MANIPULATION OF FLOWERING PERIOD AND SHOOT MULTIPLICATION IN *CLIVIA MINIATA* (LINDLEY) REGEL

by

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To my mother

Elle a fermé sa vie comme un livre d'images
Sur les mots les plus doux qui se soient jamais dits
Elle qui croyait l'amour perdu dans les nuages
Elle l'a redécouvert au creux du dernier lit

Et riche d'un sourire au terme du voyage
Elle a quitté son corps comme on quitte un bateau
En emportant la paix gravée sur son visage
En nous laissant au cœur un infini fardeau

Elle souriait de loin du cœur de la lumière
Son âme était si claire aux franges de la nuit
On voyait du bonheur jusque dans sa misère
Tout l'amour de la terre qui s'en allait sans bruit

Comme autour d'un chagrin les voix se font plus tendres
Un ecrin de silence entourait nos regards
Les yeux n'ont plus besoin de mots pour se comprendre
Les mains se parlent mieux pour se dire au revoir

Moi qui ne savait rien de la vie éternelle
J'espérais qu'au-delà de ce monde de fous
Ceux qui nous ont aimés nous restent encore fidèles
Et que parfois leur souffle arrive jusqu'à nous

Elle souriait de loin du cœur de la lumière
Et depuis ce jour là je sais que dans sa nuit
Il existe un ailleurs où l'âme est plus légère
Et que j'aurais moins peur d'y voyager aussi

Elle a fermé sa vie comme un livre d'images
Sur les mots les plus doux qui se soient jamais dits
Elle qui croyait l'amour perdu dans les nuages
Elle l'a redécouvert au creux du dernier lit

Et riche d'un sourire au terme du voyage
Elle a quitté son corps comme on quitte un ami
En emportant la paix gravée sur son visage
En nous laissant à l'âme une peine infinie

À Ma Mère
Yves Duteil

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Abstract

Clivia miniata Regel is widely cultivated as a garden ornamental and a pot plant and can be forced to flower outside the natural flowering period by applying a cold treatment. Under local conditions this could be achieved by using a treatment of 7.5 -10 °C for 14 days. This caused a significant number of the cold treated plants to flower earlier in the season with the result that the naturally short flowering period could be extended. The 14 day period of cold treatment which was required was shorter than a period of 60 days previously described.

Little information exists regarding its use as a cut flower. It was found that inflorescences could be harvested at the stage when all flowers were still closed and that more than 90% of flowers opened and developed normal colouration either in distilled water or in a commercial postharvest product (Chrysal AKC™). The product also reduced the incidence of stem splitting.

Clivia has been propagated *in vitro* with varying degrees of success but the methods are still relatively slow. Commercial protocols for propagation exist but have not been published. Therefore, the use of *in vivo* foliar applications of paclobutrazol (PAC) and Promalin™ (PRO), to stimulate branching and shoot formation, was investigated. The main effect of PAC could be seen as the stimulation of bud formation from meristematic zones on the abaxial side of leaf bases in the older, proximal axils. The mean number of shoots produced by PAC at concentrations between 250 and 25 000 ppm varied from 2.3 to 7.1 per plant, without any statistically significant difference between treatments. However, at concentrations of 5 000 ppm and higher, growth inhibition was unacceptable. PRO had the effect of stimulating bud formation from leaf bases situated near the apical meristem, in the younger distal axils. PRO also caused dichotomous branching of apical meristems. PRO applied 10 times, at 200 or 500 ppm a.i., resulted in branching of 50% of treated plants into 2 or 3 modules. However, the latter results could not be analysed statistically. The most significant benefit arising from the use of PRO was survival of the parent plant without any inhibition of vegetative growth. Both PAC and PRO had a negetive effect on flowering at the concentrations tested. The use of PAC to stimulate *in vitro* shoot formation was attempted unsuccessfully, probably due to an inappropriate medium composition.

An understanding of plant architecture is important when trying to manipulate propagation and phenology. *Clivia* has a modular growth form and exhibits sympodial branching under natural circumstances.

Keywords : Amaryllidaceae, cut flowers, flower forcing, suckers, vegetative propagation

CONTENTS

Abbreviations

a. i.	: active ingredient
CHRYS	: Chrysal AKC™
cm	: centimeter
2,4-D	: 2,4-dichlorophenoxyacetic acid
dH_2O	: distilled water
g	: gram
GA_3	: gibberellic acid
GA_4	: gibberellin no. 4
GA_7	: gibberellin no. 7
l	: litre
ml	: millilitre
mm	: millimeter
mg	: milligram
M	: Mol / litre
MS	: Murashige and Skoog
PRO	: Promalin™
PAC	: paclobutrazol
ppm	: parts per million

CHAPTER 1

Introduction

1.1 Literature review

1.1.1 Botany and history

The genus *Clivia* Lindl. belongs to the family Amaryllidaceae and comprises 4 species; *C. miniata* (Lindley) Regel, *C. nobilis* Lindl., *C. caulescens* R. A. Dyer and *C. gardenii* Hook. f. Interspecific hybrids have been created (Lötter, 2000) but no reliable reports of successful intergeneric hybrids involving *Clivia* could be found, despite attempts at such crosses having been made (Niederwieser, 2000). The earliest report of an interspecific cross in *Clivia* dates from the nineteenth century (Groenland, 1859). The most recent report on chromosome number indicates that 2n = 22 in all four species of *Clivia* (Ran, Murray & Hammet, 1999). *Clivia* spp. are indigenous to the eastern regions of southern Africa with a climatically varied habitat which includes coastal forest, secondary coastal dunes, inland forests and tree trunks (Winter, 2000). Many cultivated forms exist in shades of pink, orange (Glover, 1985), apricot (Smith, 1999), yellow (Morris, 1990), red (Lötter, 1998) and near white (McNeil, 1985). *Clivia* has a modular, sympodial growth form and inflorescences are produced terminally on modules. This pattern is similar to that seen in other amarylids such as *Hippeastrum* sp. (Rees, 1985) and *Cyrtanthus* sp. (Slabbert, 1997).

Clivia has been in cultivation in Belgium for approximately 150 years where it is still grown today (De Koster, 1998a). It also enjoys popularity in China (Nakamura, 2000), Japan (Nakamura, 1998), Australia (Smith & Henry, 1998) and North America (Koopowitz, 2000).

1.1.2 Cultivation

Investigation of optimal growing conditions for *Clivia* with respect to lighting and temperature have been undertaken in Japan (Mori & Sakanishi, 1974), and Europe (Vissers & Haleydt, 1994, De Smedt, Van Huylenbroeck & Debergh, 1996). An outline of growing conditions and stages of *Clivia* production in Belgium has also been described (De Koster, 1998b). In South Africa, however, *Clivia* production is often undertaken without control of growing temperature and flowering occurs mainly from August to September. Propagation of *Clivia* occurs largely

through seed which results in heterogeneous offspring. However, amongst certain groups or strains, elucidation of the mechanism of inheritance of flower colour makes this characteristic predictable (Morris , 1990, Lötter, 1998, Tarr, 2000). Commercial tissue culture methods for clonal propagation have been developed (Smithers, 2000, Chapman, 1999) but these protocols have not been published. No reports could be found on commercial *Clivia* cut flower production and few reports exist on postharvest treatment of cut flowers (Nowak & Rudnicki, 1990, Zhang *et al.*, 1991).

1.1.3 Biochemistry and physiology

Various biochemical and physiological studies have been conducted on *Clivia,* for example an investigation of the chemical composition of endodermal and hypodermal cell walls (Schreiber *et al.*, 1999) and water relations of the hypodermis (Casado & Heredia, 1998). The structure and function of leaf cuticles has also been studied (Dominguez & Heredia, 1999) and various secondary metabolites have been isolated (Evidente *et al.*, 1999).

1.2 Aims

The aim of this project was to determine whether manipulation of growing temperature as used in Europe could be applied under South African conditions to extend the short natural flowering period of *C. miniata*. It was then felt that if better control of the flowering period could be achieved, more attention might be given to the possibility of *Clivia* as a cut flower crop. Therefore, peduncle splitting and picking stage of *Clivia* cut flowers was investigated. Cut flower cultivation or accurately programmed cultivation requires having large quantities of clonal material available. However, the success gained by following published sources of information on *Clivia* tissue culture is limited. No reports could be found on the use of paclobutrazol to stimulate *in vitro* shoot formation and this was investigated. Nevertheless, tissue culture is not always available to growers and *Clivia* collectors may be reluctant to sacrifice plant material of superior clones. As a result, another alternative was sought. The use of foliar sprays of plant growth regulators to promote shoot formation was investigated. It was felt that an understanding of plant architecture was necessary and an investigation thereof was undertaken.

1.3 References

CASADO, C.G. & HEREDIA, A, 1998. Water permeability of hypodermis isolated from *Clivia miniata* roots. *Z. Naturforsch.* 53(11-12), 1096-1099.

CHAPMAN, A.C., 1999. *In vitro* propagation of *Clivia miniata*. BScAgric(Hons) dissertation. Department of Horticulture, University of Nottingham, England.

DE KOSTER, P., 1998a. History of *Clivia* in Belgium. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 31 -32.

DE KOSTER, P., 1998b. Selection and commercial production of *Clivia* in Europe. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 32-34.

DOMINGUEZ, E. & HEREDIA, A., 1999. Self assembly in plant barrier biopolymers. *Z. Naturforsch.* 54(1-2), 141-143.

DE SMEDT, V., VAN HUYLENBROECK, J. M. & DEBERGH, P.C., 1996. Influence of temperature and supplementary lighting on growth and flower initiation of *Clivia miniata* Regel. *Sci. Hortic.* 65, 65-72.

EVIDENTE, A., ABOU-DONIA, A., DARWISH, F.A., AMER, M.E. & KASSEM, F.F., 1999. Nobilisitine A and B, two masanane type alkaloids from *Clivia nobilis*. *Phytochemistry.* 51(8), 1151-1155.

GLOVER, W.J., 1985. Considerations in *Clivia*. *Herbertia* 41, 30-31.

GROENLAND, J., 1859. L'*Himantophyllum cyrtanthiflorum*. *Revue Horticole*. 8, 258-260.

KOOPOWITZ, H., 2000. *Clivia* names. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 30-33.

LÖTTER, W., 1998. *Clivia* mutations and colour variations. In C.Felbert, J.Winter & M.

Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 63-71.

LÖTTER, W., 2000. Advanced hybridising of Clivias. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 34-41.

MCNEIL, P.G., 1985. Hybridising *Clivia*. *Herbertia* 41, 24-29.

MORI, G. & SAKANISHI, Y., 1974. Effect of temperature on the flowering of *Clivia miniata*. *J. Japan. Soc. Hort. Sci.* 42(4), 326-332.

MORRIS, B., 1990. A true breeding strain of yellow *Clivia*. *Herbertia* 46(2), 95-96.

NAKAMURA, Y., 1998. *Clivia* breeding in Japan. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 21-23.

NAKAMURA, Y., 2000. *Clivia* in China. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 69-70.

NIEDERWIESER, J.G., 2000. Crossing *Clivia* with other amarylids. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 46-47.

NOWAK, J. & RUDNICKI, R. M., 1990. Postharvest handling and storage of cut flowers, florist greens and potted plants. Timber Press, Portland, Oregon. 35, 139.

RAN, Y., MURRAY, B.G. & HAMMET, K.R.W., 1999. Karyotype analysis of the genus *Clivia* by giemsa and fluorochrome banding and in situ hybridisation. *Euphytica*.106, 139-147.

REES, A.R., 1985. *Hippeastrum*. In Halevy, A.H.(ed). CRC handbook of flowering. CRC Press Inc., Boca Raton, Florida, USA. 295.

SCHREIBER, L., HARTMANN, K., SKRABS, M. & ZEIER, J., 1999. Apoplastic barriers in roots : chemical composition of endodermal and hypodermal cell walls. *J. Experiment. Biol.* 50(337), 1267 - 1280.

SLABBERT, M.M., 1997. Inflorescence initiation and development in *Cyrtanthus elatus*. *Scientia Hortic*. 69(1-2), 61-71.

SMITH, K.R., 2000. Named cultivars of *Clivia* in Australia. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 48-54.

SMITH, K.R. & HENRY, P., 1998. *Clivia* in Australia. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 56-59.

SMITHERS, P., 2000. The adventures of a gardener. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 13-14.

TARR, B., 2000. The great yellow *Clivia* jigsaw puzzle. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 10-12.

VISSERS, M. & HALEYDT, B., 1994. Bloeibeïnvloeding bij *Clivia*. *Verbondsnieuws Belg. Sierteelt* 38(4), 36-37.

WINTER, J., 2000. The natural distribution and ecology of *Clivia*. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 5-9.

ZHANG, W., ZHANG, H., GU, Z.P. & ZHANG, J.J.,1991. Causes of senescence of nine flowers. *Acta Botanica Sin*. 33(6), 429-436.

CHAPTER 2

Extending flowering period in *Clivia miniata* (Lindley) Regel using a cold treatment

2.1 Summary

Clivia miniata can be forced to flower out of season by applying a cold treatment which brings about emergence and development of quiescent inflorescences. Plants growing outdoors were exposed to a cold treatment of 7.5 -10 °C for 14 days, commencing during the last week of April. This caused a significant number of the cold treated plants to flower earlier in the season. The implication was that the period during which *Clivia* in flower was available for sale could be extended. The difference between the number of cold treated and control plants in flower was most noticeable in the period from 12 - 19 weeks after commencement of the cold treatment. The 14 day period of cold treatment which was required for successful forcing under local conditions was shorter than a period of 60 days previously described.

2.2 Introduction

Flower forcing is undertaken in many species in order to prolong the period in which plants are in flower or to have plants in flower for specific occasions (De Hertogh *et al.*, 1997). *Hippeastrum* sp. is an example of the Amaryllidaceae which can be successfully forced (Sandler-Ziv *et al.*, 1997). It has been shown that *Clivia miniata* can be brought into flower outside its natural flowering period by manipulating growing temperature (Mori & Sakanishi, 1974, Vissers & Haleydt, 1994, De Smedt, Van Huylenbroeck & Debergh, 1996). *Clivia* has a modular, sympodial growth form and inflorescences form terminally on modules (Chapter 5 of this dissertation). Modules are produced as two or more recurrent flushes per growing season, depending on cultural conditions. Inflorescences remain quiescent until a cold stimulus is received which stimulates elongation of the peduncle and further development of the inflorescence. It appears that the inflorescence is only able to respond to the cold stimulus once it has reached a minimum size and it is known that flower initiation is linked to the production of a certain number of leaves (De Smedt *et al.*, 1996). In South African production of *Clivia*, there is usually no control of growing temperature and flowering occurs mainly from August to September. The aim of this experiment was to determine whether a cold treatment could bring plants grown outdoors into flower earlier. It was hoped that this would extend the naturally short flowering period and ultimately increase sales and revenue to *Clivia* growers.

2.3 Materials and methods

The trial was conducted in the east of Pretoria at a nursery situated on the north facing slope of a hill where *Clivia* is grown under 70% black shade net. The experiment comprised 100 mature plants with at least 12 mature (fully elongated) leaves on each plant. Plants were growing in 6 liter plastic bags. Fifty randomly selected plants were removed from the nursery for the cold treatment and 50 plants remained as the control. The cold treatment comprised placing plants with plastic bags intact, in a dark, unventilated cold room for 14 days. The temperature was maintained between 7.5 and 10 °C and oscillated from minimum - maximum - minimum every 4 hours. The period of cold treatment was chosen after exploratory investigations indicated that 14 days was sufficient to bring plants into flower. This is in contrast to a period of 60 days at 10 °C previously described for successful forcing of *Clivia* (Mori & Sakanishi, 1974). After the cold treatment, plants were returned to the nursery. At weekly intervals, the number of plants which had reached the marketable stage was recorded for both treatments. A plant was defined as marketable when it had a normally elongated peduncle with some orange colouration in the perianth, but before any flowers were open. The air temperature in the nursery, at leaf canopy level, was recorded for the duration of the experiment using a thermograph which had been calibrated with a mercury thermometer. A Chi square test was used for the statistical analysis and required data from weekly observations to be combined into fortnightly data. The number of observations at each fortnightly interval was large enough for analysis only from week 12 onwards. A two way table of the two treatments versus time for the four fortnightly periods (effective sample size = 87) was used to test the null hypothesis which proposed that there was no relationship between treatment and time.

2.4 Results and discussion

Figure 2.1 shows the weekly minimum and maximum air temperatures in the nursery for the duration of the experiment from 24 April 2000 (week 0) to 04 September 2000 (week 19). It is apparent that maximum temperatures fluctuated between 16 and 27 ° C and minimum temperatures between 2 and 11 °C. After the cold treatment, it could be seen that cold treated plants flowered earlier than controls and that there were no negative effects following storage in the dark. Figure 2.2 shows on a weekly basis, over 19 weeks, the percentage of plants in each of the two treatments which had reached the marketable stage. Figure 2.3 (combined data) shows the percentage of plants in each treatment which had reached the marketable stage for the four fortnightly periods from week 12 - 19. The number of plants at marketable stage was

equal to zero until and including week 6. After 19 weeks, 96% of cold treated plants and 94% of controls had flowered, but Figure 2 and 3 indicate the trend whereby cold treated plants flowered before controls. Table 2.1 is the two way table of treatment versus time and shows the observed and expected values from the Chi square test for cold treated and control plants ($P < 0.0001$). It indicates that for the first two fortnightly periods (week 12 - 13 & 14 - 15), the number of cold treated plants which were marketable, was significantly higher than expected while in the control treatment the number was significantly lower than expected. Conversely, during the last two periods (week16 - 17 & 18 - 19), the number of plants at the marketable stage was significantly lower than expected for cold treated plants and significantly higher for controls. This result allowed rejection of the null hypothesis and it was concluded that the cold treatment was effective in increasing the number of plants available for sale early in the season when control plants were not marketable yet. It can therefore be deduced that one will be able to extend the period during which *Clivia* is available in flower by applying a cold treatment of relatively short duration, as described, to a portion of plants intended for sale in a specific season.

Prior to the abovementioned experiment, two exploratory investigations were carried out. In the first, a similar cold treatment was applied to outdoor grown plants at the beginning of February and flowering occurred in March and April. In the second, flowering size plants were placed in a greenhouse which was heated from the beginning of April to the end of August so that exposure to winter cold was eliminated. These plants did not flower during the natural flowering period when plants outside were in flower. A cold treatment was then applied at the end of November and brought some of the plants into flower in January and February. Therefore, it is felt that it may be possible to even further extend the flowering period in *Clivia*. However, these results could not be statistically tested and will need to be verified.

Further work could try to find out whether a cold treatment can be effectively applied earlier or later than the last week in April. The earliest and latest dates for successful forcing could also be determined. The phenomenon of negation of the effect of a cold treatment when followed by high growing temperature can also be investigated.

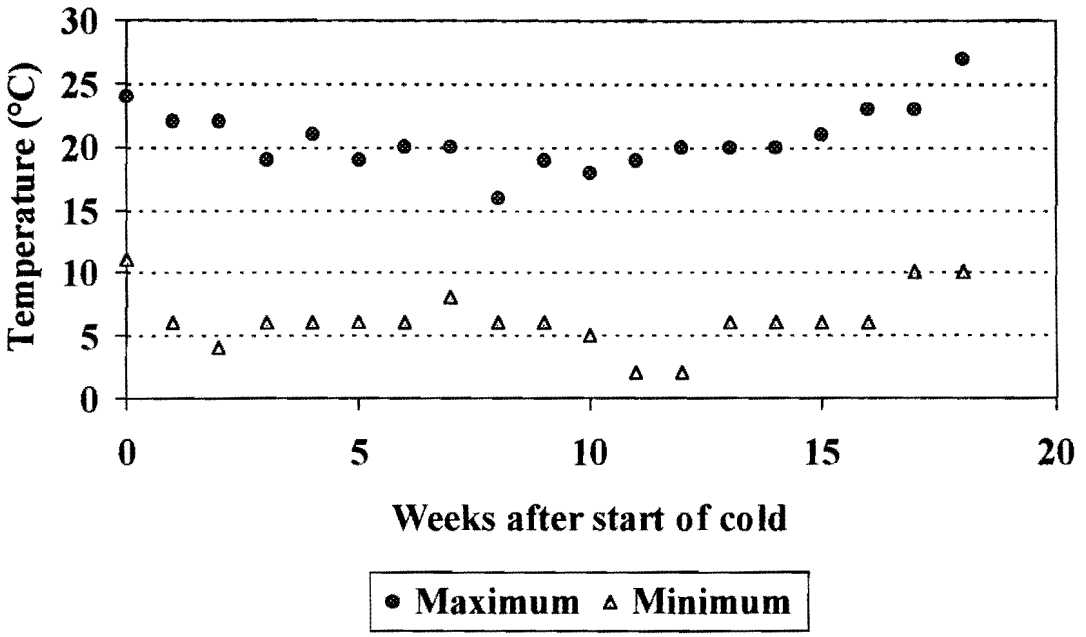


Figure 2.1 Weekly minimum and maximum air temperatures at leaf canopy level from week 0 - 19.

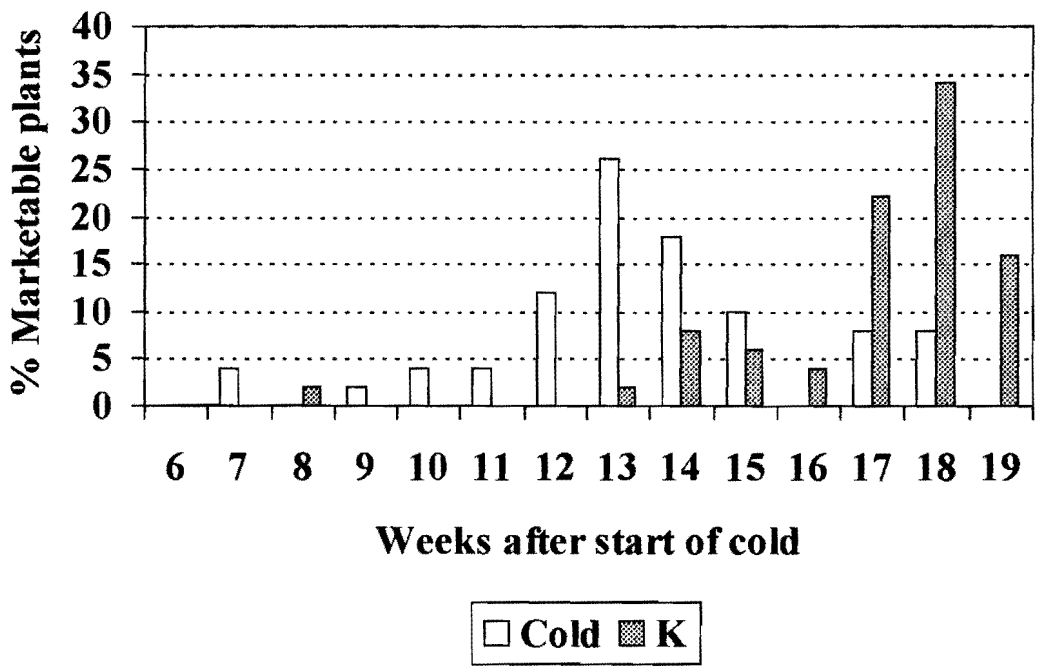


Figure 2.2 Percentage of plants at the marketable stage from week 6 - 19.

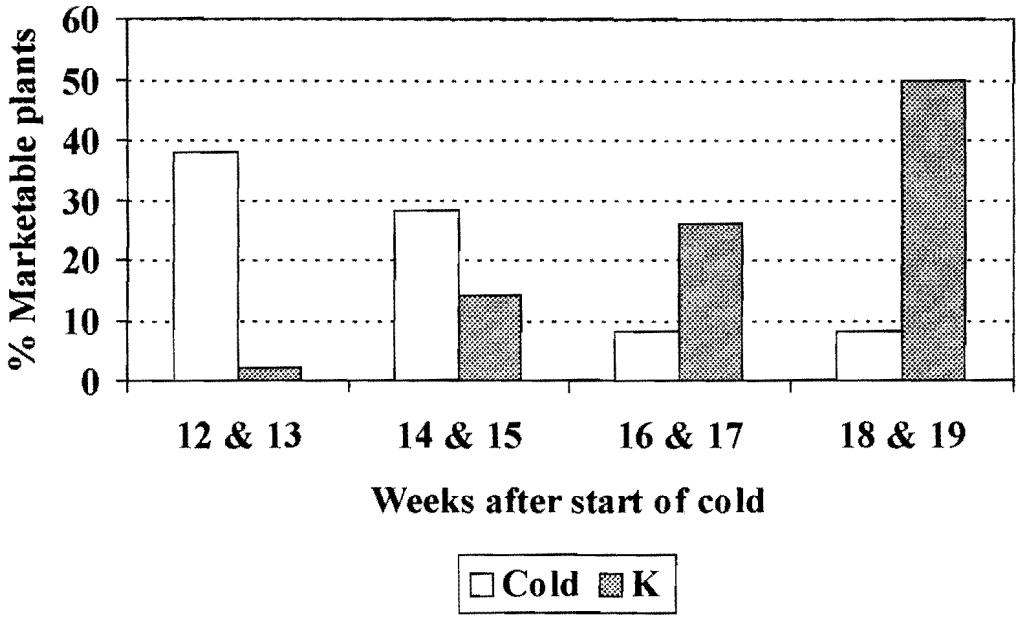


Figure 2.3 Percentage of plants at the marketable stage for the four fortnightly periods from week 12 - 19.

Table 2.1 Two way table of treatment versus time with observed and expected ( ) values for cold treated (Cold) and control (K) plants. Expected values were obtained from a Chi square test ($P<0.0001$) Effective sample size = 87.

Treatment	Week			
	12 & 13	14 & 15	16 & 17	18 & 19
Cold	19 (9)	14 (10)	4 (8)	4 (14)
K	1 (11)	7 (11)	13 (9)	25 (15)

2.5 References

DE HERTOGH, A.A., LILIEN-KIPNIS., H., BOROCHOV, A. & HALEVY, A. H., 1997. Technologies for forcing flower bulbs. *Acta Hortic.* 430, 175-182.

DE SMEDT, V., VAN HUYLENBROECK, J. M. & DEBERGH, P. C., 1996. Influence of temperature and supplementary lighting on growth and flower initiation of *Clivia miniata* Regel. *Sci. Hortic.* 65, 65-72.

MORI, G. & SAKANISHI, Y., 1974. Effect of temperature on the flowering of *Clivia miniata* Regel. *J. Japan. Soc. Hort. Sci.* 42(4), 326-332.

SANDLER-ZIV, D., COHEN, A., ION, A., EFRON, H. & AMIT, D., 1997. A two year production cycle of Israeli grown *Hippeastrum* bulbs from bulb chipping to Christmas flowering. *Acta Hortic.* 430, 361-368.

VISSERS, M. & HALEYDT, B., 1994. Bloeibeïnvloeding bij *Clivia*. *Verbondsnieuws Belg. Sierteelt* 38(4), 36-37.

CHAPTER 3

The potential of *Clivia miniata* (Lindley) Regel as a cut flower

3.1 Summary

Clivia miniata Regel is widely cultivated as a garden ornamental and a pot plant. It occurs in many attractive shades of orange, red, yellow and pink but little information exists regarding its use as a cut flower. The possibility of picking cut flowers when all the flowers on the inflorescence were still closed and forcing flowers to open in distilled water and in Chrysal AKC™ (CHRYS) was examined. A forcing temperature of 21 ± 2 °C and an irradiance of 22 ± 3.10^{-6} mol photons m^2s^{-1} was used. After 190 hours, in both distilled water and CHRYS, more than 90% of flowers had opened and developed normal colouration. However, after 96 hours, 90% of inflorescences held in distilled water and 10% of those in CHRYS had developed peduncle splitting. The mechanism involved in the prevention of splitting is unknown but it is suspected that sucrose in CHRYS may have caused this.

3.2 Introduction

Clivia miniata is widely cultivated as a garden plant in South Africa and has been cultivated as a pot plant in Europe for many years. Many attractive forms exist in shades of pink, orange, (Glover, 1985), yellow (Morris, 1990) and near white (McNeil, 1985) but few reports on *Clivia* cut flowers could be found (Drysdale, 1990, Nowak & Rudnicki, 1990). Furthermore, it appears that the flowering period of *Clivia miniata* can be manipulated to some extent by regulating growing temperature (Mori & Sakanishi 1974, De Smedt, Van Huylenbroeck & Debergh, 1996, Chapter 2 of this dissertation) and lighting (Vissers & Haleydt, 1994). Therefore, it was felt that the subject of *Clivia* cut flowers warranted further attention and that picking stage and the occurrence of peduncle splitting should be investigated. The recommended cutting stage for *Clivia* (Nowak & Rudnicki, 1990) is when 25% of the flowers on the inflorescence have already opened. In order to facilitate handling, packaging and longer vase life it was felt that it was important to determine whether flowers could be picked at a more closed stage.

3.3 Materials and methods

Flowers were picked from a suburban garden during late afternoon and transported dry to the laboratory within an hour. It follows then that the effect of extended periods of dry storage of flowers such as may occur, for example, if flowers are transported by airfreight, was not investigated. The prevailing air temperature in the laboratory was 21 ± 2 °C and the irradiance at a height of 25 cm above the inflorescences was 22 ± 3.10^{-6} mol photons m^2s^{-1}, provided by Osram™ cool white fluorescent tubes. Figure 3.1 shows the developmental stage of the inflorescences when picked; all flowers were closed but some colouration could be seen in the perianth. Ten inflorescences were placed in CHRYS (40g CHRYS / litre distilled water) and 10 in distilled water. The Chrysal product was chosen because it is used to enhance opening of carnations and other flowers cut in the bud stage. The number of open flowers was recorded as a percentage of the total number of flowers on each inflorescence over a period of 190 hours. Furthermore, the number of split peduncles which occurred in each treatment was recorded. Longitudinal sections of split peduncles which had been embedded in paraffin wax were examined. A meaningful statistical analysis was not possible due to scarcity of material and the short natural flowering period.

3.4 Results and discussion

After 190 hours (8 days), 99% of flowers kept in CHRYS and 95% of those in distilled water had opened (Table 3.1). In addition, colour development in the open flowers was normal (Figure 3.2). It was also observed that 90% of the inflorescences held in distilled water had developed peduncle splitting after 96 hours. This only occurred in 10% of stems held in CHRYS over the same period. In addition, the degree of splitting was much less severe when using CHRYS and was restricted to the tip of the peduncle. It appeared that equal numbers of flowers opened on inflorescences with split and unsplit peduncles. Figure 3.3 shows split and unsplit peduncles kept in distilled water and CHRYS, respectively. The mechanism by which splitting was prevented is unclear. However, the same problem occurs in *Hippeastrum* cut flowers due to extensive expansion of the inner parenchyma tissues. Splitting could be prevented by pulsing *Hippeastrum* stems in a 0.125 *M* sucrose or KNO_3 (potassium nitrate) solution which was thought to have conditioned the parenchyma in the basal portion of peduncles to withstand rapid expansion (Halevy & Kofranek, 1984). From sections of split *Clivia* peduncles, a difference in the size of inner and outer parenchyma cells could be seen (Figure 3.4). The chemical composition of CHRYS is a trade secret and is therefore not known.

However, it is suspected that sucrose is an ingredient and that it may have reduced the incidence of splitting.

Table 3.1 Mean number of open flowers (x) with standard deviation (s) on inflorescences held in 40g / l Chrysal AKC™ and distilled water (dH_2O) over a period of 190 hours at 21 ± 2 °C and an irradiance of 22 ± 3.10^{-6} mol photons m^2s^{-1}. The number of open flowers is expressed as a percentage of the total number of flowers on the inflorescence. n = 20

Hours		0	29	63	113	190
Chrysal	x	0	27	65	93	99
	s		7	15	8	1
dH_2O	x	0	23	48	78	95
	s		13	15	14	12

From the investigation it is concluded that it is possible to harvest *Clivia* cut flowers at a stage when all flowers are still closed and that a large proportion of the flowers will open with normal colour development under the conditions described. It seems that the problem of stem splitting can also be overcome by placing stems in CHRYS. Further work can be done to establish whether flowers will successfully open under conditions of lower irradiance than used in this experiment and to clarify the processes which cause and prevent stem splitting. The optimum treatment time and the possibility of using pulse treatments for prevention of stem splitting could also be determined. In addition, the effect of commercially available pretreatment products on the rate of flower opening and longevity could also be examined. Furthermore, the effect of dry storage of flowers on vase life, flower opening and stem splitting should be investigated.

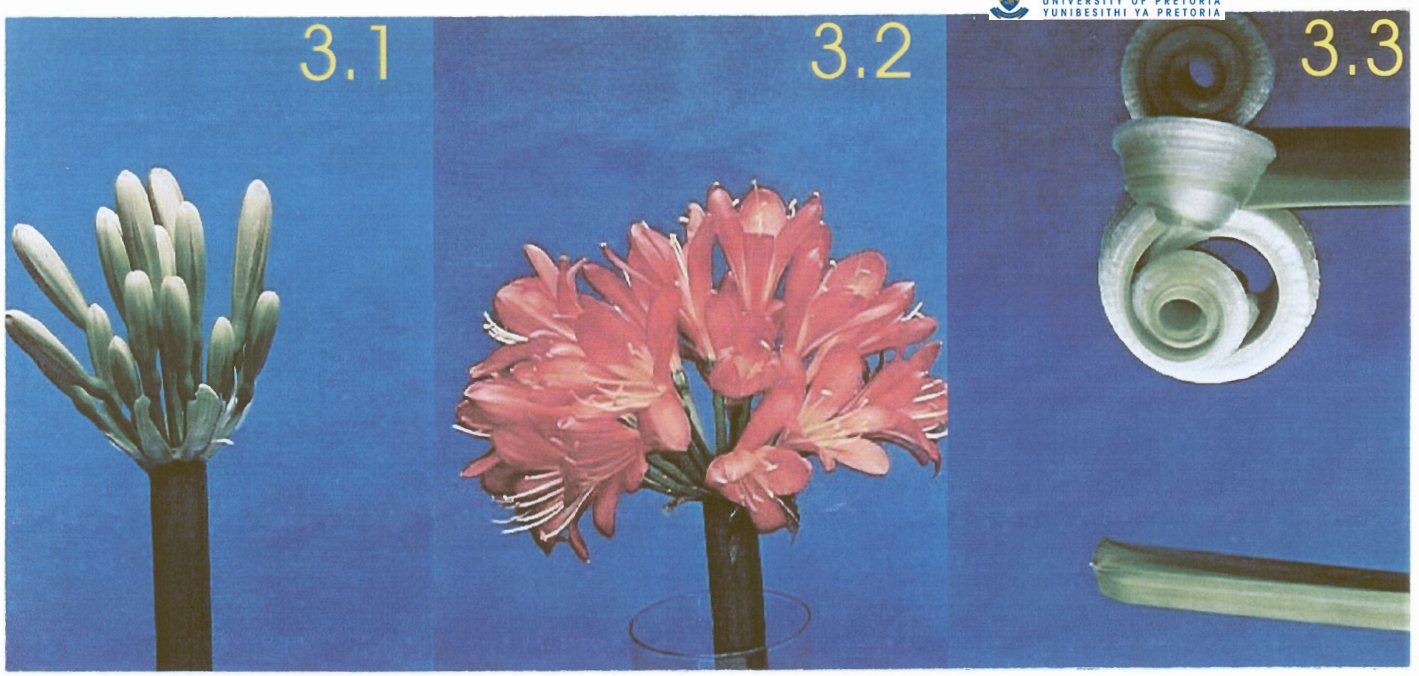


Figure 3.1 Developmental stage of *Clivia miniata* inflorescences at picking.

Figure 3.2 Colour development in *Clivia miniata* flowers on an inflorescence picked at the stage when all flowers were unopened and kept in distilled water for 6 days at 21 ± 2 °C and an irradiance of 22 ± 3.10^{-6}mol photons m^2s^{-1}.

Figure 3.3 Split (top) and unsplit (bottom) peduncles of *Clivia miniata* held in distilled water and 40g / l Chrysal AKC™, respectively.

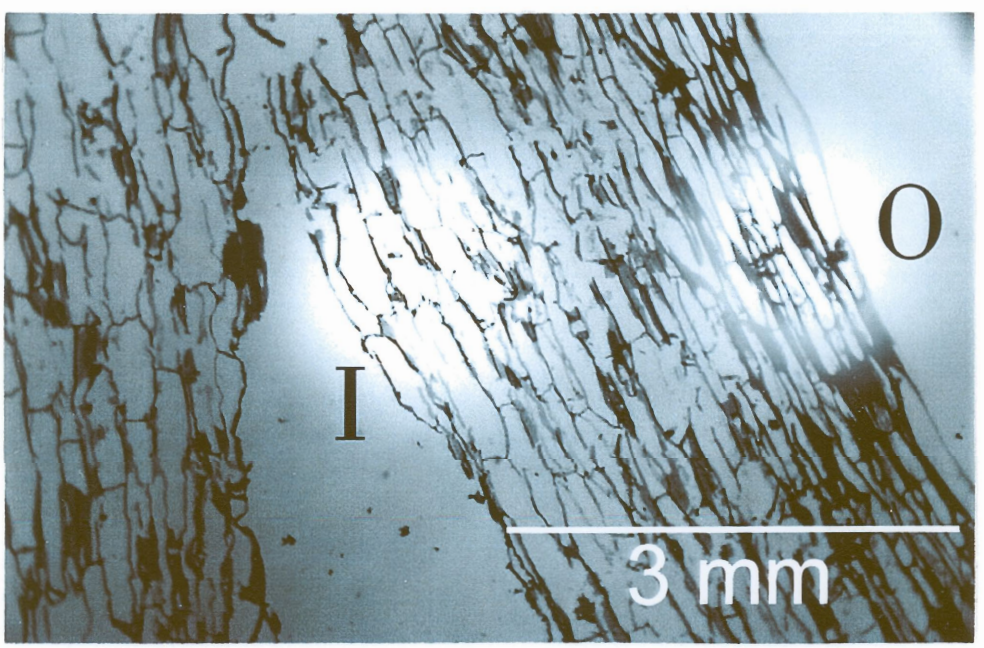


Figure 3.4 Longitudinal section of a split peduncle, showing inner (I) and outer (O) parenchyma tissues.

3.5 References

DE SMEDT, V., VAN HUYLENBROECK, J. M. & DEBERGH, P.C., 1996. Influence of temperature and supplementary lighting on growth and flower initiation of *Clivia miniata* Regel. *Sci. Hortic.* 65, 65-72.

DRYSDALE, W., 1990. E P Zimmerman, *Clivia* hybridist. *Herbertia* 46(1), 44.

GLOVER, W.J., 1985. Considerations in *Clivia*. *Herbertia* 41, 30-31.

HALEVY, A. H. & KOFRANEK A. M., 1984. Prevention of stem-base splitting in cut *Hippeastrum* Flowers. *Hortscience* 19(1), 113-114.

MCNEIL, P.G., 1985. Hybridising *Clivia*. *Herbertia* 41, 24-29.

MORI, G. & SAKANISHI, Y., 1974. Effect of temperature on the flowering of *Clivia miniata*. *J. Japan. Soc. Hort. Sci.*, 42(4), 326-332.

MORRIS, B., 1990. A true breeding strain of yellow *Clivia*. *Herbertia* 46(2), 95-96.

NOWAK, J. & RUDNICKI, R. M., 1990. Postharvest handling and storage of cut flowers, florist greens and potted plants. Timber Press, Portland, Oregon. 35, 139.

VISSERS, M. & HALEYDT, B., 1994. Bloeibeïnvloeding bij *Clivia*. *Verbondsnieuws Belg. Sierteelt* 38(4), 36-37.

CHAPTER 4

Promotion of shoot formation in *Clivia miniata* (Lindley) Regel with paclobutrazol and Promalin™

4.1 Summary

Propagation of superior clones of *Clivia* occurs through division of basaly produced suckers which is relatively slow or by tissue culture which is not always readily available to small scale growers. This study investigates the use of paclobutrazol (PAC) and Promalin™ (PRO) (benzyl adenine + GA_4 + GA_7) as a foliar spray to promote shoot formation. It was found that PAC promoted bud formation in the axils of older proximal leaf bases and the mean number of shoots produced at concentrations between 250 and 25 000 ppm PAC varied from 2.3 to 7.1 without any statistically significant difference among treatments, 7 months after application. However, at concentrations of 5 000 ppm and higher, growth inhibition was unacceptable and death of the parent plant occurred in some individuals due to abortion of the apical meristem. GA_3 applied 3 times fortnightly, at 500 ppm, appeared to be useful in alleviating growth inhibition caused by PAC, but the effect could not be quantified. It was found that PRO also promoted branching, but from the axils of younger, distal leaf bases. PRO also resulted in dichotomous branching of apical meristems. When applied 10 times, at either 200 or 500 ppm active ingredient, PRO resulted in acrotonic branching of 50% of treated plants into 2 or 3 modules. However, the latter results could not be analysed statistically. The most significant benefit arising from the use of PRO was survival of the parent plant without any inhibition of growth.

4.2 Introduction

Clivia miniata can be vegetatively propagated by division of suckers. The rate at which suckers are produced varies from clone to clone and is often not satisfactory. Published tissue culture methods are still imperfect, resulting in varying degrees of success (Wang, LI & Yang, 1995, Finnie, 1998, Chapman, 1999). In addition, tissue culture facilities are not within reach of many amateur growers who are in possession of desirable clones. This investigation was undertaken to determine whether the plant growth regulators paclobutrazol and Promalin™ could be used as a foliar spray to promote shoot formation and branching in *Clivia*.

Paclobutrazol (PAC) has been used in *in vitro* propagation to stimulate shoot formation in a range of plants such as *Nerine* sp. (Ziv, Kahany & Lilien-Kipnis, 1994), *Gladiolus* sp. (Nagaraju, Parthasarathy & Bhowmik, 1997) and *Tulipa* (Kuijpers & Langens-Gerrits, 1997). PAC applied as a foliar spray resulted in production of side shoots in *Cordyline* sp. (Higiladi & Watad, 1992) and when applied to *Clivia* to reduce plant size, caused the same effect (Van Huylenbroeck, 1998). However, in the latter work, a wide range of concentrations was not tested and the yield of shoots was relatively low. PAC is a plant growth regulator which inhibits the synthesis of gibberellic acid (Grossmann, 1990). However, in *in vitro* culture of potato, growth inhibition could be reversed by using GA_3 in the medium (Simko, 1993).

Benzyl adenine (BA) can also be used to stimulate shoot formation. This was achieved in *Cordyline* sp. (Maene & Debergh, 1982) and geranium (Foley & Keever, 1992). In *Spathyphyllum* sp. grown *in vitro*, shoot induction by BA was dramatically enhanced in the presence of imidazole fungicides. As with PAC, the latter effect was obtained by inhibition of GA_3 production (Werbrouck & Debergh, 1995, Werbrouck *et al.*, 1996). PRO (GA_4 + GA_7 + BA; 19g / l active ingredient) is registered in South Africa for the promotion of branching in apples (Vermeulen, Grobler & Van Zyl, 1997).

4.3. Materials and methods

4.3.1 The effect of paclobutrazol

PAC (Cultar™; 250g / l active ingredient) was applied to flowering size plants as a foliar spray until run off, at concentrations of 1, 2, 4, 10, 20, 50 and 100ml Cultar™ / l, corresponding respectively to 250, 500, 1 000, 2 500, 5 000, 12 500 and 25 000 ppm a.i. Eight replicates per treatment were used. The number of shoots formed was then recorded and the statistical analysis comprised a regression analysis of the number of shoots formed as a function of concentration. An analysis of variance (ANOVA) using Tukey's least significant difference was done to determine whether the mean number of shoots differed significantly among the treatments ($\alpha = 0.05$).

4.3.2 Alleviation of PAC induced growth inhibition by GA_3

A small number of plants (8) were used to determine if the growth retarding effect of PAC could be alleviated by GA_3. Ten months following a PAC spray at 25 000 ppm, GA_3 (Berelex™

100g / kg a.i.), at a concentration of 500 ppm, was applied to 4 of the plants treated with PAC. Three applications were made; a single spray until runoff, every fortnight, for six weeks. The plants treated with PAC had produced a large number of stunted, basal shoot primordia.

4.3.3 Promalin™ application through the leaves

In a second set of flowering size plants, a foliar spray of PRO until runoff, was applied during April when plants were not in flower. Concentrations of 5.3, 10.4 and 26.3 ml PRO / l were used (corresponding respectively to 100, 200 & 500 ppm a.i.). Ten applications were made; one application every second day, for 20 days. Eight replicates of each treatment were used. The number of plants which reacted was then recorded.

After observation of the effects of the first PRO application, PRO was applied to a third set of plants for the purpose of an anatomical investigation (section 4.3.5). A concentration of 375ml PRO / l (7 125 ppm a.i.) was applied to small number of seedlings at the six leaf stage. The solution was painted onto the crown and leaf axils of each plant with a paint brush, wetting the areas until runoff. Two application regimes were used; a single application per plant and three applications per plant. In the case of three applications, one application was given fortnightly, for six weeks. At various intervals, plants were harvested for anatomical investigation.

4.3.4 Promalin™ application through the roots

In a forth set of plants, seedlings at the 6-8 leaf stage, an attempt was made to apply PRO via the root system. All soil was washed from the roots before plants were placed in an aerated water medium (hydroculture). The nutrient solution comprised Chemicult™ hydroponic nutrient powder at the rate of 0.5g / l. After 1 month, a PRO spray was applied to the roots at concentrations of 0, 4 750 and 7 125 ppm a.i. comparing one and three applications. In the case of three applications, roots were sprayed once every evening, for three days. After removing plants from hydroculture in the early evening and allowing them to dry for 10 minutes, PRO was sprayed onto roots until runoff. After spraying, roots were covered in a plastic bag to prevent evaporation, left overnight and returned to hydroculture in the morning. After the last spray, plants were replanted in a decomposed bark medium. Each treatment was replicated eight times.

4.3.5 Anatomical investigation

An anatomical investigation was conducted to elucidate shoot architecture and to identify the origin of shoots produced after PRO and PAC treatments. Material was prepared by fixation in formalin acetic acid alcohol followed by dehydration in sequential alcohol and alcohol xylene mixtures. After embedding in paraffin wax, microtome sections were cut and stained with toluidine blue (O'Brien & McCully, 1981).

4.4 Results and discussion

4.4.1 The effect of paclobutrazol

Ten months after the application of a 2 500 ppm PAC foliar spray, a clearly visible reduction in plant height could be seen (Figure 4.1). This was accompanied by branching in the older proximal axils of leaf bases (basitonic branching) which was especially prolific at higher concentrations. At and above concentrations of 5 000 ppm PAC, complete and near complete disintegration of the parent plant and much of the root system occurred in some individuals (Figure 4.2). However, it follows that the viability and probability of survival of shoots on plants compromised to this extent would be much reduced. It was interesting to note that PAC caused the death of the apical meristem, but stimulated bud formation in the meristematic zones in the axils of basal leaves (Figure 4.10).

Seven months after PAC application, a relationship between PAC concentration and the number of shoots formed could be found and was given by $S = 2.799 + 0.179\ k - 0.001\ k^2$ where S and k represented the number of shoots and PAC concentration, in ml Cultar / l water, respectively (Figure 4.3). All three terms were highly significant ($Pr > t$; <0.0001, <0.0007 and <0.0044 respectively). A low value for R^2 (0.22) was obtained and indicates that there were other factors which also played a role in the number of shoots formed. Since the plants used were raised from seed, genetic variation is likely to have played a role. This was true for the response of different cultivars of *Gladiolus* to PAC (Nagaraju *et al.*, 1997). The ANOVA indicated that the number of shoots formed at all PAC concentrations differed significantly from the control, but not from one another (Table 4.1).

The effect of PAC on flowering was negative because of death of the apical meristem. Furthermore, existing inflorescences disintegrated completely at concentrations of 5 000 ppm and higher and where this did not occur, peduncles were unacceptably short.

Figure 4.1 Reduction in plant height 11 months after application of 2 500 ppm paclobutrazol (front) compared to the untreated control (back).

Figure 4.2 Disintegration of the parent plant and root system 13 months after application of 25 000 ppm paclobutrazol.

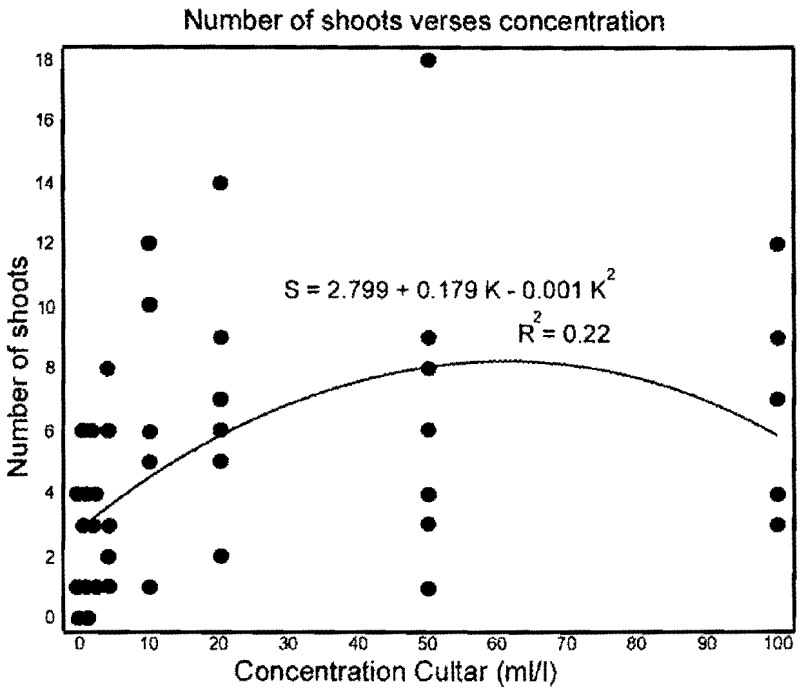


Figure 4.3 Regression analysis of number of shoots as a function of paclobutrazol concentration (ml Cultar™ / l water). $R^2 = 0.22$.

Table 4.1 Mean number of shoots as a function of paclobutrazol (PAC) concentration (ml Cultar™ / l), seven months after application. Means with the same letter do not differ significantly. $\alpha = 0.05$, Tukey, n = 64.

ml Cultar™ / l	0	1	2	4	10	20	50	100
Mean number of shoots	1	2.3[a]	3.3[a]	3.5[a]	7.1[a]	6.5[a]	6.6[a]	6.1[a]

4.4.2 Alleviation of PAC induced growth inhibition by GA_3

It appeared that the 3 applications of GA_3 at 500 ppm alleviated the inhibition caused by PAC to some extent (Figure 4.4). It was felt that the use of GA_3 could have practical benefits but its effect could not be quantified or statistically analysed.

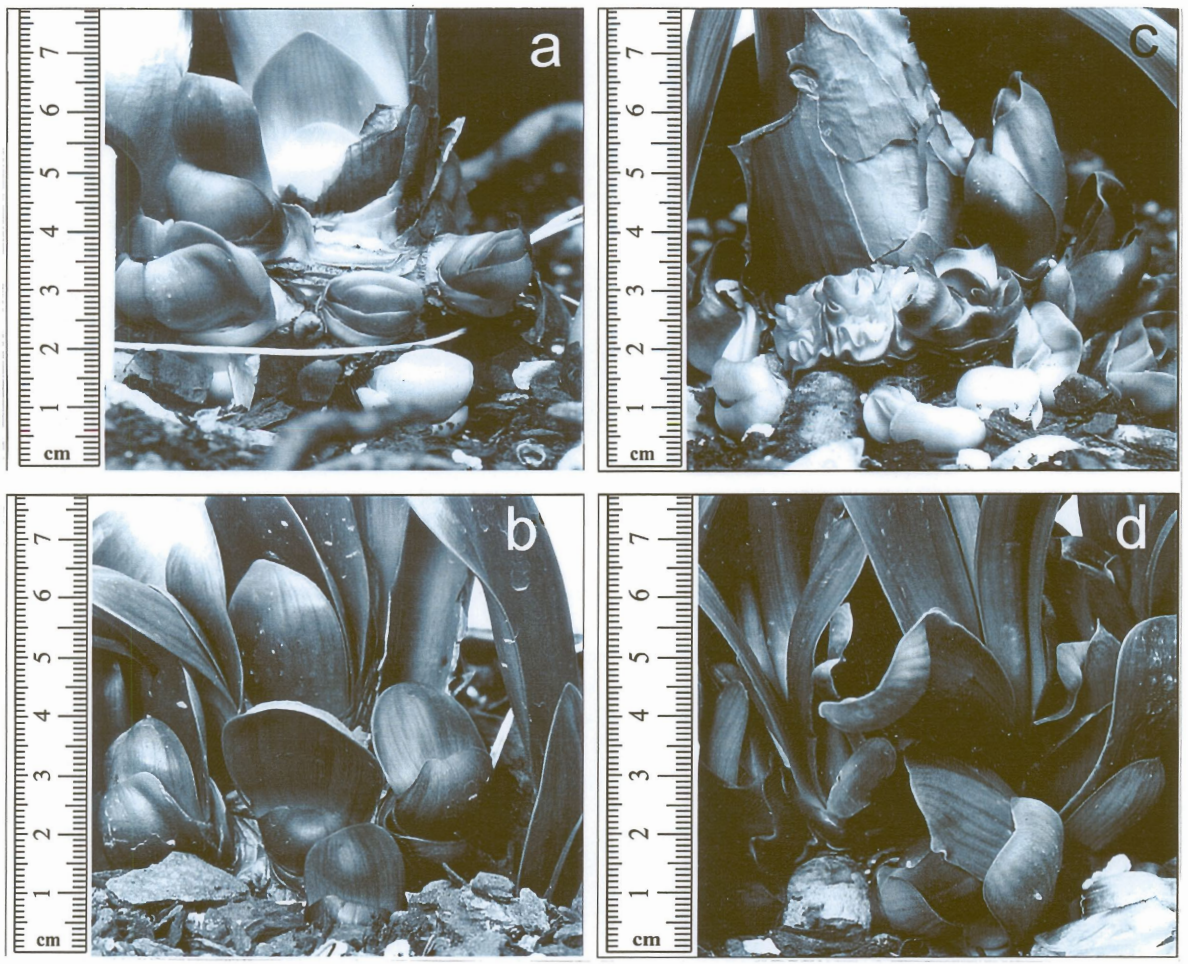


Figure 4.4 Alleviation of growth inhibition after application of 25 000 ppm paclobutrazol. Ten months after application of PAC, following formation of shoot primordia, 3 fortnightly applications of GA_3 at 500 ppm were made. Photo taken 2 months after the last application of GA_3.

a: Plant no.1 before, without GA_3. **c:** Plant no. 2 before, with GA_3.

b: Pant no.1 after, without GA_3. **d:** Plant no. 2 after, with GA_3.

4.4.3 Promalin™ application through the leaves

The result of PRO application was branching of treated plants in the region around the apical meristem. Shoots formed in this way were different in morphology to those formed by PAC in the sense that leaf size and shape were normal (Figure 4.5). One year following the 10 applications of PRO at 0, 100, 200 and 500 ppm, the percentage of plants exhibiting formation of additional shoots was 0%, 25%, 50% and 50% respectively. However, these results could not be statistically analysed. The most significant benefit which arose from the use of PRO was that there was no inhibition of vegetative growth or destruction of the parent plant. However, flowering was negatively affected and deformed flowers could be observed when using 100, 200, 300 or 7 125 ppm PRO (Figure 4.6). The excessive formation of green tissue in the perianth probably occurred because cytokinins promote chloroplast development and chlorophyl synthesis (Salisbury & Ross, 1992) while the abnormal thickness of the tissue could be due to cell proliferation and expansion caused respectively by BA and gibberellins in PRO. A degree of green colouration sometimes occurs normally in certain individuals of *Clivia*.

4.4.4 Promalin™ application through the roots

PRO at 7 125 ppm a.i. proved to be phytotoxic when applied the roots. When applied at 7 125 ppm a.i., 100 % of individuals exhibited necrosis of the root system within two weeks, followed by gradual death of the entire plant. Phytotoxicity could also be observed, to a lesser extent, at 4 750 ppm a.i. Table 4.2 shows the number of plants surviving at the various application rates, 4 months after the last PRO application. From the first experiment in which PRO was applied through the leaves (section 4.3.3), it was apparent that visible branching could only be detected approximately 1 year after application. At the date of publication it was not possible to determine whether PRO application through the roots had achieved this.

Table 4.2 Percentage of plants surviving 4 months after application of PRO to the roots once (1x) and 3 times (3x) at concentrations of 4 750 and 7 125 ppm a.i.

PRO (ppm a.i.)	0	4 750; 1x	4 750; 3x	7 125; 1x	7 125; 3x
% survival	100	100	62.5	87.5	0

Figure 4.5 New (1 & 3) and original (2) modules, 1 year following10 applications of 500 ppm a.i. Promalin™.

Figure 4.6 Abnormal flower development (bottom) compared to control (top), after a single foliar application of 7 125 ppm a.i. Promalin™, before emergence of the inflorescence.

4.4.5 Anatomical investigation

In an untreated mature plant, Figure 4.7 depicts the development of a new module adjacent to the old one which has terminated in an inflorescence. This suggests a modular, sympodial plant architecture (Chapter 6). Potentially meristematic zones are located in leaf axils, on the abaxial side of leaf bases, but no differentiated axillary buds are present (Figure 4.8). These meristematic zones are believed to be the source of new modules produced when a PAC treatment is applied. This would be consistent with the findings that PAC enhanced meristem formation on stem explants of *Tulipa* (Kuijpers & Langens-Gerrits, 1997). Figure 4.9 indicates these zones accentuated in a seedling, 5 months after treatment with 5 000 ppm PAC while Figure 4.10 indicates a new bud forming in the most proximal axil of another seedling following the same treatment. It is evident that the bud is orientated downwards and this is consistent with the "U" shape which can be observed in suckers attached to the parent plant. At high PAC concentrations, it is believed that bud formation from proximal axillary meristems occurs continuously in subsequent modules and that this is responsible for the prolific regeneration of shoots not characterised by any distinguishable pattern (Figure 4.3).

It is believed that the action of PRO is different to that of PAC. Modules formed in response to PRO also appear to arise from axillary meristems but from those directly adjacent to the apical meristem. This is illustrated in a seedling, 3 months after application of PRO at 7 125 ppm a.i., where axillary meristems have given rise to a new module on either side of the original apical meristem (Figure 4.11). In addition, it appears that following the application of PRO to seedlings, dichotomous branching of the apical meristem may occur (Figure 4.12). The occurrence of both dichotomous and axillary branching in the same individual has been observed in a seedling (Figure 4.13).

In mature plants, axillary branching would explain the formation of the shoots in Figure 4.5. It is thought that dichotomy also occurs in mature plants because leaf pairs, fused along the abaxial surface, were seen emerging from the apical meristem after treatment with PRO. These leaves were followed by the emergence of two new modules of similar size; one next to each adaxial surface of the fused leaf pair. Furthermore, it is proposed that in mature plants, pseudo-dichotomous branching may be the result of an early switch or reversion of the terminal bud from a reproductive to a vegetative stage. (In an untreated plant the reproductive bud would have given rise to the inflorescence.) Following reversion, the new vegetative bud and the axillary bud which was to form the new module after flowering, may then develop further, in

the form of 2 vegetative buds of more or less equal size.

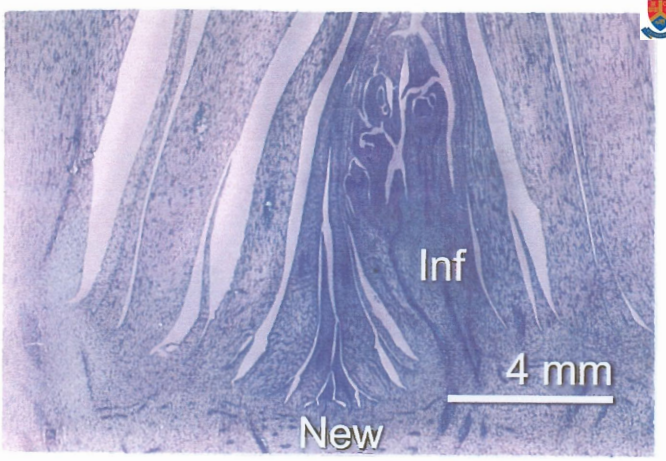


Figure 4.7 Longitudinal section of an untreated mature shoot showing termination of the old module in an inflorescence (Inf) and formation of the new module (New).

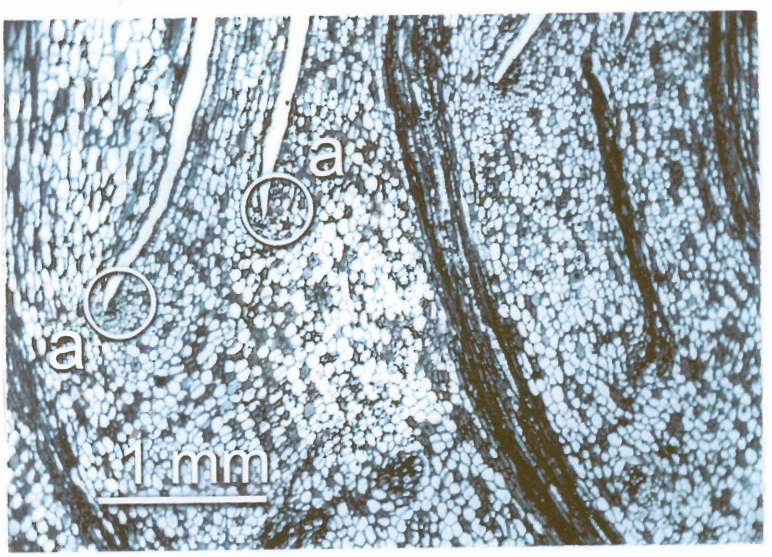


Figure 4.8 Longitudinal section of an untreated seedling at the 6 leaf stage, showing potentially meristematic zones (a) located on the abaxial side of leaf bases.

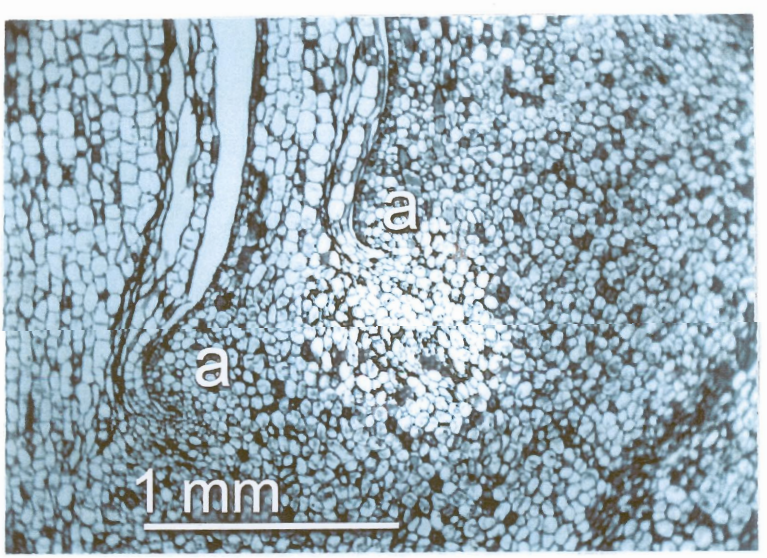


Figure 4.9 Longitudinal section of a seedling, 5 months after treatment at the six leaf stage, with 5 000 ppm paclobutrazol, indicating accentuated meristematic zones (a).

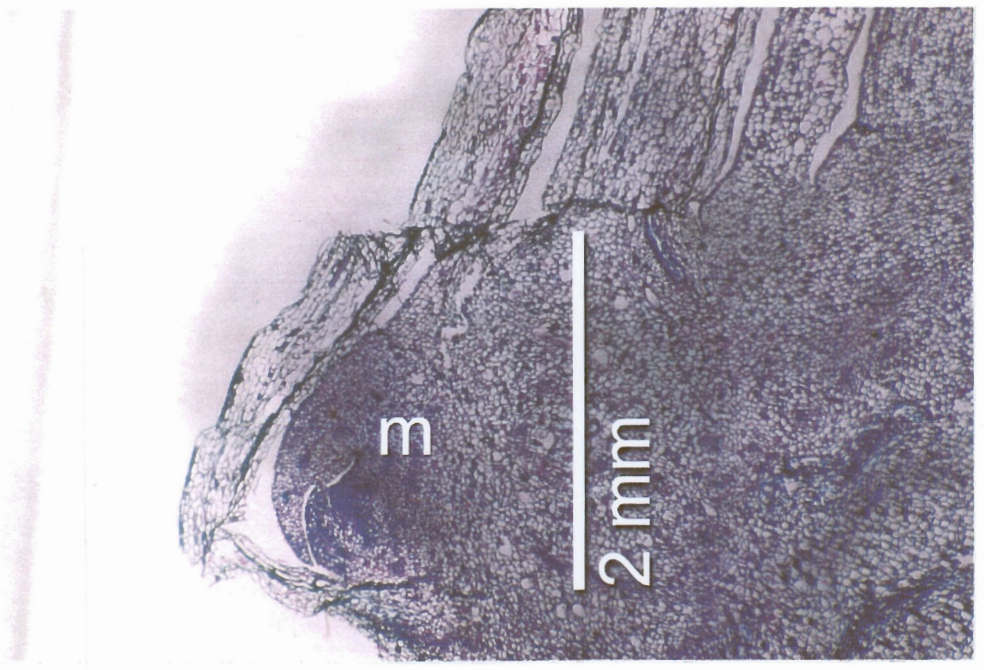


Figure 4.10 Longitudinal section of the most proximal axil of a seedling, 5 months after treatment at the six leaf stage, with 5 000 ppm paclobutrazol, indicating bud formation (m).

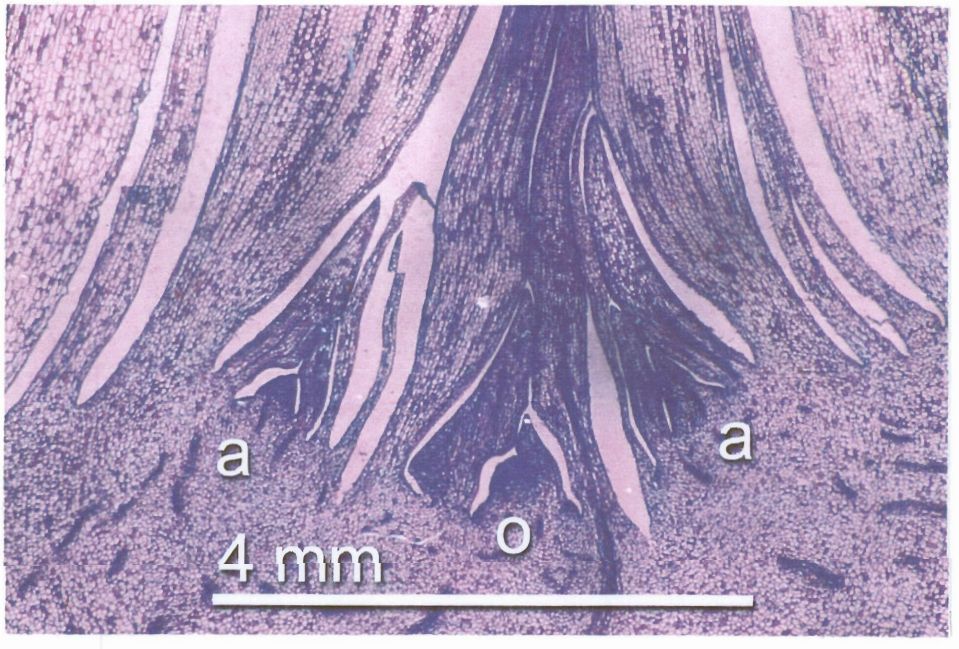


Figure 4.11 Longitudinal section of the apical meristem of a seedling, 3 months after treatment at the six leaf stage, with 7 125 ppm a.i. Promalin™, indicating new modules (a) adjacent to the original apical meristem (o). Note adnation of the buds (a) to the abaxial, basal part of the leaves.

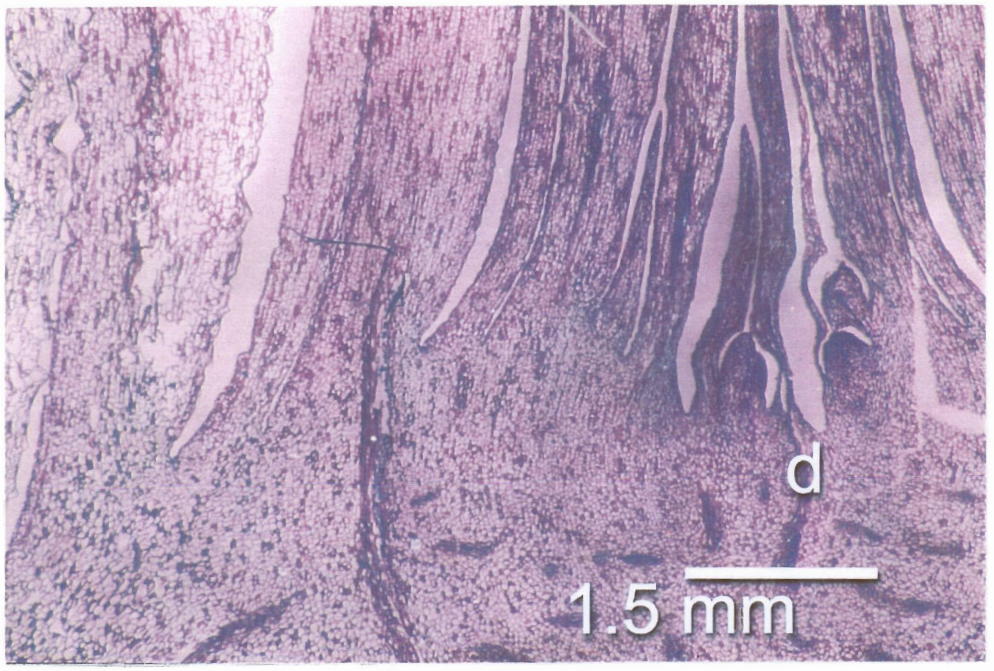


Figure 4.12 Longitudinal section of the apical meristem of a seedling, 2 months after treatment at the six leaf stage, with the first of 3 fortnightly applications of 7 125 ppm a.i. Promalin™. Dichotomous branching (d) of an apical meristem is shown.

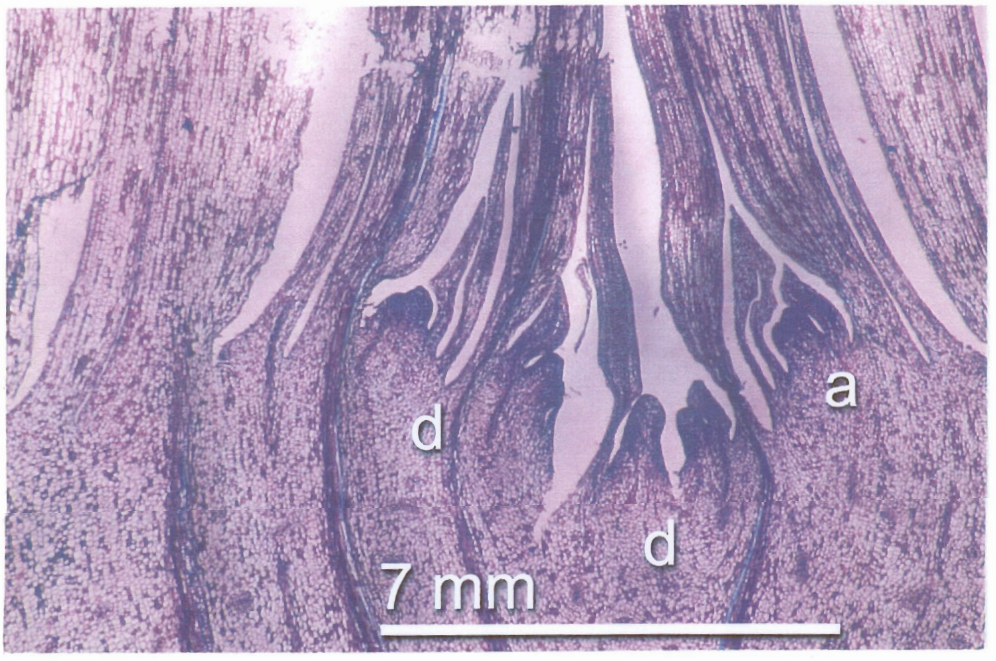


Figure 4.13 Longitudinal section of the apical meristem of a seedling, 3 months after treatment at the six leaf stage, with 7 125 ppm a.i. Promalin™, indicating dichotomous (d) and axillary (a) branching.

The use of paclobutrazol to stimulate multiple shoot formation in *Clivia miniata* (Lindley) Regel *in vitro*

5.1 Summary

Clivia has been propagated *in vitro* with varying degrees of success using embryos and fruit wall material as explants but the methods are relatively slow. However, commercial protocols for propagation exist, but have not been published. After observing the stimulatory effect which paclobutrazol (PAC) had on shoot formation *in vivo,* the same technique was attempted *in vitro*. Seeds were germinated on a MS medium supplemented with 20g / l sucrose, 3g / l agar, 0.6g / l Gelrite™, 1g / l myo inositol, 1.4mg / l 2,4-D, 2mg / l benzyl adenine and 3mg / l kinetin. After seven months, the seedlings were dipped in paclobutrazol (PAC) at concentrations of 125 - 1 000 ppm before being placed on fresh medium. Five months following application of PAC, there was no evidence of multiple shoot formation and mortality was approximately 70%.

5.2 Introduction

Moderate success has been achieved with the *in vitro* propagation of *Clivia*. Zygotic embryos from seeds could be used as the explant material for *C. miniata* (Wang, Li & Yang, 1995, Wang, 1998) and for *C. nobilis* (Min & Jinsheng, 1984). For purposes of propagating a mature plant, fruit wall material could be used (Finnie, 1998). However, seasonal availability of fruit walls at the right developmental stage together with slow plantlet regeneration hampered the development of a commercial protocol. The same conclusion was reached in another study (Chapman, 1999). Nevertheless, it appears that *Clivia* is successfully propagated *in vitro* on a commercial scale in Japan (Smithers, 2000) but the protocols used have not been published. The aim of this study was to examine whether paclobutrazol (PAC) could be used for generation of multiple shoots *in vitro*.

5.3 Materials and methods

Seeds were used as the explant material and were germinated *in vitro* following surface sterilisation. Disinfestation occurred in a 2.5% NaOCl solution (JIK™) for 10 minutes followed

by rinsing 3 times in sterile distilled water. A Murashige & Skoog (MS) medium supplemented with 20g / l sucrose, 3g / l agar, 0.6g / l Gelrite™, 1g / l myo inositol, 1.4mg / l 2,4-D, 2mg / l benzyl adenine and 3mg / l kinetin, adjusted to pH 5.6, was used. The higher relative cytokinin concentration was chosen in order to promote development of shoots and to suppress root growth. After seven months, plants were dipped in autoclaved PAC solutions and placed on fresh medium. PAC concentrations of 0,125, 250, 500 and 1000 ppm were used with 20 replicates of each treatment.

5.4 Results and discussion

After 5 months of culture there were no significant signs of new growth or any indication of formation of multiple shoots. Approximately 70 % of explants exhibited necrosis of the shoot in all treatments, including the control. The cause of the low survival rate was probably related to the medium composition, since much better growth was achieved when explants from the same batch were grown under the same conditions but with a different medium (Swanevelder, 2000). Furthermore, the formulation of PAC (Cultar™, 250g / l active ingredient) could not be filter sterilised and therefore necessitated autoclaving. The effect which this may have had on its efficacy is not known.

5.5 References

CHAPMAN, A.C., 1999. *In vitro* propagation of *Clivia miniata*. BScAgric(Hons) dissertation. Department of Horticulture, University of Nottingham, England.

FINNIE, J.F., 1998. *In vitro* culture of *Clivia miniata*. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 7-10.

MIN, L. & JINSHENG, S., 1984. Tissue culture of Kaffirlily (*Clivia nobilis*). *Acta Hortic. Sin.* 11(1), 47 - 49.

SMITHERS, P., 2000. The adventures of a gardener. In M. Dower, C.Felbert & J.Winter (eds). Clivia yearbook 2. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 13-14.

SWANEVELDER, Z.H., 2000. Tissue culture of *Clivia miniata*. BSc(Hons) dissertation. Department of Botany, University of Pretoria, South Africa.

WANG, L.C., LI, M.Q. & YANG, Y.H., 1995. *In vitro* culture of the young embryos of *Clivia miniata* Regel. *Journal of the Southwest Agricultural University*. 17(5), 396-398.

WANG, X.L., 1998. Studies on Kaffirlily embryo culture and speed multiply technique. *Journal of Jilin Agricultural University*. 20(2), 20-21.

CHAPTER 6

Illustration of the natural and manipulated plant architecture of *Clivia miniata* (Lindley) Regel.

6.1 Summary

Schematic representations of the natural and manipulated plant architecture of *Clivia* can be used to better understand its phenology and propagation. It is proposed that *Clivia* has a modular growth form in which inflorescences are borne terminally. Under natural conditions, sympodial branching occurs after flower initiation in a module. The number of inflorescences which are produced per season depends on the number of modules produced and this can be manipulated by modifying cultural conditions. The effect of paclobutrazol on architecture could be seen in highly repetitive, basitonic axillary branching while the effect of PRO was to stimulate less repetitive, acrotonic axillary branching and / or dichtomous division of the apical meristem.

6.2 Introduction

The juvenile period in *Clivia*, during which no inflorescences are initiated, ends after the production of 12 -13 leaves and may be as short as 12 months depending on growing conditions and genotype. In general, initiated inflorescences develop up to a certain stage and then enter a dormant period before exposure to a low temperature causes their emergence. Emergence of the inflorescence follows about one year after initiation. Following initiation of the first inflorescence, further inflorescences are produced, on average, after every set of 4-5 leaves. (De Smedt, Van Huylenbroeck & Debergh, 1996). However, no mention is made of modules in the former work and this chapter describes the growth of *Clivia* in terms thereof.

6.3 Materials and methods

A thorough morphological study of *Clivia* is not discussed in this dissertation and results obtained in previous chapters were interpreted in terms of existing terminology (Hallé, Oldeman & Tomlinson, 1978, Bell & Bryan, 1991).

6.4 Results and discussion

6.4.1 Natural architecture

It is here demonstrated that *Clivia* has a modular growth form and that it exhibits sympodial branching under natural conditions. After the juvenile phase, a module consists of about four leaves and a terminal inflorescence. Following initiation of the inflorescence, growth of the flowering module ceases and a new module arises in the axil of a leaf base, adjacent to the inflorescence (Figure 6.1., corresponding to Figure 4.7). When the juvenile stage is ended at the 12-13 leaf stage (De Smedt *et al.*, 1996), it is not known whether the 12-13 leaves present are the product of 3 successive modules with aborted terminal buds or of a single module.

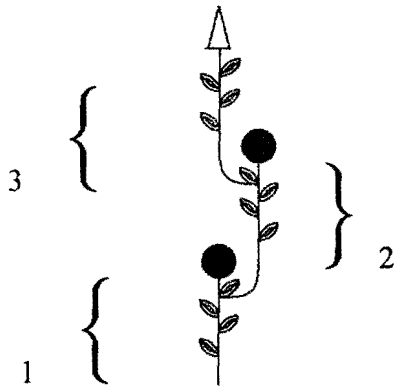


Figure 6.1 Schematic representation of sympodial branching of *Clivia* under natural conditions indicating a plant with 3 modules (1-3).

No axillary buds could be seen in *Clivia* and it is interesting to note that instead, meristematic zones which give rise to new buds and modules are situated on the abaxial surface of leaf bases (Figure 4.8 & 4.9). The position of the bud can be described as being displaced in an acropetal direction and adnation to the abaxial surface of the above leaf base occurs (Bell & Bryan, 1991).

More than one module can be produced per year if cultural conditions are favourable, with the result that there may be up to three inflorescences within a plant at a given moment. Two of these may be sufficiently developed to emerge together, or within close succession of each other, once exposed to either a natural or an artificial cold stimulus. Such plants have been referred to as 'twins'. However, if cultural conditions are not favourable, the production of

leaves and therefore inflorescences may be slow and plants may not flower every year. Damage by pests or diseases may result in death of the inflorescence at an early stage with the result that not every inflorescence which has been initiated will emerge.

A specific temperature regime is important in the initiation of flowers in a wide range of plants and it seems likely that this may also be true for *Clivia*. From the information available, it appears that at a temperature of 20 °C, both initiation of flowers and the rate of production of leaves is satisfactory (Mori & Sakanishi, 1974, De Smedt *et al.*, 1996, De Koster, 1998). However, emergence of quiescent inflorescences relies on exposure to temperatures below 20 °C. The effect of higher temperatures on flower initiation, as may occur under outdoor tropical or subtropical climates, is not known.

6.4.2 Manipulated architecture

The effect of paclobutrazol could be seen in highly repetitive, basitonic axillary branching from leaf bases in the proximal, older axils. This is illustrated in Figure 6.2 (corresponding to Figure 4.2 & 4.10).

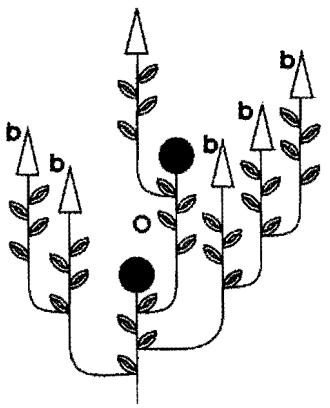
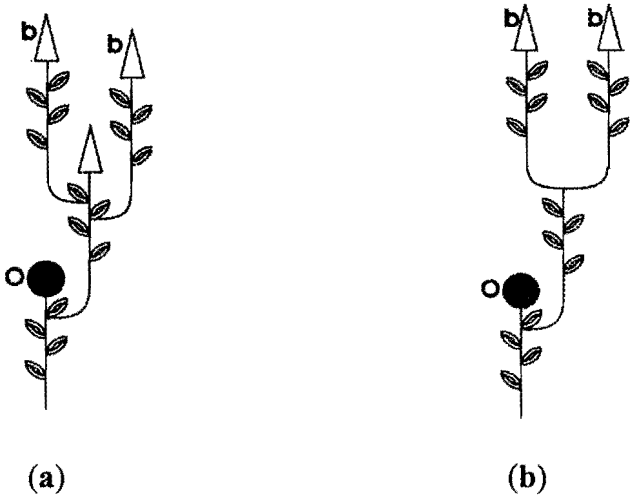


Figure 6.2 Schematic illustration of repetitive basitonic branching in *Clivia* caused by paclobutrazol. The original branch consisting of 3 modules (o) and new branches (b) are indicated.

By contrast, the effect of PRO sprays could be seen in less repetitive, acrotonic axillary branching from leaf bases in the younger, distal axils (Figure 6.3a, corresponding to Figure 4.5 & 4.11). PRO also caused dichotomous branching (a symmetrical split or division) of apical meristems (Figure 6.3b, corresponding to Figure 4.12). The fact that the response to PAC and PRO occurred in specific regions could probably be explained by the different balance of plant hormones which occurs in the different locations of activity.



(a) (b)

Figure 6.3 Acrotonic (**a**) and dichotomous (**b**) branching caused by Promalin™. The original module (o) and new branches (b) are indicated.

It follows from the above discussion that there is an interaction between the physiology, plant morphology and phenology of *Clivia* and that an understanding thereof will influence the success with which these can be manipulated.

6.5 References

BELL, A.D. & BRYAN, A.,1991. An illustrated guide to flowering plant morphology. Oxford University Press, Oxford. England. 230, 288-289.

DE KOSTER, P., 1998. Selection and commercial production of of *Clivia* in Europe. In C.Felbert, J.Winter & M. Dower (eds). Clivia yearbook 1998. Clivia Club, Kirstenbosch Botanical Gardens, South Africa. 32-34.

DE SMEDT, V., VAN HUYLENBROECK, J. M. & DEBERGH, P.C., 1996. Influence of temperature and supplementary lighting on growth and flower initiation of *Clivia miniata* Regel. *Sci. Hortic.* 65, 65-72.

HALLÉ, F., OLDEMAN, R.A. & TOMLINSON, P.B., 1978. Tropical trees and forests. An architectural analysis. Springer-Verlag, Berlin, Germany. 86, 87.

MORI, G. & SAKANISHI, Y., 1974. Effect of temperature on the flowering of *Clivia miniata*. *J. Japan. Soc. Hort. Sci.* 42(4), 326-332.

CHAPTER 7

General conclusions

Rich and diverse variation exists in the genus *Clivia*. Therefore, methods which will enable more rapid clonal multiplication would enable propagation of plants with desirable characteristics. This would stimulate further research into cultural aspects such as the control of phenology in commercial production. Once further work has been conducted on the specific cultural requirements of particular clones, their commercialisation will lead to development of more new cultivars either through conventional breeding or the use of modern biotechnology. In combination, successful control of propagation and phenology will lead to increased popularity of *Clivia* in a market which constantly demands new and improved products which are attractive to consumers and profitable for producers. Although the propagation techniques presented in this study may not be adequate for commercial scale propagation, it is felt that they will be useful in allowing wider distribution of desirable genotypes which are in the possession of individual collectors or a small number of growers.

Since significant advances have been made in the cultivation and breeding of *Clivia* in Japan and Europe, a concerted research effort will need to be applied locally in order to gain any competitive advantage. It is hoped that the results of this study will serve to illustrate what may be achieved if further work is conducted in the areas which were examined and where preliminary results were obtained.

APPENDIX

Statistical analyses

Two way table of treatment versus time (Section 2.3)

Frequency
Expected
Cell Chi - Square
Percent
Row Pct
Col Pct

	1	2	3	4	
1	19	14	4	4	41
	9.4253	9.8966	8.0115	13.667	
	9.7625	1.7014	2.0086	6.8374	
	21.84	16.09	4.6	4.6	47.13
	46.34	34.15	9.76	9.76	
	95.00	66.67	23.53	13.79	
2	1	7	13	25	46
	10.575	11.103	8.9885	15.333	
	8.6693	1.5165	1.7903	6.0942	
	1.15	8.05	14.94	28.74	52.87
	2.17	15.22	28.26	54.32	
	5.00	33.33	76.47	86.21	
Total	20	21	17	29	87
	22.99	24.14	19.54	33.33	100.00

Statistic	DF	VALUE	PROB
Chi - square	3	38.3442	<.0001
Likelihood Ratio Chi - Square	3	43.8267	<.0001
Mantel - Haenszel Chi - Square	1	36.3627	<.0001
Phi Coefficient		0.6639	
Contingency Coefficient		0.5531	
Cramer's V		0.6639	

Effective Sample size = 87
Frequency missing = 8

Regression analysis of number of shoots as a function of concentration paclobutrazol (Section 4.3)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	191.24356	95.62178	8.37	0.0006
Error	61	696.50644	11.41814		
Corrected Total	63	887.75000			

Root MSE	3.37907	R-Square	0.2154
Dependant Mean	4.56250	Adj R-Sq	0.1897
Coeff Var	74.06189		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard error	t Value	Pr > |t|
Intercept	Intercept	1	2.79884	0.60343	4.64	<.0001
V4	Conc	1	0.17897	0.04993	3.58	0.0007
SQV4	Hormone	1	-0.00149	0.00050303	-2.96	0.0044

Analysis of variance (Section 4.3)

The ANOVA Procedure
Tukey's Studentised Range (HSD)

Alpha	0.05
Error DF	56
Error Mean Square	0.066855
Critical Value of Studentised Range	4.4523
Minimum Significant Difference	0.407

Means with the same letter are not significantly different

Tukey Grouping			Mean	N	V4
	A		0.8521	8	10
B	A		0.8200	8	20
B	A		0.8153	8	100
B	A		0.7974	8	50
B	A	C	0.6076	8	4
B	A	C	0.5935	8	2
B		C	0.4188	8	1
		C	0.2124	8	0