similar results. It was demonstrated that more axillary buds grew from increasingly smaller fragments of rhizome, when released from the dominance effect of the apex. This, it was suggested, was due to the lack of competition from or dominance by, any nearby axillary buds. It was also shown, that on multi-node fragments many axillary buds may start to grow after removal of the apex. However, within a few days only one, or very few, axillary buds were still seen to be actively growing. This effect was attributed to the establishment of dominance over the majority of the axillary buds by those that remained in active growth. Pierik *et al.* (1988) also found with *Alstroemeria* that the fewer the number of shoots present on a rhizome fragment, the greater the multiplication rate. However, in that study, when explants consisting of one single shoot were used, this was a mixture of explants with axillary rhizome buds and sometimes a rhizome apex. It was also not made clear whether explants with more than one shoot were similar mixtures of some with and some without a rhizome apex.

Between the cultivars used in the present study, differences could be seen in the overall trends when the shoots remained on the explant. This was

especially true of the cultivar 'Valiant'. The cultivars 'Eleanor' and 'Parade' are known to produce strong shoots *in vivo*, whereas those of the cultivar 'Valiant' are somewhat weaker. If these characters are reflected *in vitro*, then as apical dominance seems to be caused, to an extent, by the directed movement of metabolites to the apices, this may influence multiplication rates *in vitro*. Consequently, if the shoots of the cultivar 'Valiant' are causing less redirection of metabolites due to their inherent weak growth, more lateral rhizomes may be produced than would have been expected, as more nutrients are reaching them. This could be the reason for the higher than expected number of lateral rhizomes produced by the cultivar 'Valiant', with the explants at subculture consisting of single enlarged basal internodes with shoots.

Another difference between the cultivars was that those which are more vigorous *in vitro* produced higher numbers of lateral rhizomes in response to the treatments provided. It is possible that the weaker cultivars could have achieved the same levels of lateral rhizome production, if they had been assessed over longer periods of time. Hence, this observation was considered to be a reflection of the different growth rates of the cultivars.

This strong apical dominance *in vitro* does seem to be reflected *in vivo*, especially if the low amount of suitable rhizome material for propagation purposes, is considered against the high number of potential sites for lateral rhizome production present after the two year period a plant spends in a production bed (Parigo Horticultural Co., personal communication). The large disparity between these numbers can then be regarded as reflecting the strength of apical dominance *in vivo*. However, the results produced from this experiment can not necessarily be extrapolated to a consideration of apical dominance in *Alstroemeria in vivo*, as movement of metabolites within each system may differ. *In vivo* translocation of metabolites is essentially within the shoots and down into the rhizome and roots, whereas *in vitro* the movement of metabolites is from the medium to the shoots via the rhizome. Therefore, it may be that *in vitro*, nutrients reach the axillary buds on the

way to being directed towards the shoots rather than after redirection has taken place. If this is so, then it may influence the degree of apical dominance occurring within the two systems.

7.4.2 Chemical Control of Apical Dominance

7.4.2.1 TIBA

The absence of any effect of TIBA on the number of lateral rhizomes produced in *Alstroemeria* is supported by work on *Hippeastrum hybridum* (Bhattacharjee, 1983), where it was found that soaking bulbs in solutions of TIBA had no effect on the number of bulbs produced. For spring barley (*Hordeum distichum*) (Woodward and Marshall, 1988), a foliar application of TIBA caused the number of elongating tillers to increase but did not increase the total number of tillers. The addition of TIBA to the culture medium for ryegrass (*Lolium multiflorum*) (Dalton and Dale, 1981) also produced increases in tillering that were not significant. By contrast, *Senecio* x *hybridus* (Gertsson, 1988) and *Acer* species (Marks and Wiltshire, 1984) *in vitro*, produced significantly more shoots following the application of TIBA. However, the shoots produced in the *Acer* cultures were of inferior quality for subsequent culture.

Van Aartrijk and Blom-Barnhoorn (1983, 1984) reported increases in bulblet formation of *Lilium speciosum in vitro*, in the presence of TIBA. Conversely, Flint and Alderson (1986) found that fewer bulblets were formed from *Narcissus* chip propagules when TIBA was applied in a conventional chipping system. It appears therefore, that the effects of TIBA on the multiplication rates of plants vary greatly, depending on the plant being used and the system employed, as differences may be seen between results produced *in vivo* and *in vitro*.

7.4.2.2 Thidiazuron

Phenylurea derivatives with cytokinin activity, including thidiazuron, have been used successfully in the last few years to increase proliferation within *in vitro* shoot multiplication systems, mainly with woody plants. Thidiazuron has been used with several ornamental and fruit tree species, including *Tilia cordata*, *Sorbus aucuparia* and *Robinia pseudoacacia* (Chalupa, 1987), *Acer* x *freemanii* (Kerns and Meyer, 1986), *Celtis occidentalis* (Meyer and Kerns, 1986), azaleas (*Rhododendron* sp.) (Briggs *et al.*, 1988), *Syringa* x *hyacinthiflora* (Einset and Alexander, 1984) and apple (*Malus domestica*) (Nieuwkerk *et al.*, 1986; Wang *et al.*, 1986). The compound N-(2-chloro-4-pyrimidyl)-N'-phenylurea (4PU) has also been used with *Morus alba* (Ohyama and Oka, 1982). Higher rates of proliferation have been achieved compared with those for most cytokinins.

Application of thidiazuron to the tree *Oxydendrum arboreum* (Banko and Stefani, 1989) and to muskmelon (*Cucumis melo*) (Niedz *et al.*, 1989) failed to produce any significant increase in the number of shoots produced. Similarly, in the present study, thidiazuron did not increase the number of lateral rhizomes, shoots or roots produced by the *Alstroemeria* cultivars. There are comparatively few reports of work with thidiazuron on other monocotyledonous plants. When applied to the corms of *Ixia flexuosa*, thidiazuron induced the formation of multiple adventitious buds. However, these buds were less vigorous than those produced on BAP supplemented medium (Meyer and van Staden, 1988). Recently, Henny and Fooshee (1990) reported that thidiazuron, applied as a solution to the base of *Alocasia* plants, significantly increased the number of basal buds. However, most of these buds were formed beneath the substrate surface and after 12 weeks only a small proportion had penetrated the surface.

Read *et al.* (1987) found that dipping plant material in thidiazuron was more effective than having it present continuously in the medium. This was attributed to the high activity of the compound, which they found to be

deleterious to plant growth with prolonged exposure.

7.4.2.3 BAP/NAA

The absence of any effect of auxin on the production of lateral rhizomes contrasts with the results of several other reports. Shoot multiplication has been enhanced for *Plantago ovata* (Barna and Wakhlu, 1988) and *Stylidium* species (McComb, 1985) following the incorporation of both cytokinin and auxin in the culture medium. Similar results have been achieved with some monocotyledonous species, including *Costus speciosus* (Chaturvedi *et al.*, 1984) and several species in the bromeliad genus *Aechmea* (Dijck *et al.*, 1988; Jones and Murashige, 1974). Pierik (1987) also reported the synergistic effect of cytokinin and auxin on the increase in multiplication rate for the bromeliad *Vriesea.* However, it should be noted that the reaction of bromeliads *in vitro* is often very variable, even if the starting material appears to be homogenous (Pierik, personal communication).

The present result with *Alstroemeria* does, however, support the observations of Dalton and Dale (1985) who found that the rate of tiller induction in rye grass (*Lolium multiflorum*) was not affected by the addition of auxin to the medium with cytokinin.

7.4.2.4 GA_3

Catalano and Hill (1969) and Sachs and Thimann (1964) suggested that GA_3 may promote the action of kinetin on releasing axillary buds from apical dominance. However, when GA_3 was applied with or without BAP to *Alstroemeria* cultivars *in vitro*, no significant changes in the multiplication rate of the rhizome explants were observed.

The application of GA_3 onto plants of English ivy (*Hedera helix*) (Lewnes and Moser, 1976; Frydman and Wareing, 1973) produced significant increases in axillary bud growth and subsequent branching of the plants. The addition of GA_3 to the culture medium for embryos of American lotus (*Nelumbo lutea*)

(Kane *et al.*, 1988) has been shown to produce significant increases in rhizome growth and node number. The GA_3 also stimulated rhizome branching, which was not seen to occur in the control treatment.

GA_3 has now been applied to field grown, monocotyledonous crops in attempts to hasten their growth and increase yields. Field grown ginger (*Zingiber officinale*) (Furutani and Nagao, 1986), following GA_3 application, exhibited inhibition of shoot emergence and decreases in rhizome yield, whereas tumeric (*Curcuma longa*) (Randhawa and Mahey, 1984) exhibited no significant differences in plant height or tiller number. Foliar application of GA_3 on spring barley (*Hordeum distichum*) (Woodward and Marshall, 1988) has been seen to reduce the number of elongating tillers and to restrict tiller bud growth. A similar treatment of the bulbous *Iris* cultivar 'Wedgewood', caused a reduction in bulb yield (Halevy and Shoub, 1964). Similar results have been reported for Easter lily (*Lilium longiflorum*) (Zieslin and Tsujita, 1988), where application of GA_3 caused no change in stem length. In *Hyacinthus orientalis*, addition of GA_3 to the culture medium inhibited both bulblet regeneration and bulblet weight on excised bulb scale segments (Pierik and Steegmans, 1975).

7.4.2.5 Paclobutrazol

The ability of paclobutrazol and other compounds to retard the growth of pot grown tulips and lilies, producing more compact and manageable plants, has been well established (Hanks and Menhenett, 1983; Menhenett and Hanks, 1982, 1982/3). Application of paclobutrazol to freesias has been shown to cause more reduction of the flower stem length than of the leaf length, thereby placing the flowers too close to the leaves and making them unmarketable as potted plants (Berghoef and Zevenbergen, 1990). However, these reports make no mention of any effect on the growth and development of the bulbs of the treated plants. When applied to the grass *Lolium perenne* (Hebblethwaite *et al.*, 1982, 1986), paclobutrazol changed the amount of tillering but the effect was very variable. This was considered to be due to effects of the

environment, as these trials were conducted in the field.

Multiplication rates of shoot systems *in vitro* have been enhanced by the stimulation of axillary bud development in *Asparagus officinalis* (Chin, 1982) by ancymidol (α-cyclopropyl-α(4-methoxyphenyl)-5-pyrimidine-methanol) and in *Aechmea fasciata* (Ziv *et al.*, 1986) by paclobutrazol. In *Lapageria rosea* (McKinless *et al.*, 1988) paclobutrazol, by decreasing shoot elongation, produced a structure similar to that of a short rhizome, from which roots were initiated *de novo*. This has been used as part of a protocol for increasing rooted plant production *in vitro*. Enhanced cormlet production of gladiolus has also been achieved in liquid cultures supplemented with paclobutrazol (Ziv, 1989, 1990). In contrast to these results, Khunachak *et al.* (1987) observed no changes in shoot growth of *Asparagus officinalis* following application of paclobutrazol.

With *Alstroemeria*, paclobutrazol had no effect on the rhizome multiplication rate, however, the changes in shoot and root morphology which occurred may be of benefit prior to the planting out of propagated material. Paclobutrazol has also been shown to increase root size in *Asparagus officinalis* (Khunachak, 1987) and to promote tuber growth in the potato (*Solanum tuberosum*) (Balamani and Poovaiah, 1985). If paclobutrazol were to be added to the rooting medium of *Alstroemeria*, then the subsequent changes in root, shoot and leaf morphology, could be important in increasing water uptake and decreasing transpiration, during the weaning and establishment of plants *ex vitro*. Other effects of paclobutrazol on the water use of plants, including changes in stomatal conductivity (Atkinson, 1986) and epicuticular wax (Smith *et al.*, 1990), could have important roles to play in the weaning and establishment of plants.

7.5 CONCLUSION

It would appear that the shoot apices and the root apex exert a very strong dominance effect *in vitro* on the second axillary bud of the shoots of *Alstroe-*

meria. This axillary bud is the one which can grow into a lateral rhizome and is the only other axillary bud on a shoot, apart from the one which carries on the growth of the main rhizome. These axillary buds may interact and inhibit the growth of each other. These conclusions are supported by the fact that the highest multiplication rate was achieved only when both types of apex were excised and the expanded basal internodes subdivided. With the rhizome apex being recultured, essentially one more plant was produced in each of the treatments where it was removed.

Attempts to control the apical dominance present in *Alstroemeria* by the application of PGRs, apart from the previously reported effects of cytokinins, especially BAP, produced no significant effects on the multiplication rate of the rhizome explants *in vitro.* This may indicate a high degree of inhibition of axillary bud growth, controlled and maintained by the apices, the mechanism of which is not yet fully understood.

Further work is therefore needed to elucidate the dominance mechanisms working in *Alstroemeria* and to assess the effects of a wider range of PGRs. Investigation of the vitrification which occurred in the present study, in order to establish methods to circumvent this problem, would also be useful.

Chapter 8: SUMMARY AND CONCLUSIONS

8.1 GENERAL

Two main areas of interest were considered in this thesis. The first was the assessment of the effects of environmental factors on the growth of the rhizome of *Alstroemeria in vivo* and *in vitro*.

Clear differences were seen between the cultivars used in this study, in their morphology and in their responses to environmental factors *in vivo* and *in vitro*. These differences have been considered to reflect the characters and adaptations possessed by the various parental species included in the breeding programmes of the cultivars.

Temperature and irradiance *in vivo* had significant effects on the dry weight of plant parts produced but little effect on their number. Changes in daylength had little effect on any of the parameters studied. The changes in dry weight were thought to be due to alterations in the rate of photosynthesis produced by (i) changes in reaction rates within the plants caused by the different temperatures and (ii) changes in the total light energy received by the plants, due to the variation in irradiance and daylength. However, daylength had a profound effect on the time of flowering.

The results from the work *in vivo* indicated that for maximum rhizome production a temperature of between 13 and 18°C, a high irradiance and a short daylength are required. However, as the latter two tend to be inhibitory to flowering, high summer irradiances are usually reduced by shading the plants and the daylength is often maintained at about 12 hours.

Temperature *in vitro* produced significant responses for all of the parameters studied, apart from that of root production. Irradiance and daylength produced no significant differences in any parameter *in vitro*. This was attributed to the change from an autotrophic to a heterotrophic mode of nutrition. Very low irradiances, however, caused etiolation of the shoots. It was considered that a shorter daylength may also have produced a similar effect, as it influences the total light integral received by the explant. Consequently, due to this interaction, it is difficult to assess which may be the more important factor of the light environment.

From the *in vitro* investigations, it is suggested that for maintenance of a good multiplication rate of the rhizome and for production of healthy shoots, the requirements for the culture environment were a temperature of 15°C, an irradiance of at least 5 W m^{-2} and a daylength of no more than 8 hours in a 24 hour day/night cycle.

Initially it was thought that temperature was influencing the establishment *in vivo* of newly potted plant material. Later work, however, showed that the important factor in this process appeared to be the condition of the rhizome and tubers, particularly regarding any physical damage that may have occurred during the preparation of 'splits' for planting. It was also noted that if no tubers were present when the 'splits' were planted, all of the plants failed to establish. This suggested that the tubers contained a factor important for plant establishment. Further work *in vitro*, showed that extracts from the tubers, when added to the medium, could cause dramatic increases in shoot growth. The nature of this factor is still, however, a matter of speculation. This phenomenon could be linked to the plant's need in its native habitats, for phases of fast, active growth, in order to become re-established after periods of dormancy.

Overall, the study on the effect of temperature, *in vivo* and *in vitro*, on *Alstroemeria* showed that the optimum temperature for growth is approximately the same in both systems. However, when comparing the effects of

irradiance and daylength between the *in vivo* and *in vitro* systems, marked differences can be seen in the requirements for growth.

The second area of interest was the inhibition of lateral rhizome growth by apical dominance. It has been clearly demonstrated that the rhizome and shoot apices exert very strong inhibitory controls on the growth of the lateral rhizomes. An interaction, probably competitive, also occurs between the axillary buds, with a tendency for one or a few to become dominant and inhibit the growth of the others. The greatest multiplication rate of the rhizome was achieved when the rhizome and shoot apices were removed and the enlarged basal internodes were subdivided.

Attempts to control apical dominance with PGRs produced no significant responses. This suggests, that either the method of application of the PGRs was not appropriate for *Alstroemeria in vitro* or that the mechanism of apical dominance in the plant is more complex than has been considered previously.

Commercially, a change in subculturing practices for *Alstroemeria* to that mentioned above, could produce substantial improvements in the multiplication rates achieved. However, the extra manipulations required may decrease the efficiency of the operator, by causing a reduction in the number of explants subcultured per unit of working time. Consequently, if this is not offset by an increase in multiplication, such a change would not be cost effective. If a chemical method were able to produce the same effect then the efficiency of the micropropagation system should not be affected and providing that the PGR was not too expensive, a profitable increase in the multiplication rate of *Alstroemeria* could be achieved.

8.2 SUGGESTIONS FOR FUTURE WORK

Commercially, there may be some value in establishing the minimum amount of light required to give a good multiplication rate, in order to minimise the cost of the lighting. As this would be considering the total light energy received, determining the minimum time necessary for its accumulation may

also be of value.

The effect of light quality on rhizome multiplication of *Alstroemeria* was not studied and there appear to be no reports in the literature on this interaction. As the quality of light is thought to be fundamental to many morphogenetic and physiological aspects of plant development, information in this area for *Alstroemeria* may help to enhance the multiplication rate of the rhizome and the growth of the explant in general.

The application of PGRs to the explants for short periods of time and then replacing them on unsupplemented *Alstroemeria* production medium, i.e. 'pulsing', is worth studying. Such an approach may initiate the required effect, without generating problems such as vitrification, which are often considered to result from prolonged exposure to some PGRs. As a consequence of using PGRs to influence the growth of explants, it will be necessary to carry out growth and flowering trials, to see if any of the plant's characters, e.g. morphology, flower colour or time to flowering, have been modified.

With a plant such as *Alstroemeria*, which appears to have complex interactions between the lateral buds, rhizome apices and the shoots, and between the lateral buds themselves, a more fundamental understanding of these interactions would lead to a better appreciation of the limitations associated with the multiplication of the rhizome and how they may be overcome.

References

AARTRIJK, J. VAN, & BLOM-BARNHOORN, G.J. (1983). Adventitious bud formation from bulb-scale explants of *Lilium speciosum* Thunb. *in vitro*. Effects of wounding, TIBA and temperature. *Zeitschrift für Pflanzenphysiologie*, **110**, 355-363.

AARTRIJK, J. VAN, & BLOM-BARNHOORN, G.J. (1984). Adventitious bud formation from bulb-scale explants of *Lilium speciosum* Thunb. *in vitro*. Interacting effects of NAA, TIBA, wounding and temperature. *Journal of Plant Physiology*, **116**, 409-416.

ADAMS, P.A., MONTAGUE, M.G., TEPFER, M., RAYLE, D.L., IKUMA, H. & KAUFMAN, P.B. (1975). Effects of gibberellic acid on the plasticity and elasticity of *Avena* stem segments. *Plant Physiology*, **56**, 757-760.

ADANIYA, S., SHODA, M. & FUJIEDA, K. (1989). Effects of daylength on flowering and rhizome swelling in ginger (*Zingiber officinale* Roscoe). *Journal of the Japanese Society for Horticultural Science*, **58(3)**, 649-656.

ALI, A. & FLETCHER, R.A. (1971). Hormonal interaction in controlling apical dominance in soybeans. *Canadian Journal of Botany*, **49**, 1727-1731.

ANON. (1980). *Alstroemeria. In Grower guide No.18., New cut flower crops*, 1-12. London, Grower Books.

ANON. (1984). *Paclobutrazol plant growth regulator for ornamentals*. Technical Information, ICI, Plant Protection Division, U.K..

ANON. (1986). *Dropp (thidiazuron) cotton defoliant*. Technical Information, Schering Agrochemicals, Berlin.

ANON. (1987). *Levington makes the grade*. Technical Information, Fisons plc Horticulture Division, Ipswich.

ANON. (1989). The cut flower export industry in Colombia. *International Floriculture Quarterly Report*, **1(3)**, 21-31.

ANON. (1990). Analysis of cut flowers auction returns mid. 1987 to mid. 1989. *International Floriculture Quarterly Report*, **1(4)**, 61-63.

ARNDT, F., RUSH, R., STILLFRIED, H.K., HANISCH, B. & MARTIN, W.C. (1976). SN 49537: A new cotton defoliant. *Plant Physiology*, **57**, suppl. 99.

ATKINSON, D. (1986). Effects of some plant growth regulators on water use and uptake of mineral nutrients by tree crops. *Acta Horticulturae*, **179**, 395-404.

ATKINSON, D. & CHAUHAN, J.S. (1987). The effect of paclobutrazol on the water use of fruit plants at two temperatures. *Journal of Horticultural Science*, **62(4)**, 421-426.

BALAMANI, V. & POOVAIAH, B.W. (1985). Retardation of shoot growth and promotion of tuber growth of potato plants by paclobutrazol. *American Potato Journal*, **62**, 363-369.

BALDWIN, B.C. & WIGGINS, T.E. (1984). Action of fungicidal triazoles of the diclobutrazol series on *Ustilago maydis*. *Pesticide Science*, **15**, 156-166.

BANKO, T.J. & STEFANI, M.A. (1989). *In vitro* propagation of *Oxydendrum arboreum* from mature trees. *Hortscience*, **24(4)**, 683-685.

BARNA,K.S. & WAKHLU, A.K. (1988). Axillary shoot induction and plant regeneration in *Plantago ovata* Forsk. *Plant Cell, Tissue and Organ Culture*, **15**, 169-173.

BAYER, E. (1987). Die Gattung *Alstroemeria* in Chile. *Mitteilungen der Botanischen Staatssammlung München*, **24(0)**, 1-362.

BERGHOEF, J. & ZEVENBERGEN, A.P. (1990). The effect of precooling, environmental factors and growth-regulating substances on plant height of freesia as pot plant. *Acta Horticulturae*, **226**, 251-257.

BEYER, E.M. JR. (1973). Abscission, support for a role of ethylene modification of auxin transport. *Plant Physiology*, **52**, 1-5.

BHATTACHARJEE, S.K. (1983). Influence of growth regulating chemicals on *Hippeastrum hybridum* Hort. *Gardens' Bulletin (India)*, **36(2)**, 237-242.

BIK, R.A. & VAN DEN BERG, TH.J.M. (1981). Nitrogen and potassium fertilization of *Alstroemeria* cultivars 'Orchid' and 'Carmen' grown on peat. *Acta Horticulturae*, **126**, 287-292.

BLOM, T.J. & PIOTT, B.D. (1990). Constant soil temperature influences flowering of Alstroemerias. *Hortscience*, **25(2)**, 189-191.

BRIDGEN, M.P. (1986). Somatic embryogenesis and organogenesis of *Alstroemeria*. *Hortscience*, **21**, 306 (abstract).

BRIDGEN, M.P. & WINSKI, P.J. (1989). Influence of environmental factors on stage II proliferation of *Alstroemeria*. *Hortscience*, **24**, 139 (abstract).

BRIGGS, B.A., MCCULLOCH, S.M. & EDICK, L.A. (1988). Micropropagation of azaleas using thidiazuron. *Acta Horticulturae*, **227**, 330-333.

BROERTJES, C. & VAN HARTEN, A.M. (1978). Cut flowers, *Alstroemeria*. *In Developments in Crop Science 2. Application of Mutation Breeding Methods in the Improvement of Vegetatively Propagated Crops*, 152-156. Amsterdam, Elsevier Scientific Publishing Co..

BROERTJES, C. & VAN HARTEN, A.M. (1988). Cut flowers, *Alstroemeria*. *In Developments in Crop Science 12. Applied Mutation Breeding for Vegetatively Propagated Crops*, 165-169. Amsterdam, Elsevier Scientific Publishing Co..

BROERTJES, C. & VERBOOM, H. (1974). Mutation breeding of *Alstroemeria*. *Euphytica*, **23**, 39-44.

BURG, S.P. & BURG, E.A. (1967). Inhibition of polar auxin transport by ethylene. *Plant Physiology*, **42**, 1224-1228.

BURG, S.P. & BURG, E.A. (1968). Ethylene formation in pea seedlings; its relation to the inhibition of bud growth caused by indole-3-acetic acid. *Plant Physiology*, **43**, 1069-1074.

BUXBAUM, F. (1951). Die grundachse von *Alstroemeria* und die Einheit ihres morphologischem Typus mit dem der echten Liliaceen. *Phytomorphology*, 1, 170-184.

CAPELLE, S.C., MOK, D.W.S., KIRCHNER, S.C. & MOK, M.C. (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of $N^6 - (\Delta^2$-isopentenyl)[8-^{14}C]adenosine in callus tissues of *Phaseolus lunatus* L.. *Plant Physiology*, **73**, 796-802.

CATALANO, M. & HILL, T.A. (1969). Interaction between gibberellic acid and kinetin in overcoming apical dominance, natural and induced by IAA in tomato (*Lycopersicon esculentum*). *Nature*, **222**, 985-986.

CHALUPA, V. (1987). Effect of benzylaminopurine and thidiazuron on *in vitro* shoot proliferation of *Tilia cordata* Mill., *Sorbus aucuparia* L. and *Robinia pseudoacacia* L. *Biologia Plantarum* (Praha), **29(6)**, 425-429.

CHANCELLOR, R.J. (1974). The development of dominance amongst shoots arising from fragments of *Agropyron repens* rhizomes. *Weed Research*, **14**, 29-38.

CHATFIELD, J.M. & ARMSTRONG, D.J. (1986). Regulation of cytokinin oxidase activity in callus tissues of *Phaseolus vulgaris* L. cv 'Great Northern'. *Plant physiology*, **80**, 493-499.

CHATURVEDI, H.C., MISRA, P. & JAIN, M. (1984). Proliferation of shoot tips and clonal multiplication of *Costus speciosus* in long-term culture. *Plant Science Letters*, **35**, 67-71.

CHEPKAIROR, M. & WAITHAKA, K. (1988). Growth and flowering of *Alstroemeria*. *Acta Horticulturae*, **218**, 115-120.

CHIN, C-K. (1982). Promotion of shoot and root formation in asparagus *in vitro* by ancymidol. *Hortscience*, **17(4)**, 590-591.

CHUANG, C.C., OUYANG, T.W., CHIA, H., CHOU, S.M. & CHING, C.K. (1978). A set of potato media for wheat anther culture. *Proceedings of the Symposium on Plant Tissue Culture*, 51-56. Peking, Science Press.

COUSSON, A., TOUBART, P. & TRAN THANH VAN, K.M. (1989). Control of morphogenetic pathways in thin cell layers of tobacco by pH. *Canadian Journal of Botany*, **67(3)**, 650-654.

COX, M.J. & MCMASTERS, M.M. (1947). Starch from *Alstroemeria. Herbertia*, **14**, 71-80.

DALTON, S.J. & DALE, P.J. (1981). Induced tillering of *Lolium multiflorum in vitro. Plant Cell, Tissue and Organ Culture*, **1**, 57-64.

DALTON, S.J. & DALE, P.J. (1985). The application of *in vitro* tiller induction in *Lolium multiflorum. Euphytica*, **34**, 897-904.

DALZIEL, J. & LAWRENCE, D.K. (1984). Biochemical and biological effects of kaurene oxidase inhibitors such as paclobutrazol . *In* Menhenett, R. and Lawrence, D.K. (Eds.). *Biochemical Aspects of Synthetic and Naturally Growing Plant Growth Regulators*, 43-57. British Plant Growth Regulator Group, Monograph 11.

DAMBRE, P. (1987). Kortedagbehandling bij *Alstroemeria. Verbondsnieuws voor de Belgische Sierteelt*, **31(15)**, 911-921.

DAVIES, P.J. & RUBERY, P.H. (1978). Components of auxin transport in stem segments of *Pisum sativum* L.. *Planta*, **142**, 211-219.

DEEN, J. (1986). Licence to make money. *Horticulture Week*, **July 11**, 1986.

DEPTA, H., EISELE, K.H. & HERTEL, R. (1983). Specific inhibitors of auxin transport: action on tissue segments and *in vitro* binding to membranes from maize coleoptiles. *Plant Science Letters*, **31**, 181-192.

DEPTA, H. & RUBERY, P,H. (1984). A comparative study of carrier participation in the transport of 2,3,5-triiodobenzoic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid by *Cucurbita pepo* L. hypocotyl segments. *Journal of Plant Physiology*, **115**, 371-387.

DIJCK, R. VAN, DE PROFT, M. & DE GREEF, J. (1988). Role of ethylene and cytokinins in the initiation of lateral shoot growth in bromeliads. *Plant Physiology*, **86**, 836-840.

EGGINGTON, P. (1986). *Alstroemeria* moves into ayr with winter flowering types. *Grower*, **106(1)**, 22-25.

EINSET, J.W. & ALEXANDER, J.H. (1984). Multiplication of *Syringa* species and cultivars in tissue culture. *Combined Proceedings of the International Plant Propagators Society*, **34**, 628-632.

EVENSON, J.P., BRYANT. P.J. & ASHER, C.J. (1978). Germination and early growth of ginger (*Zingiber officinale* Roscoe). I - Effects of constant and fluctuating soil temperature. *Tropical Agriculture*, **55(1)**, 1-7.

FELLMAN, C.D., READ, P.E. & HOSIER, M.A. (1987). Effects of thidiazuron and CPPU on meristem formation and shoot proliferation. *Hortscience*, **22(6)**, 1197-1200.

FLINT, G.J. & ALDERSON, P.G. (1986). *Narcissus* propagation by chipping: effect of a range of plant growth regulators on bulbil yield and length. *Acta Horticulturae*, **177**, 315-322.

FORTANIER, E.J., BRENK, G. VAN. & WELLENSIEK, S.J. (1979). Growth and flowering of *Nerine flexuosa alba*. *Scientia Horticulturae*, **11**, 281-290.

FRYDMAN, V.M. & WAREING, P.F. (1974). Phase change in *Hedera helix* L.. *Journal of Experimental Botany*, **25**, 420-429.

FURUTANI, S.C. & NAGAO, M.A. (1986). Influence of daminozide, gibberellic acid and ethephon on flowering, shoot growth and yield of ginger. *Hortscience*, **21(3)**, 428-429.

GABRYSZEWSKA, E. & HEMPEL, M. (1984). Propagation of *Alstroemeria* sp. *in vitro*. *Plant Tissue and Cell Culture Application to Crop Improvement*, 523-524. Proceedings of the International Symposium, Olomovic, Czechoslovakia.

GABRYSZEWSKA, E. & HEMPEL, M. (1985). The influence of cytokinins and auxins on *Alstroemeria* in tissue culture. *Acta Horticulturae*, **167**, 295-300.

GALSTON, A.W. & DAVIES, P.J. (1969). Hormonal regulation in higher plants. *Science*, **163**, 1288-1297.

GEHLOT, H.S., UPADHYAYA, A., DAVIES, T.D. & SANKHLU, N. (1989). Growth and organogenesis in moth bean callus as affected by paclobutrazol. *Plant Cell Physiology*, **30(6)**, 933-936.

GEORGE, E.F. & SHERRINGTON, P.D. (1984). *Plant Propagation by Tissue Culture*. Basingstoke, Exegetics.

GERTSSON, U.E. (1988). Influence of macronutrient composition, TIBA and dark treatment on shoot formation and nitrogen content in petiole explants of *Senecio* x *hybridus*. *Journal of Horticultural Science*, **63(3)**, 497-502.

GIANFANGA, T.J. (1987). Natural and synthetic growth regulators and their use in horticulture and agronomic crops. *In* Davies, P.J. (Ed.). *Plant Hormones and their Role in Growth and Development*, 614-635. Dordrecht, Martinus Nijhoff.

GOEMANS, J.A.M. (1962). Breeding of Alstroemerias. *Journal of the Royal Horticultural Society*, **87**, 282-284.

GOLDSMITH, M.H.M. (1977). The polar transport of auxin. *Annual Review of Plant Physiology*, **28**, 439-478.

GONZALEZ BENITO, M.E. & ALDERSON, P.G. (1990). Regeneration from *Alstroemeria* Callus. *Acta Horticulturae*, **280**, 135-138.

GRAEBE, J.E. (1987). Gibberellin biosynthesis and control. *Annual Review of Plant Physiology*, **38**, 419-465.

GROSSMAN, K., KWAITKOWSKI, J., SIEBECKER, H. & JUNG, J. (1987). Regulation of plant morphology by growth retardants. Effects on phytohormone levels in soybean seedlings determined by immunoassay. *Plant Physiology*, **84**, 1018-1021.

GROUT, B.W.W. & ASTON, M.J. (1978). Transplanting of cauliflower plants regenerated from meristem culture. II. Carbon dioxide fixation and the development of photosynthetic ability. *Horticultural Research*, **17**, 65-71.

GROUT, B.W.W. & MILLAM, S. (1985). Photosynthetic development of micropropagated strawberry plantlets following transplanting. *Annals of Botany*, **55**, 129-131.

GOULSTON, G.H. & SHEARING, S.J. (1985). Review of the effects of paclobutrazol on ornamental plants. *Acta Horticulturae*, **167**, 339-348.

GUERN, J. & USCIATI, M. (1972). The present status of the problem of apical dominance. *In* Kaldeway, H. and Varder, Y. (Eds.). *Hormonal Regulation in Plant Growth and Development*, 383-400. Weinheim, Verlag Chemie.

GUZMAN, C.C. DE & FUENTE, R.K. DELA. (1984). Polar calcium flux in sunflower hypocotyl segments. *Plant Physiology*, **76**, 347-352.

HAKKAART, F.A. & VERSLUIJS, J.A.M. (1985). Viruses of *Alstroemeria* and preliminary results of meristem culture. *Acta Horticulturae*, **164**, 71-75.

HALEVY, A.H. (1986). Factors effecting the induction of contractile roots in *Gladiolus*. *Acta Horticulturae*, **177**, 323-330.

HALEVY, A.H. & SHOUB, J. (1964). The effect of cold storage and treatment with gibberellic acid on flowering and bulb yields of Dutch *Iris*. *Journal of Horticultural Science*, **39**, 120-129.

HANG, A. & TSUCHIYA, T. (1988). Chromosome studies in the genus *Alstroemeria* II. Chromosome constitutions of eleven additional cultivars. *Plant Breeding*, **100**, 273-279.

HANKS, G.R. & MENHENETT, R. (1983). Responses of potted tulips to novel growth retarding chemicals and interactions with time of rooting. *Scientia Horticulturae*, **21**, 73-83.

HARRISON, M. (1982). The role of cytokinin and auxin transport in apical dominance. *Plant Physiology*, **69**, suppl. 206 (abstract).

HEALY, W.E. & LANG, D. (1985). *Alstroemeria* planting density. *Research Bulletin, Colorado Greenhouse Growers Association*, **415**, 2-3.

HEALY, W. & LANG, D. (1989). Postharvest handling of *Alstroemeria*. *Hortscience*, **24(4)**, 641-643.

HEALY, W.E. & WILKINS, H.E. (1979). Flowering requirements of *Alstroemeria hybrida* 'Regina'. *Hortscience*, **14(3)**, 395 (abstract).

HEALY, W.E. & WILKINS, H.F. (1981). Alstroemerias show promise as energy-efficient crop. *Florists' Review*, **169(4370)**, 16, 40-45.

HEALY, W.E. & WILKINS, H.F. (1982a). Responses of *Alstroemeria* 'Regina' to temperature treatments prior to flower-inducing temperatures. *Scientia Horticulturae*, **17**, 383-390.

HEALY, W.E. & WILKINS, H.F. (1982b). The interaction of temperature on flowering of *Alstroemeria* 'Regina'. *Journal of the American Society for Horticultural Science*, **107(2)**, 248-251.

HEALY, W.E. & WILKINS, H.F. (1985). *Alstroemeria*. *In* Halevy, A.H. (Ed.) *Handbook of Flowering* **1**, 419-424. CRC Press, Boca Raton, Florida.

HEALY, W.E. & WILKINS, H.F. (1986a). *Alstroemeria* culture. *Herbertia*, **24**, 16-20.

HEALY, W.E. & WILKINS, H.F. (1986b). Influence of light treatments before and after induction treatment on flowering of *Alstroemeria* 'Regina'. *Hortscience*, **21(6)**, 1390-1392.

HEALY, W.E. & WILKINS, H.F. (1986c). Relationship between rhizome temperatures and shoot temperatures for floral initiation and cut flower production of *Alstroemeria* 'Regina'. *Journal of the American Society for Horticultural Science*, **111(1)**, 94-97.

HEALY, W.E., WILKINS, H.F. & CELUSTA, M. (1982). Role of light quality, photoperiod and high intensity supplemental lighting on flowering of *Alstroemeria* 'Regina'. *Journal of the American Society for Horticultural Science*, **107(6)**, 1046-1049.

HEBBLETHWAITE, P.D., BATTS, G.R., BARRETT, S.K. & WILTSHIRE, J.J.J. (1986). Growth and seed yield responses to the timing of paclobutrazol (PP333) application in *Lolium perenne* L.. *Journal of Applied Seed Production*, 4, 52-56.

HEBBLETHWAITE, P.D., HAMPTON, J.G. & MCLAREN, J.S. (1982). The chemical control of growth, development and yield of *Lolium perenne* grown for seed. *In* McLaren, J.S. (Ed.) *Chemical Manipulation of Crop Growth and Development*, 505-524. University of Nottingham, 33rd Easter School in Agricultural Science, 505-524.

HEDDEN, P. & GRAEBE, J.E. (1985). Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. *Journal of Plant Growth Regulation*, **4**, 111-122.

HEINS, R.D. & WILKINS, H.F. (1976). *Alstroemeria* cultural research 1975-1976. University of Minnesota, the influence of night interruption with incandescent light and shoot pruning techniques. *Florists' Review*, **159(4123)**, 34-35, 80-81.

HEINS, R.D. & WILKINS, H.F. (1979). Effect of soil temperature and photoperiod on vegetative and reproductive growth of *Alstroemeria* 'Regina'. *Journal of the American Society for Horticultural Science*, **104(3)**, 359-365.

HENNY, R.J. & FOOSHEE, W.C. (1990). Thidiazuron stimulates basal bud and shoot formation in *Alocasia* x *chantrieri* André. *Hortscience*, **25(1)**, 124.

HEPLER, P.K. & WAYNE, R.O. (1985). Calcium and plant development. *Annual Review of Plant Physiology*, **36**, 397-439.

HERTEL, R. & LEOPOLD, A.C. (1963). Versuche zur analyse des auxintransports in der koleoptile von *Zea mays* L.. *Planta*, **50**, 535-562.

HILLMAN, J.R. (1984). Apical dominance. *In* Wilkins, M.B. (Ed.) *Advanced Plant Physiology*, 127-148. Pitman, London.

HUANG, H., YIN, W.S. & ZHANG, G.F. (1989). The effect of paclobutrazol on watermelon growth. *Scientia Horticulturae*, **39**, 9-14.

HUSSEY, G. (1976). *In vitro* release of axillary shoots from apical dominance in monocotyledonous plantlets. *Annals of Botany*, **40**, 1323-1325.

HUSSEY, G., HILTON, J. & LUMSDEN, P. (1980). *In vitro* propagation of *Alstroemeria*. *Annual Report of the John Innes Institute, 1979*, **17**, 56.

HUSSEY, G. (1980). Propagation of some members of the Liliaceae, Iridaceae, and Amaryllidaceae, by tissue culture. *In* Brickel, C., Cutler, D.F. and

Gregory M. (Eds.) *Petaloid Monocotyledons, Horticultural and Botanical Research*. 33-42. Linnaean Society, Symposium Series 8, London, Academic Press.

HUTCHINSON, J. (1959). *The Families of Flowering Plants Vol. II, Monocotyledons*, 2nd edition. London, Oxford University Press.

JACOBS, M. & GILBERT, S.F. (1983). Basal localizaton of the presumptive auxin transport carrier in pea stem cells. *Science*, **220**, 1297-1300.

JACOBS, W.P. & CASE, D.B. (1965). Auxin transport, gibberellin and apical dominance. *Science*, **148**, 1729-1731.

JIAO, J., TSUJITA, M.J. & MURR, D.P. (1986). Effects of paclobutrazol and A-Rest on growth and flowering, leaf carbohydrate and leaf senescence in 'Nellie White' Easter lily (*Lilium longiflorum*) Thunb. *Scientia Horticulturae*, **30**, 135-141.

JONES, J.B. & MURASHIGE, T. (1974). Tissue culture propagation of *Aechmea fasciata* Baker and other bromeliads. *Combined Proceedings of the International Plant Propagators Society*, **24**, 117-126.

KANE, M.E., SHEEHAN, T.J. & FERWERDA, F.H. (1988). *In vitro* growth of American lotus embryos. *Hortscience*, **23(6)**, 611-613.

KANG, B.G., NEWCOMB, W. & BURG, S.P. (1971). Mechanism of auxin-induced ethylene production. *Plant Physiology*, **47**, 504-509.

KAUFMAN, P.B. (1965). The effects of growth substances on intercalary growth and cellular differentiation in developing internodes of *Avena sativa*. *Physiologia Plantarum*, **18**, 703-724.

KAUFMAN, P.B. (1968). Role of gibberellins in the control of intercalary growth and cellular differentiation in developing *Avena* internodes. *Annals of the New York Academy of Sciences*, **144**, 191-203.

KEIL, L. (1986). *Alstroemeria* culture. *Research Bulletin*, **436**, Colorado Greenhouse Growers' Association, Inc., in cooperation with Colorado State University.

KEIL-GUNDERSON, L.S., GOLDSBERRY, K.L. & CHAPMAN, P.L. (1989). Air and substrate temperatures for 'Atlas' and 'Monika' *Alstroemeria*. *Hortscience*, **24(4)**, 613-616.

KERNS, H.R. & MEYER, M.M. JR. (1986). Tissue culture propagation of *Acer* x *freemanii* using thidiazuron to stimulate shoot tip proliferation. *Hortscience*, **21(5)**, 1209-1210.

KHUNACHAK, A., CHIN, C., LE, T. & GIANFANGA, T. (1987). Promotion of asparagus shoot and root growth by growth retardants. *Plant Cell, Tissue and Organ Culture*, **11**, 97-110.

KIM, Y.S., SAKIYAMA, R. & TAZUKE, A. (1989). Effect of temperature on the elongation and the estimation of weight of asparagus spears. *Journal of the Japanese society for Horticultural Science*, **58(1)**, 155-160.

KING, J.J. & BRIDGEN, M.P. (1987). *In vitro* induction of axillary and adventitious shoots in *Alstroemeria*. *Hortscience*, **22**, 1149, (abstract 799).

KROGT, T.M. VAN DER (1985). Autumn supplementary lighting of *Alstroemeria* leads to winter flowering. *Vakblad voor de Bloemisterij*, **40(36)**, 50-57.

LAKSHMI, N. (1980). Cytotaxanomical studies in eight genera of Amaryllidaceae. *Cytologia*, **45**, 663-673.

LAU, O. & YANG, S.F. (1973). Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production. *Plant Physiology*, **51**, 1011-1014.

LEAKEY, R.R.B., CHANCELLOR, R.J. & VINCE-PRUE, D. (1975). Parental factors in dominance of lateral buds on rhizomes of *Agropyron repens* (L.) Beauv.. *Planta* (Berl.), **123**, 267-274.

LEAKEY, R.R.B., CHANCELLOR, R.J. & VINCE-PRUE, D. (1978). Regeneration from rhizome fragments of *Agropyron repens* (L.) Beauv. III. Effects of nitrogen and temperature on the development of dominance amongst shoots on multi-node fragments. *Annals of Botany*, **42**, 197-204.

LELIVELD, H.P.J. (1973). Early planting of Alstroemerias is necessary. *Vakblad voor de Bloemisterij*, **28(33)**, 11.

LENTON, J.R., HEDDEN, P. & GALE, M.D. (1987). Gibberellin insensitivity and depletion in wheat. Consequences for plant development. *In* Hoad, G.V., Lenton, J.R., Jackson, M.B. and Atkin, R.K. (Eds.) *Hormone Action in Plant Development - A Critical Appraisal*, 133-144. London, Butterworth.

LEWNES, M.A. & MOSER, B.C. (1976). Growth regulator effects on apical dominance in English ivy. *Hortscience*, **11(5)**, 484-485.

LEVER, B.G., SHEARING, S.J. & BATCH, J.J. (1982). PP333 - A new broad spectrum growth retardant. *Proceedings 1982 British Crop Protection Conference - Weeds*, 3-10.

LIN, W.C. (1984). The effect of soil cooling and high intensity supplementary lighting on flowering of *Alstroemeria* 'Regina'. *Hortscience*, **19(4)**, 515-516.

LIN, W.C. (1985). Influence of soil cooling and high intensity lighting on the growth and flowering of *Alstroemeria* 'Regina'. *Hortscience*, **20(3)**, 378-380.

LIN, W.C. & MOLNAR, J.M. (1983). Effect of photoperiod and high intensity supplementary lighting on flowering of *Alstroemeria* 'Orchid' and 'Regina'. *Journal of the American Society for Horticultural Science*, **108(6)**, 914-917.

LIN, W.C. & MONETTE, P.L. (1987). *In vitro* propagation of *Alstroemeria* 'Alsaan'. *Plant Cell and Organ Culture*, **9**, 29-35.

LIU, P.B.W. & LOY, J.B. (1976). Action of gibberellic acid on cell proliferation in the subapical shoot meristem of watermelon seedlings. *American Journal of Botany*, **63(5)**, 700-704.

LUMSDEN, P.J., PRYCE, S. & LEIFERT, C. (1990). Effect of mineral nutrition on the growth and multiplication of *in vitro* cultured plants. *In* Nijkamp, H.J.J., Van Der Plas, L.H.W. and Van Aartrijk, J. (Eds.) *Progress in Plant Cellular and Molecular Biology*, 108-113. Proceedings of the VIIth International Congress on Plant Tissue and Cell Culture, Amsterdam, 1990.

LURSSEN, K. (1987). The use of inhibitors of gibberellin and sterol biosynthesis to probe hormone action. *In* Hoad, G.V., Lenton, J.R., Jackson, M.B. and Atkins, R.K. (Eds.) *Hormone Action in Plant Development - A Critical Appraisal*, 133-144. London, Butterworth.

MABBERLEY, D.J. (1987). *The Plant Book*. Cambridge University Press.

MARKS, T.R. & WILTSHIRE, S. (1984). *In vitro* Culture of *Acer* spp. *Annual Report East Malling Research Station*, 90-91.

MCARTHUR, D.A.J. & EATON, G.W. (1987). Effect of fertilizer, paclobutrazol and chlormequat, on strawberry. *Journal of the American Society for Horticultural Science*, **112(2)**, 241-246.

MCCOMB, J.A. (1985). Micropropagation of the rare species *Stylidium coroniforme* and other *Stylidium* species. *Plant Cell, Tissue and Organ Culture*, **4**, 151-158.

MCINTYRE, G.I. (1969). Apical dominance in the rhizome of *Agropyron repens*. Evidence of competition for carbohydrate as a factor in the mechanism of inhibition. *Canadian Journal of Botany*, **47**, 1189-1197.

MCINTYRE, G.I. (1971). Studies on bud development in the rhizome of *Agropyron repens*. II. The effect of the nitrogen supply. *Canadian Journal of Botany*, **50**, 393-401.

MCINTYRE, G.I. (1977). The role of nutrition in apical dominance. *Symposia of the Society for Experimental Biology*, **31**, 251-273.

MCINTYRE, G. & DAMSON, E. (1988). Apical dominance in *Phaseolus vulgaris*. The triggering of shoot decapitation and leaf excision on growth of lateral buds. *Physiologia Plantarum*, **74**, 607-614.

McKinless, J., Alderson, P.G. & Rice, R.D. (1988). Rooting of *Lapageria rosea* shoots multiplied *in vitro*. *Acta Horticulturae*, **226**, 73-79.

Menhenett, R. (1981). Studies with plant growth regulators. *Report Glasshouse Crops Research Institute 1980*, 76-77.

Menhenett, R. & Hanks, G.R. (1982).New retardant shows promise for pot grown lilies and tulips. *Grower*, **97**, 17-20.

Menhenett, R. & Hanks, G.R. (1982/1983). Comparisons of a new triazole retardant PP333 with ancymidol and other compounds on pot grown tulips. *Plant Growth Regulation*, **1**, 173-181.

Metraux, J-P. (1987). Gibberellins and plant cell elongation. *In* Davies, P.J. (Ed.) *Plant Hormones and their Role in Plant Growth and Development*, 296-317. Dordrecht, Martinus Nijhoff.

Meyer, H.J. & van Staden, J. (1988). *In vitro* multiplication of *Ixia flexuosa*. *Hortscience*, **23(6)**, 1070-1071.

Meyer, M.M. & Kerns, H.R. (1986). Thidiazuron and *in vitro* shoot proliferation of *Celtis occidentalis* L.. *VIth International Congress of Plant Tissue and Cell Culture*, 149 (abstract).

Miller, W.B. & Langhans, R.W. (1989). Reduced irradiance affects dry weight partitioning in Easter Lily. *Journal of the American Society for Horticultural Science*, **114(2)**, 306-309.

Mitchell, K.J. (1953a). Influence of light and temperature on the growth of ryegrass (*Lolium spp.*). I. Pattern of vegetative development, *Physiologia Plantarum*, **6**, 21-47.

Mitchell, K.J. (1953b). Influence of light and temperature on the growth of ryegrass (*Lolium spp.*). II. The control of lateral bud development. *Physiologia Plantarum*, **6**, 425-443.

Mok, M.C. & Mok, D.W.S. (1985). The Metabolism of [^{14}C]-thidiazuron in callus tissues of *Phaseolus lunatus*. *Physiologia Plantarum*, **65**, 427-432.

Mok, M.C., Mok, D.W.S., Armstrong, D.J., Shudo, K., Isogai, Y. & Okamoto, T. (1982). Cytokinin activity of N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (thidiazuron). *Phytochemistry*, 21(7), 1509-1511.

Mok, M., Mok, D., Turner, J. & Mujer, C. (1987). Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture systems. *Hortscience*, **22(6)**, 1194-1197.

Molnar, J. (1975). *Alstroemeria* - A promising new cut flower. *Ohio Florists' Association, Bulletin*, **553**.

MORGAN, P.W., BEYER. E. JR. & GAUSMAN, H.W. (1968). Ethylene effects on auxin physiology. *In* Wightman, F. and Setterfield, G. (Eds.) *Biochemistry and Physiology of Plant Growth Substances*, 1225-1273. Ottowa, Runge.

MORRIS, D.A., KADIR. G.O. & BARRY, A.J. (1973). Auxin transport in intact pea seedlings (*Pisum sativum* L.). The inhibition of transport by 2,3,5-triiodobenzoic acid. *Planta*, **110**, 173-182.

MURASHIGE. T. & SKOOG. F. (1962). A revised medium for rapid growth and bioassays with tobacco cell cultures. *Physiologia plantarum*, **15**, 473-497.

NIEDERGANG-KAMIEN. E. & SKOOG, F. (1956). Studies on polarity and auxin transport in plants. I. Modification of polarity and auxin transport by triiodobenzoic acid. *Physiologia Plantarum*, **9**, 60-73.

NIEDZ, R.P., SCHILLER SMITH. S., DUNBAR, K.B., STEPHENS, C.T. & MURAKISHI. H.H. (1989). Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo*. *Plant Cell, Tissue and Organ Culture*. **18(3)**. 313-319.

NIEUWKERK. J.P. VAN. ZIMMERMAN, R.H. & FORDHAM, I. (1986). Thidiazuron stimulation of apple shoot proliferation *in vitro*. *Hortscience*, **21(3)**, 516-518.

NOORDEGRAAF. C.V. (1972). Advancing flowering of Alstroemerias. *Vakblad voor de Bloemisterij*. **27(40)**, 13.

NOORDEGRAAF. C.V. (1975). Temperature and daylength requirements of *Alstroemeria*. *Acta Horticulturae*, **51**, 267-274.

OHYAMA, K. & OKA. S. (1982). Multiple shoot formation from mulberry (*Morus alba* L.) hypocotyls by N-(2-chloro-4-pyridyl)-N'-phenylurea. *In* Fujiwara. A. (Ed.) *Plant Tissue Culture 1982* (Proceedings of the Fifth International Congress on Plant Tissue and Cell Culture), 149-150. Tokyo, International Association for Plant Tissue Culture.

OKUBO, H. & UEMOTO,S. (1985). Changes in endogenous gibberellin and auxin activities during first internode elongation in tulip flower stalk. *Plant Cell Physiology*. **26(4)**. 709-719.

OSBORNE, D.J. & MULLINS. M.G. (1969). Auxin, ethylene and kinetin in a carrier protein model system for the polar transport of auxins in petiole segments of *Phaseolus vulgaris*. *New Phytologist*, **68**, 977-991.

PALMER, J.H. & HALSALL. D.M. (1969). Effect of transverse gravity stimulation, gibberellin and indoleacetic acid upon polar transport of IAA^{C14} in the stem of *Helianthus annuus*. *Physiologia Plantarum*, **22**, 59-67.

PHILLIPS, I.D.J. (1975). Apical dominance. *Annual Review of Plant Physiology*, **26**, 341-367.

PIERIK, R.L.M. (1987). *In Vitro Culture of Higher Plants*. Dordrecht, Martinus Nijhoff.

PIERIK, R.L.M. & STEEGMANS, H.H.M. (1975). Effect of auxins, cytokinins, gibberellins, abscisic acid and ethephon on regeneration and growth of buiblets on exised bulb scale segments of hyacinth. *Physiologia Plantarum*, **34**, 14-17.

PIERIK, R.L.M., VAN VOORST, A., BOOY, G., VAN ACKER, C.A.M., LELIVELT, C.L.C. & DE WIT, J.C. (1988). Vegetative propagation of *Alstroemeria* hybrids *in vitro*. *Acta Horticulturae*, **226**, 81-89.

PILET, P.E. (1965). Action of gibberellic acid on auxin transport. *Nature*, **208**, 1344-1345.

POWELL, M.C. & BUNT, A.C. (1984). Periodicity of growth in the *Alstroemeria* cultivars 'Campfire', 'Red Sunset' and 'Zebra'. *Scientia Horticulturae*, **24**, 359-367.

POWELL, M.C. & BUNT, A.C. (1986). The effects of propagation date on flower production in *Alstroemeria* 'Campfire' and 'Red Sunset'. *Scientia Horticulturae*, **28**, 147-157.

PRIESTLY, J.H., SCOTT, L.I. & GILLETT, E.C. (1935). The development of the shoot in *Alstroemeria* and the unit of shoot growth in monocotyledons. *Annals of Botany*, **49**, 161-179.

PUNTIERI, J.G. & GOMEZ, I.A. (1988). Análysis del crecimiento vegetativo del amancay (*Alstroemeria aurantiaca* D. Don.). *Revista Chilena de Historia Natural*, **61(2)**, 177-185.

QUINLAN, J.D. & RICHARDSON, P.J. (1984). Effects of paclobutrazol (PP333) on apple shoot growth. *Acta Horticulturae*, **146**, 105-111.

RANDHAWA, G.S. & MAHEY, R.K. (1984). Effects of chemicals on emergence of tumeric (*Curcuma longa* L.). *Journal of Research, Punjab Agricultural University*, **21(3)**, 470-471.

RASKIN, I. & KENDE, H. (1984). Role of gibberellin in the growth response of submerged deep water rice. *Plant Physiology*, **76**, 947-950.

REES, A.R. (1972). *The growth of bulbs*. Academic Press, London.

REES, A.R., MENHENETT, R & HANKS, G.R. (1982). Growth retardants for pot plant production of tulips and mid-century hybrid lilies. *21st International Horticultural Congress*, 1701, (abstract 2).

RICHARDS, J.H., MUELLER, R.J. & MOTT, J.J. (1988). Tillering in tussock grasses in relation to defoliation and apical bud removal. *Annals of Botany*, **62**, 173-179.

ROBERTS, J.A. & HOOLEY, R. (1988). *Plant Growth Regulators*. London, Blackie.

ROBINSON, G.W. (1963). *Alstroemeria*. *Journal of the Royal Horticultural Society*, **88**, 490-494.

RUBERY, P.H. (1979). The effects of [(3-phenyl-1,2,4-thiadiazol-5-yl)-thio]acetic acid, a synthetic growth regulator, on auxin uptake by tobacco stem segments and crown gall suspension culture cells. *Plant Science Letters*, **14**, 365-371.

RUBERY, P.H. (1980). The mechanism of transmembrane auxin transport and its relation to the chemiosmotic hypothesis of polar transport of auxin. *In* Skoog, F. (Ed.) *Plant Growth Substances*, 50-60. Berlin, Springer.

RUBERY, P.H. (1987a). Auxin transport. *In* Davies, P.J. (Ed.) *Plant Hormones and their Role in Plant Growth and Development*, 341-362. Dordrecht, Martinus Nijhoff.

RUBERY, P.H. (1987b). Manipulation of hormone transport in physiological and developmental studies. *In* Hoad, G.V., Lenton, J.R., Jackson, M.B. and Atkin, R.K. (Eds.) *Hormone Action in Plant Development - A Critical Appraisal*. 161-174. London, Butterworth.

SACHS, T. & THIMANN, K.V. (1964). Release of lateral buds from apical dominance. *Nature*, **201**, 939-940.

SACHS, T. & THIMANN, K.V. (1967). The role of auxins and cytokinins in the release of buds from dominance. *American Journal of Botany*, **54(1)**, 136-144.

SAKAI, S. & IMASEKI, H. (1971). Auxin-induced ethylene production by mungbean hypocotyl segments. *Plant and Cell Physiology*, **12**, 349-359.

SALINGER, J.P. (1985). *Alstroemeria*. *Commercial Flower Growing*, 115-118. Wellington, Butterworths Horticultural books.

SALTVEIT, M.E. & FONTENO, W.C. (1983). Auxin transport in *Dracaena marginata* stems. *Journal of the American Society for Horticultural Science*, **108(2)**, 183-186.

SCOTT, T.K., CASE, D.B. & JACOBS, W.P. (1967). Auxin-gibberellin interaction in apical dominance. *Plant Physiology*, **42**, 1329-1333.

SHANKS, J.B. (1980). Chemical dwarfing of several ornamental greenhouse crops with PP333. *Proceedings of the 7th Meeting of the Plant Growth Regulators Working Group*, 46-52. Plant Growth Regulator Society of America.

SHEARING, S.J. & BATCH, J.J. (1982). Amenity grass retardation - some concepts challenged. *In* McLaren, J.S. (Ed.) *Chemical Manipulation of Crop Growth and Development*, 467-483. University of Nottingham, 33rd Easter School in Agricultural Science.

SHELDRAKE, A.R. & NORTHCOTE, D.H. (1968). The production of auxin by tobacco internode tissues. *New Phytologist*, **67**, 1-13.

SIMS, T.V. (1985). Alternative cut flower crops. *In Project No. 2 in the Agricultural Service Overseas Study Tour Programme 1984/1985*, 6-10. Ministry of Agriculture, Fisheries and Food.

SKUTERUD, R. (1984). Growth of *Elymus repens* (L.) Gould and *Agrostis gigantea* Roth. at different light intensities. *Weed Research*, **24**, 51-57.

SMITH, D.R. & LANGHANS, R.W. (1962). The influence of photoperiod on the growth and flowering of Easter Lily (*Lilium longiflorum* Thunb. var. 'Croft'). *Proceedings of the American Society for Horticultural Science*, **80**, 599-604.

SMITH, E.F., ROBERTS, A.V. & MOTTLEY, J. (1990). The preparation *in vitro* of chrysanthemum for transplantation to soil. 2. Improved resistance to dessication conferred by paclobutrazol. *Plant Cell, Tissue and Organ Culture*, **21**, 133-140.

SMITH, M. & BRIDGEN, M.P. (1989). Effect of calcium and iron on the *in vitro* growth of *Alstroemeria*. *Hortscience*, **24**, 94 (abstract 278).

STINCHCOMBE, G.R., COPAS, E., WILLIAMS, R.R. & ARNOLD, G. (1984). The effects of paclobutrazol and daminozide on the growth and yield of cider apple trees. *Journal of Horticultural Science*, **53(3)**, 323-327.

STINSON, H.L. (1952). Amaryllid culture - advances in *Alstroemeria* culture. *Herbertia*, **8**, 99-105.

STODDART, J.L. (1987). Biochemical considerations in developmental studies. *In* Hoad, G.V., Lenton, J.R., Jackson, M.B. and Atkin, R.K. (Eds.) *Hormone Action in Plant Development - A Critical Appraisal*, 275-286. London, Butterworth.

STOWE, B.B. & YAMAKI, T. (1957). The history and physiological action of the gibberellins. *Annual Review of Plant Physiology*, **8**, 181-216.

STUART, D.A. & JONES, R.L. (1977). Roles of extensibility and turgor in gibberellin- and dark-stimulated growth. *Plant Physiology*, **59**, 61-68.

SUTTLE, J.C. (1984a). Effect of the defoliant thidiazuron on ethylene evolution from mungbean hypocotyl segments. *Plant Physiology*, **75**, 902-907.

SUTTLE, J.C. (1984b). Effects of the defoliant thidiazuron on leaf abscission and ethylene evolution from cotton seedlings. *In* Fuchs, Y. and Chalutz, E. (Eds.) *Ethylene: Biochemical, Physiological and Applied Aspects*, 277-278. Dordrecht, Martinus Nijhoff.

SUTTLE, J.C. (1985). Involvement of ethylene in the action of the cotton defoliant thidiazuron. *Plant Physiology*, **78**, 272-276.

TAKAHASHI, S., SHUDO, K., OKAMOTO, T., YAMADA, K. & ISOGAI, Y. (1978). Cytokinin activity of N-phenyl-N'-(4-pyridyl)urea derivatives. *Phytochemistry*, **17**, 1201-1207.

TAKATORI, F.H. (1985). *Asparagus officinalis*. *In* Halevy, A.H. (Ed.) *Handbook of Flowering*, **1**, 517-520. CRC Press, Boca Raton, Florida.

TAMAS, I.A. (1987). Hormonal regulation of apical dominance. *In* Davies, P.J. (Ed.) *Plant Hormones and their Role in Plant Growth and Development*. 393-410. Dordrecht, Martinus Nijhoff.

THOMSON, K-S., HERTEL, R., MÜLLER, S. & TAVARES, J.E. (1973). 1-N-Naphthylphthalamic acid and 2,3,5-triiodobenzoic acid: *in vitro* binding to particulate cell fractions and action on auxin transport in corn coleoptiles. *Planta*, **109**, 337-352.

THOMAS, J.C. & KATTERMAN, F.R.H. (1986). Cytokinin activity induced by thidiazuron. *Plant Physiology*, **81**, 681-683.

TORREY, J.G. (1973). Hormonal regulation of plant growth and morphogenesis. *Science*, **181**, 1075-1076.

TRAN THANH VAN, K.M. (1981). Control of morphogenesis in *in vitro* systems. *Annual Review of Plant Physiology*, **32**, 291-311.

TROMP, J. (1987). Growth and flower-bud formation in apple as affected by paclobutrazol, daminozide and tree orientation in combination with various gibberellins. *Journal of Horticultural Science*, **62(4)**, 433-440.

TSUCHIYA, T., HANG, A., HEALY, W.E. & HUGHES, H. (1987). Chromosome studies in the genus *Alstroemeria*. 1. Chromosome numbers in 10 cultivars. *Botanical Gazette*, **148(3)**, 519-524.

TSUCHIYA, T. & HANG, A. (1987). Chromosome studies in the genus *Alstroemeria*. *Acta Horticulturae*, **205**, 281-287.

TUCKER, D.J. (1976). Endogenous growth regulators in relation to side shoot development in the tomato. *New Phytologist*, **77**, 561-568.

TUCKER, D.J. (1977). Hormonal regulation of lateral bud outgrowth in the tomato. *Plant Science Letters*, **8**, 105-111.

TUCKER, D.J. (1978). Apical dominance in the tomato: the possible roles of auxin and abscisic acid. *Plant Science Letters*, **12**, 273-278.

UPHOF, J.C.T. (1952). A review of the genus *Alstroemeria*. *Plant Life* (*Herbertia* ed.), **8**, 36-53.

VERBOOM, H. (1979). Alstroemerias and some other flower crops for the future. *Scientific Horticulture*, **31**, 33-42.

WANG, S.Y., STEFFENS, G.L. & FAUST, M. (1986). Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. *Phytochemistry*, **25(2)**, 311-317.

WANG, T.L. & WAREING, P.F. (1979). Cytokinins and apical dominance in *Solanum andigena*: lateral shoot growth and endogenous cytokinin levels in the absence of roots. *New Phytologist*, **82**, 19-28.

WARDLE, K., DALSOU, V., SIMPKINS, I. & SHORT, K.C. (1983a). Redistribution of rubidium in plants of *Chrysanthemum morifolium* Ram. cv. 'Snowdon' derived from tissue cultures and transferred to soil. *Annals of Botany*, **51**, 261-264.

WARDLE, K., DIXON, P.A. & SIMPKINS, I. (1981). Sodium accumulation by leaves of cauliflower plantlets and the effect of the mode of plant formation. *Annals of Botany*, **47**, 653-659.

WARDLE, K., DOBBS, E.B. & SHORT, K.C. (1983b). *In vitro* acclimatization of aseptically cultured plantlets to humidity. *Journal of the American Society for Horticultural Science*, **108**, 386-389.

WARDLE, K., QUINLAN, A. & SIMPKINS,I. (1979). Abscisic acid and the regulation of water loss in plantlets of *Brassica oleracea* L. var. *botrytis*, regenerated through apical meristem culture. *Annals of Botany*, **43**, 745-752.

WEBSTER, A.D. & QUINLAN, J.D. (1984). Chemical control of tree growth of plum (*Prunus domestica* L.). I. Preliminary studies with the growth retardant paclobutrazol. *Journal of Horticultural Science*, **59(3)**, 367-375.

WEILER, T.C. (1973). Cold and daylength requirements for flowering in a *Lilium speciosum* Thunb. cultivar. *Hortscience*, **8(3)**, 185.

WEILER, T.C. & LANGHANS, R.W. (1972). Growth and flowering responses of *Lilium longiflorum* Thunb. 'Ace' to different daylengths. *Journal of the American Society for Horticultural Science*, **97(2)**, 176-177.

WHITE, P.R. (1963). *The Cultivation of Animal and Plant Cells*, 2nd edition. New York, Roland Press.

WIDHOLM, J.M. & SCHAFFER, R. (1971). The effect of TIBA and 2,4-D on growth and metabolic processes of soybean hypocotyl sections. *Phytochemistry*, **10**, 1213-1222.

WILKINS, H.F., HEALY, W.E. & GILBERTSON-FERRIS, T.L. (1980). Comparing and contrasting the control of flowering in *Alstroemeria* 'Regina', *Freesia* x *'hybrida'* and *Lilium longiflorum*. *In* Brickel, C., Cutler, D.F. and Gregory, M. (Eds.) *Petaloid Monocotyledons, Horticultural and Botanical Research*, 51-63. Linnaean Society Symposium Series 8, London, Academic Press.

WILKINS, H.F. & HEINS, R.D. (1976). *Alstroemeria* general culture. *Florists' Review*, **159(4121)**, 30-31, 78-80.

WILLIS, J.C. (1985). *A Dictionary of the Flowering Plants and Ferns*, 8th edition. Cambridge University Press.

WILSON, J. (1979). *Alstroemeria* - The flower crop of the 80's. *Horticulture Industry*, July 1979, 14-15.

WINSKI, P.J. & BRIDGEN, M.P. (1988). Embryo rescue of *Alstroemeria*. *Hortscience*, **23**, 758 (abstract).

WOODWARD, E.J. & MARSHALL, C. (1988). Effects of plant growth regulators and nutrient supply on tiller bud outgrowth in barley (*Hordeum distichum* L.). *Annals of Botany*, **61**, 347-354.

WOOLLEY, D.J. & WAREING, P.F. (1972). The interaction, movement and metabolism of a cytokinin in rootless cuttings. *New Phytologist*, **71**, 781-793.

YAMADA, Y., SATO, F. & HAGIMORI, M. (1978). Photoautotropism in green cultured cells. *In* Thorpe, T.A. (Ed.) *Frontiers of Plant Tissue Culture, 1978*, 453-462. International Association for Plant Tissue Culture.

YEANG, H.Y. & HILLMAN, J.R. (1981). Control of lateral bud growth in *Phaseolus vulgaris* L. by ethylene in the apical shoot. *Journal of Experimental Botany*, **32**, 395-404.

YEANG, H.Y. & HILLMAN, J.R. (1982). Lateral bud growth in *Phaseolus vulgaris* L. and the levels of ethylene in bud and adjacent tissue. *Journal of Experimental Botany*, **33**, 111-117.

YIP, W-K. & YANG, S.F. (1986). Effect of thidiazuron, a cytokinin-active urea derivative, in cytokinin-dependent ethylene production systems. *Plant Physiology*, **80**, 515-519.

YOSHII, H. & IMASEKI, H. (1981). Biosynthesis of auxin-induced ethylene. Effects of indole-3-acetic acid, benzyladenine and abscisic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. *Plant and Cell Physiology*, **22(3)**, 369-379.

ZEISLIN, N. & TSUJITA, M.J. (1988). Regulation of stem elongation of lilies by temperature and the effect of gibberellin. *Scientia Horticulturae*, **37**, 165-169.

ZIV, M. (1989). Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. *Plant Cell, Tissue and Organ Culture*, **17**, 101-110.

ZIV, M. (1990). Morphogenesis of gladiolus buds in bioreactors - implication for scaled-up propagation of geophytes. *In* Nijkamp, H.J.J., van der Plas, L.H.W. and van Aartrijk, J. (Eds.) *Progress in Plant Cellular and Molecular Biology*, 119-124. Proceedings of the VIIth International Congress on Plant Tissue and Cell Culture, Amsterdam, 1990.

ZIV, M., KANTEROVITZ, R. & HALEVY, A.H. (1973). Vegetative propagation of *Alstroemeria in vitro*. *Scientia Horticulturae*, **1**, 271-277.

ZIV, M., YOGEV, T. & KREBS, O. (1986). Effects of paclobutrazol and chlormequat on growth pattern and shoot proliferation of normal and variant *Aechmea fasciata* Baker plants regenerated *in vitro*. *Israel Journal of Botany*, **35**, 175-182.

Appendix A: Formulation of Murashige and Skoog (MS) Revised Medium

Stock solution	Stock concentration ($g\,l^{-1}$)	Volume of stock solution for 1 l of medium (ml)	Final concentration ($mg\,l^{-1}$)
$CaCl_2.2H_2O$	44.0000	10	440.000
KNO_3	76.0000	25	1900.000
NH_4NO_3	165.0000	10	1650.000
KH_2PO_4	17.0000	10	170.000
$MgSO_4.7H_2O$	37.0000	10	370.000
$MnSO_4.4H_2O$	2.2300	10	22.300
H_3BO_3	0.6200	10	6.200
$ZnSO_4.7H_2O$	0.8600	10	8.600
$Na_2MoO_4.2H_2O$	0.0250	10	0.250
$CoCl_2.6H_2O$	0.0025	10	0.025
KI	0.0830	10	0.830
$CuSO_4.5H_2O$	0.0025	10	0.025
$FeSO_4.7H_2O$	2.7850	10	27.850
Na_2EDTA	3.7250	10	37.250
Inositol	10.0000	10	100.000
Glycine	0.2000	10	2.000
Nicotinic acid	0.0500	10	0.500
Pyridoxine-HCl	0.0500	10	0.500
Thiamine-HCl	0.0100	10	0.100

Na_2EDTA - ethylenediaminotetraacetic acid, disodium salt.

Other components: sucrose $30g\,l^{-1}$ and agar (Difco Bacto) $10g\,l^{-1}$.

From Murashige and Skoog, 1962.

Appendix B: Formulation of *Alstroemeria* Production Medium

All the components, methods of formulation and storage, are the same as in MS, except for the concentrations of the vitamins, sucrose and agar.

Stock solution	Stock concentration ($g\,l^{-1}$)	Volume of stock solution for 1 l of medium (ml)	Final concentration ($mg\,l^{-1}$)
Nicotinic acid	0.1	10	1
Pyridoxine-HCl	0.1	10	1
Thiamine-HCl	0.1	10	1

Other components: sucrose 40 $g\,l^{-1}$ and agar 8 $g\,l^{-1}$.

From Parigo Horticultural Co., (personal communication).

Appendix C: Stock Solutions of Growth Regulators

- **6-Benzylaminopurine (BAP).**

  100 mg l^{-1}: initially dissolved in 0.1 M hydrochloric acid solution, then made up to final volume with distilled water.

- **α-Naphthaleneacetic acid (NAA), and gibberellic acid (GA_3).**

  100 mg l^{-1}: initially dissolved in 0.1 M potassium hydroxide solution, then made up to final volume with distilled water.

- **2,3,5-Triiodobenzoic acid (TIBA).**

  100 mg l^{-1}: dissolved and made up to final volume in distilled water.

- **Thidiazuron** (Schering Agrochemicals Ltd).

  10 mg l^{-1}: dissolved and made up to final volume in distilled water.

- **Paclobutrazol** (ICI Ltd).

  100 mg l^{-1}: mixed with and made up to final volume in distilled water.

Stock solutions were stored at 5°C in the dark.

Appendix D: Preparation of Extracts from *Alstroemeria* Tubers

Soil was removed from the tubers by washing under cold running water. The clean tubers were then homogenized in a blender with distilled water, 100 g of tuber being added to every 200 ml of distilled water. The resulting mixture was strained through cloth to remove the majority of the plant debris. The liquid produced was centrifuged for 15 minutes at 3500 revolutions per minute (r.p.m.) to remove remaining small amounts of plant debris. The supernatant was decanted from the pellet and stored frozen until required.

This procedure was performed as rapidly as possible on ice, using distilled water chilled to 2 to 4°C, to minimize the natural degradative reactions brought about as a response to the homogenization of the plant tissues.

This method was adapted from Chuang *et al.* (1978).