Axillary bud development in chrysanthemum

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Stellingen

1. De kwaliteit van chrysantenstekken wordt voor het grootste gedeelte beïnvloed tijdens de uitgroei van de okselknoppen en niet tijdens de aanleg van de okselknoppen.
   Dit proefschrift

2. Bij het gedeeltelijk ontbladeren van niet te lange scheuten van chrysant is het voor de uitgroei van de bovenste okselknop niet van belang welke bladeren worden verwijderd.
   Dit proefschrift

3. Een verdere toename van de omvang van het merg is bij chrysant het gevolg van een toename van het aantal mergcellen en niet meer van een toename van de celgrootte.
   Dit proefschrift

4. De waarneming dat bij een gelijkblijvend bladoppervlak en een vermindering van het aantal okselknoppen de apicale dominantie in chrysant verloren gaat, steunt de opvatting dat de apicale dominantie afhankelijk is van de beschikbaarheid van voedingsstoffen.
   Dit proefschrift

5. De kwaliteit van chrysantenstekken kan verbeterd worden door het verminderen van het aantal okselknoppen per moederplant.
   Dit proefschrift

6. Zowel de praktijk als het onderzoek zullen gebaat zijn bij een eenduidige definitie van stekkwaliteit.

7. De Nederlandse tuinbouw dient zich minder te richten op opbrengstverhoging en meer op winstmaximalisatie.

8. 'Positieve discriminatie' wordt door vrouwen niet altijd als positief ervaren.

9. Als men zegt 'ik heb geen tijd' bedoelt men te zeggen 'ik leg mijn prioriteiten anders'.

10. Bij werkbesprekingen verdient de opvatting van Paul Valéry dat de gedachten die je voor jezelf houdt verloren gaan, de aandacht.

11. De opvatting dat met minder geld de kwaliteit van het onderwijs verbeterd kan worden is een utopie.

12. De invloed van humor op het werk wordt onderschat.

Stellingen behorende bij het proefschrift:
'Axillary bud development in chrysanthemum'

Henrieke A. de Ruiter
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Abstract

De Ruiter, H.A. 1996. Axillary bud development in chrysanthemum. Dissertation Wageningen Agricultural University, Wageningen, The Netherlands. 103 pp.; English and Dutch summaries.

Each chrysanthemum cutting originates from an axillary bud. For an improvement of the cultivation of cuttings or more specific their quality, it is necessary that the development of an axillary bud can be controlled as good as possible. Axillary bud development can be distinguished into axillary bud formation and axillary bud outgrowth. The effect of assimilates, position and age of axillary buds, and temperature on formation and outgrowth of the axillary buds and the subsequent cutting quality was studied. Measured quality parameters of the cuttings were: fresh- and dry weight, diameter, number of leaves, leaf area, length, number of pith cells in a cross section and diameter of the pith.

The effect of assimilates and temperature on axillary bud formation and subsequent cutting quality was only small. Differences that occurred were mainly due to differences in developmental stage and degree of inhibition of the axillary buds.

On the other hand, axillary bud outgrowth and subsequent cutting quality can be influenced. An increase in number of leaves (assimilates) per axillary bud, by removing axillary buds, increased cutting quality. Position and age of the axillary buds also affected cutting quality when the outgrowth of the bud took place on the plant. However, the outgrowth of axillary buds separated from the plant was not influenced by age and position of the buds. Finally, decrease in temperature reduced axillary bud outgrowth but favoured subsequent cutting quality.

The best way to improve cutting quality is increasing the amount of assimilates per outgrowing axillary bud and/or decreasing the temperature. Unfortunately, increasing cutting quality in these ways decreases the number of produced cuttings. An economic optimum for quality and number of cuttings should be found.

Key words: age, assimilate supply, axillary bud, chrysanthemum, *Chrysanthemum morifolium*, cutting quality, development, *Dendranthema grandiflora*, formation, outgrowth, pith, position, temperature.

Contents

Account

The Chapters 3, 4.1, 4.2, 4.3, 4.4 and 5.1 have been submitted for publication in international journals. The following Chapters have already been published or accepted for publication.

Chapter 3: De Ruiter, H.A. 1996. Chrysanthemum cuttings as affected by age and position of the axillary buds. Ann. Bot. 77: 99-104.

Chapter 4.1: De Ruiter, H.A. 1993. Improving cutting quality in chrysanthemum by stock plant management. Scientia Hort. 56: 43-50.

Chapter 4.2: De Ruiter, H.A. and Tromp, J. 1996. The growth and quality of axillary shoots of chrysanthemum as affected by number and position. J. Hort. Sci. (in press)

1. General introduction

History

From old Chinese sources can be derived that chrysanthemums were found in China 500 years before Christ and a few hundred years later, transported from China to Japan. In Japan hybridizations were made between different species, but which species is not known. Today chrysanthemums are the final product of more than 2000 years of hybridization (Anonymous, 1985).
In 1688 the first chrysanthemums reached Europe, but those sent to The Netherlands got lost. In 1789, the French Captain Blanchard took three species to Marseille, that, probably, formed the starting material for the cultivation in Europe (Zimmer et al., 1991). Chrysanthemum cultivation in The Netherlands started in the beginning of the 20th century. At first chrysanthemums were only grown in summer and autumn dependent on the short days that they needed for flowering. In the sixties technical improvement made year round production possible (Anonymous, 1985). The main chrysanthemum species used for culture purposes and accordingly used in this research is known as '*Chrysanthemum morifolium* Ramat' but its official name is '*Dendranthema grandiflora* Tzvelev'.

Cultivation

The chrysanthemum cultivation is one of the most advanced and controlled glass cultivations in The Netherlands. Chrysanthemums are vegetatively propagated by means of cuttings taken from stock plants. In practice a stock plant is made by removing 5 cm shoot from above the sixth leaf (counting from the soil surface) of a rooted cutting. The axillary buds in the axils of the remaining six leaves are able to grow out into shoots and at the moment there is 5 cm shoot standing above the fourth leaf (counting from the base of the new formed shoots) this 5 cm shoot (the cutting) is picked (Fig. 1.1). This procedure is repeated until the stock plants are about three months old, whereafter the stock plant is discarded. Thus each cutting originates from an axillary bud. An axillary bud grows out, if the apical meristem of the shoot on which the bud is located is removed. Axillary bud development can be divided into axillary bud formation (before topping) and axillary bud outgrowth (after topping). Little is known about factors influencing bud formation; therefore, the distinction between bud formation and bud outgrowth will not be elaborated in

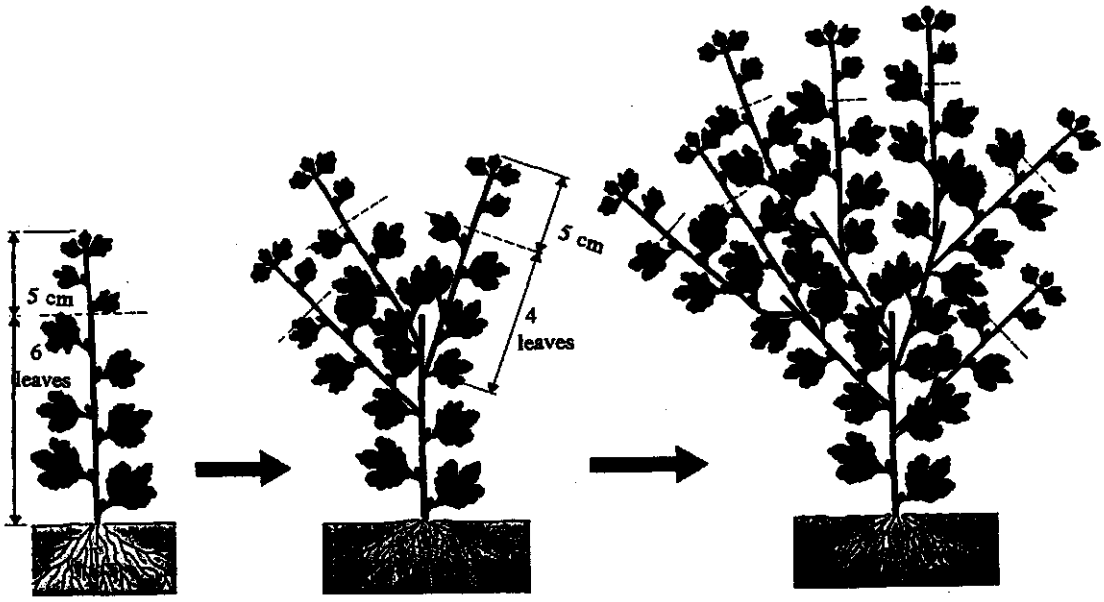

Fig. 1.1: Production of a chrysanthemum stock plant.

this Chapter.

For a further improvement of the cultivation of cuttings or more specific their quality, it is necessary to control development and quality of cuttings in a high degree. Several factors are known to have an influence on axillary bud development. A distinction can be made between endogenous and nutritional factors on the one and environmental factors on the other hand. With respect to quality a number of parameters is used, viz. fresh weight and diameter (Anderson and Carpenter, 1974; Agustsson and Canham, 1981; Eng et al., 1985), dry weight (Wott and Tukey, 1969), the number of roots after propagation (Röber, 1976) and hardness of the cutting (Chan, 1955). A second not lesser important aspect of quality is uniformity, which, as a matter of course, should be so great as possible. Uniform cuttings are important for a uniform flower production (Van der Hoeven, 1989; Van Vliet, 1989). Grading the cuttings in different weight categories enlarged the uniformity of the flowering plants (Van Vliet, 1990; De Greef, 1989). Cuttings with a higher fresh- and dry weight prior to propagation had a higher dry weight and produced more and larger roots after propagation than cuttings of lower initial dry weight (Wott and Tukey, 1969).

Endogenous factors

bud age and position

Axillary buds along a shoot differ from each other in age and position. Furthermore, it should be realized that bud formation and bud outgrowth do not necessarily occur at the same environmental conditions. Commercial companies take cuttings from different lengths and from different ages, which explains part of the variation between cuttings (Furuta and Kiplinger, 1955). In an experiment where chrysanthemum shoots were pruned above the basal four or eight nodes, the highest axillary bud produced the maximum number of cuttings (total of generations) and the lowest produced the minimum (Heins and Wilkins, 1979).

nutrition

Nitrogen and potassium nutrition of the stock plants influences the quality of the cuttings. Röber (1978) found for chrysanthemum that total weight of the cuttings produced by one stock plant increased with increasing N and K from the first to the fourth picking but decreased thereafter. Roughly speaking the number of roots per cutting and the weight per cutting showed a similar course, an increase from the first to the second generation and after that it had no effect at all. Good and Tukey (1967) also had the opinion that the nutrition of the stock plants manifests itself in the cuttings. Machin (1973), reported that picking cuttings is more difficult at a low N and K nutrition because fibre development occurs closer to the apical meristem. Applying NPK (20-2-11 mg l^{-1} substrate) every week considerably increased cutting yield (Krause, 1981).

Environmental factors

leaf position

There is some information that leaf position with regard to the developing buds affects bud outgrowth. When removing the five uppermost leaves from a chrysanthemum shoot with a total of ten leaves, more lower axillary buds sprout and less upper ones. Removing the five lower leaves has no effect (Keppeler, 1968).

hormones

Amo ([4-hydroxy-5 isopropyl-2 methylphenyl] trimethylammonium chloride, 1-piperidine carboxylate), CCC (2-chloroethyl trimethylammonium chloride) and Phosfon (2,4-dichlorobenzyl, tributylphosphonium chloride) all inhibit stem elongation, resulting in a thickening of the stem. This inhibition is reversed or prevented by application of GA (gibberellin). GA-treated stems are considerably thinner than those of the control plants, having fewer and smaller cells across the pith, cortical and vascular tissues. According to Sachs and Kofranek (1963) apparently there is an inverse relationship between longitudinal and transverse growth: if the one is promoted, the other is inhibited.

temperature

Hughes and Cockshull (1972) reported that in the range of 24°C-30°C, the growth of cuttings increases with temperature. Stefanis and Langhans (1982) growing cuttings at 21°C, 27°C and 32°C found the highest dry weight at 27°C; during the subsequent rooting, temperature has no effect on dry weight. Berg and Cutter (1969) state that, in general, after removing the apical meristem the two uppermost axillary buds produce 1.4-1.6 leaves per day during the first nine to ten days. Thereafter, that number drops to 0.7-0.8. Cockshull et al. (1981) reported that the rate of leaf initiation and the shape of the leaf is influenced by temperature. The higher the temperature the more leaves are initiated and the longer the internodes (the maximum temperature investigated was 20°C). According to Klapwijk (1987) the leaf initiation rate is constant during the summer period (1.0 leaf per day), decreases linearly to midwinter (0.3 leaf per day), and increases linearly again to the end of April. Stem length responded in the same way. Leaves initiated at relatively high temperatures (27°C) show less notches than leaves initiated at low (17°C) temperatures (Schwabe, 1959). Low day temperatures reduce the increase of the leaf surface (Cockshull et al., 1981). According to Keppeler (1968), high temperatures promote the growth of the upper buds.

light intensity and quality

Especially light intensity plays an important role in axillary bud outgrowth (Machin, 1973). At increasing light intensity, more lower buds sprout (Keppeler, 1968). According to Schwain (1964), extra artificial illumination increases dry weight of cuttings. He further found that from September to January the number of cuttings

increased when extra light was given whereas from January to April there was no effect. At a light intensity of 100 W m^{-2} given by Multivapor and Lucalox lamps stock plants grown from September 30 to May 15 produced more cuttings than those receiving only seasonal daylight + photoperiod lighting; in addition cutting quality was improved reflected in higher fresh- and dry weight and thicker stems (Anderson and Carpenter, 1974). According to Moe (1985) an increase in irradiation (given by fluorescent lamps) from 5 to 15 W m^{-2} raised the yield of cuttings by 58%. Hughes and Cockshull (1971a, b) reported that from January to April chrysanthemum plants profited more by an increase in light intensity and CO_2 concentration than in the period September to December. Dry weight was higher, leaf surface larger and lateral shoots longer. By splitting the total sum of light in low light intensity for many hours and high intensity for a few hours, keeping the same total amount of light, dry weight, leaf surface, leaf weight and water content of the stem were higher at low light intensity for many hours (Hughes, 1973a and b).
In addition to light intensity, light quality also is an important factor. If chrysanthemums are grown under red light the axillary buds sprout quicker, especially the ones in the middle part of the stem (Heins and Wilkins 1979). According to Keppeler (1968) the upper buds grow more rapidly in red light.

Relative humidity

Keppeler (1968) found that chrysanthemum buds sprout more rapidly when standing under mist. He also found that a high relative humidity promoted the growth of the upper axillary buds.

CO_2

In a study of Molitor and Hentig (1987) carbon dioxide enrichment during stock plant production of chrysanthemum (up to 1600 μl $litre^{-1}$) promoted cutting fresh- and dry weight.

Aim of the study

The aim of the present study was to enlarge the knowledge of the development (formation and outgrowth) of axillary buds of chrysanthemum. A number of factors were investigated in their effect on axillary bud formation (before topping) and axillary bud outgrowth (after topping). More knowledge of the development of an axillary bud will enlarge the possibilities to influence development and quality of a

cutting. As a result cuttings of better quality may become available for stock plant and flower production. Used quality parameters are: fresh weight, dry weight, number of leaves, area of leaves, diameter of the cutting, length, number of pith cells and amount of pith.

Outline of the study

In Chapter 2 the formation of an axillary bud is described. In order to get a better insight in the moment axillary bud development begins, axillary buds are studied using a scanning electron microscope.
In Chapter 3 the effect of age and position of axillary buds on cutting quality is unravelled.
Chapter 4 focuses on some factors influencing axillary bud outgrowth. Successively, the effect of different stock plant management systems (Chapter 4.1), assimilate supply (Chapter 4.2), temperature (Chapter 4.3) and age and number of leaves (Chapter 4.4) on axillary bud outgrowth will be evaluated.
In Chapter 5 the attention is directed on axillary bud formation. In Chapter 5.1 the effect of assimilate supply on axillary bud formation is discussed and in Chapter 5.2 the effect of temperature.
Finally, in Chapter 6 an attempt is made to integrate the results of the previous Chapters.

2. Formation of axillary buds

Introduction

Chrysanthemums are vegetatively propagated by means of cuttings taken from stock plants. Every cutting originates from an axillary bud situated in the axil of a leaf.
For a number of plants mature buds, whether terminal or axillary, contain bud primordia in the axils of their leaf primordia. These two generations of buds are referred to as 'primary' and 'secondary', the former term being applied to the mature bud, the latter applied to the young embryonic buds that are developing within the larger bud (Garrison, 1949a and b; Majumdar and Datta, 1946; Marcelis-van Acker, 1994a).
In an apex of *Chrysanthemum morifolium* 'Improved Albatross III', leaf primordia appear in a certain pattern (Fig. 2.1). A newly formed axillary bud first becomes evident because of a "shell zone" (Clowes, 1961). This is a pattern of cell walls that topographically sets off a pocket of meristematic cells in the axil of a leaf primordium. In *Chrysanthemum morifolium* 'Albatross', this shell zone first appears in middle to late P4 (Fig. 2.1). By the time the primordium becomes P9, a hump of cells (representing the secondary axillary bud) is very evident in cross sections (Berg, 1970). Koch (1893) reported that in angiosperms, buds generally arise in connection with the third or fourth pair of leaf primordia behind the shoot apex. Gifford found that in *Drimys winteri* var. chilensis the first axillary bud activity is first perceptible in connection with the fourth or fifth leaf primordium of the primary axillary bud.
As a part of our research project on influencing axillary bud development in chrysanthemum, the present study was set up to analyze the sequence of appearance of the different leaf primordia and the first secondary axillary bud in the cultivar 'Cassa'.

Materials and methods

Unrooted cuttings (5 cm long) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev) cultivar 'Cassa' were obtained from Fides nurseries, De Lier. They were rooted in a mixture of sand and peat (1:1 by volume) and after 14 days planted into 14 cm-square plastic pots containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:25 by volume) (Lentse potgrond, number

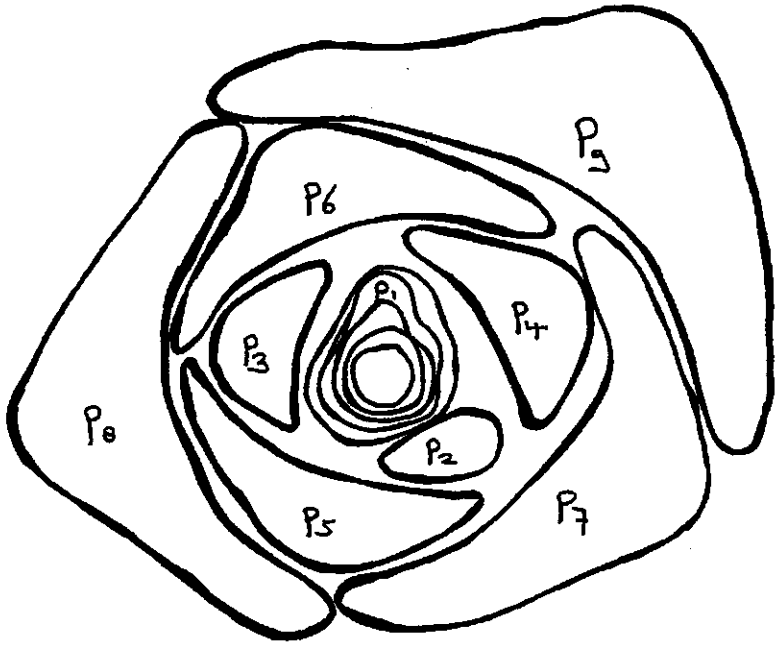

Fig. 2.1: An apex of *Chrysanthemum morifolium* 'Improved Albatross III', P9 is the oldest leaf primordium and P1 the youngest (according to Berg and Cutter, 1969).

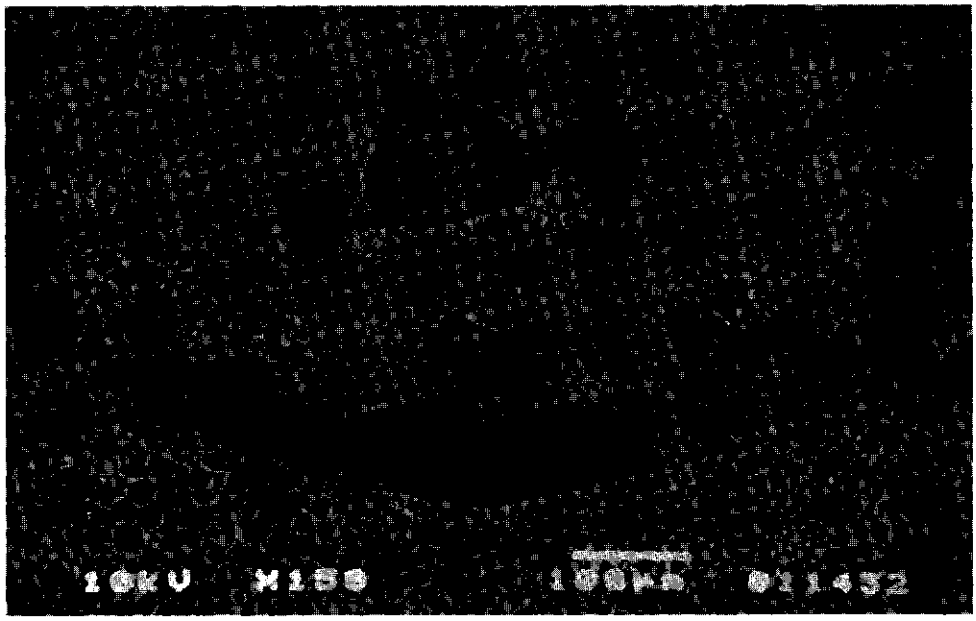

Fig. 2.2: An apex of *Chrysanthemum morifolium* cultivar 'Cassa', P5 is the oldest leaf primordium and P1 the youngest.

4). Thereafter they were transferred to a controlled environment room with a day/night temperature of 18°C, a relative humidity of 70%, a light intensity of 30 W m^{-2} and a day length of 16 hours. The plants were fed once every two weeks with alternately a solution containing: N, P and K (18:18:18) and N, P, K and Mg (15:3:15:5). At the time 5 cm stem had developed above the sixth leaf (counting from the soil surface), this 5 cm stem was picked. The six axillary buds of the topped plants were now able to develop into shoots. From the topmost shoot, axillary buds in the axils of the fourth leaf (counting from the base of the shoot) were taken at different times, i.e. at different developing stages. The axillary buds were fixed in 2% glutaraldehyde for two hours. After dehydration through a graded ethanol series buds were critical point dried in liquid CO_2 using a Balzers union CPD 020. The buds were mounted on stubs and cut with a razor blade. After this, they were sputter coated with a gold/palladium mixture. The axillary buds were studied and photographed using a Jeol JSM 5200 scanning electron microscope at 10 or 15 kV

Results

An axillary bud of *Chrysanthemum morifolium* cultivar 'Cassa' has the same pattern of leaf initiation as an axillary bud of *Chrysanthemum morifolium* cultivar 'Improved Albatross III' (Fig. 2.2). In Fig. 2.2 it can also be seen that in longitudinal sections, the leaf primordium next to leaf primordium 3 is leaf primordium 1.

At the moment five leaf primordia were initiated inside a primary axillary bud, a sharp axil between leaf primordium 1 and leaf primordium 3 could be observed (Fig. 2.3). The distance between the primary axillary bud and the apical meristem at that time was about 1 cm. A little later (still five leaf primordia were initiated inside a primary axillary bud) the sharp axil changed into a blunt axil (Fig. 2.4). There were no signs of a secondary axillary bud yet. The distance between the primary axillary bud and the apical meristem was about 1.5 cm. At the time six leaf primordia were developed inside the primary axillary bud, the first signs of a secondary axillary bud were visible. The distance between the primary axillary bud and the apical meristem was about 2 cm.

At the time the shoots were topped (5 cm between the primary axillary bud and the apical meristem) about 16 leaf primordia were initiated and several secondary axillary buds were present inside the primary axillary bud.

Fig. 2.3: A sharp axil (arrow) between leaf primordium 1 and leaf primordium 3.

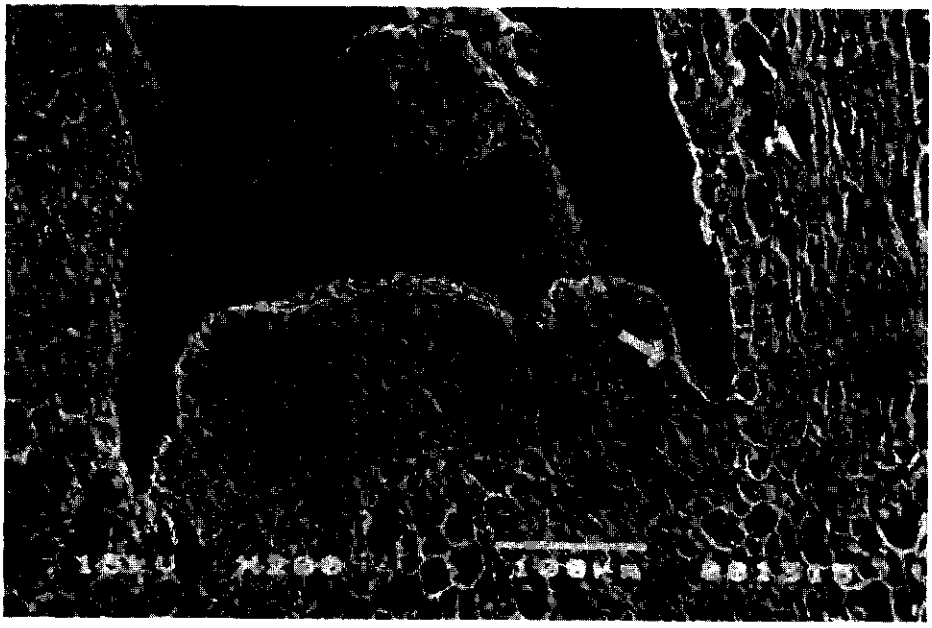

Fig. 2.4: A blunt axil (arrow) between leaf primordium 1 and leaf primordium 3.

Discussion

According to our results the formation of the first secondary axillary bud inside a primary axillary bud begins in connection with the fifth or sixth leaf primordium. The sharp axil between leaf primordium 1 and 3 changes into a blunt axil and soon the first signs of a secondary axillary bud are visible.

According to Berg (1970) the first shell zone in *Chrysanthemum morifolium* cultivar 'Albatross' is visible in middle to late P4, by the time the primordium becomes P9, a hump of cells (representing the axillary bud) is very evident. In Fig. 2.5 this hump of cells can already be seen in P6. However, we must keep in mind that the plants Berg (1970) was working with were of a different cultivar and grown at different circumstances. All studies on leaf initiation rates have shown that leaves are formed at a constant rate over long periods of time under constant environmental conditions (Berg and Cutter, 1969; Schwabe, 1959; Klapwijk, 1987). Although we have studied several primary axillary buds of one particular stage of development, we must keep in mind that in the organization of a vegetative shoot apex of chrysanthemum an

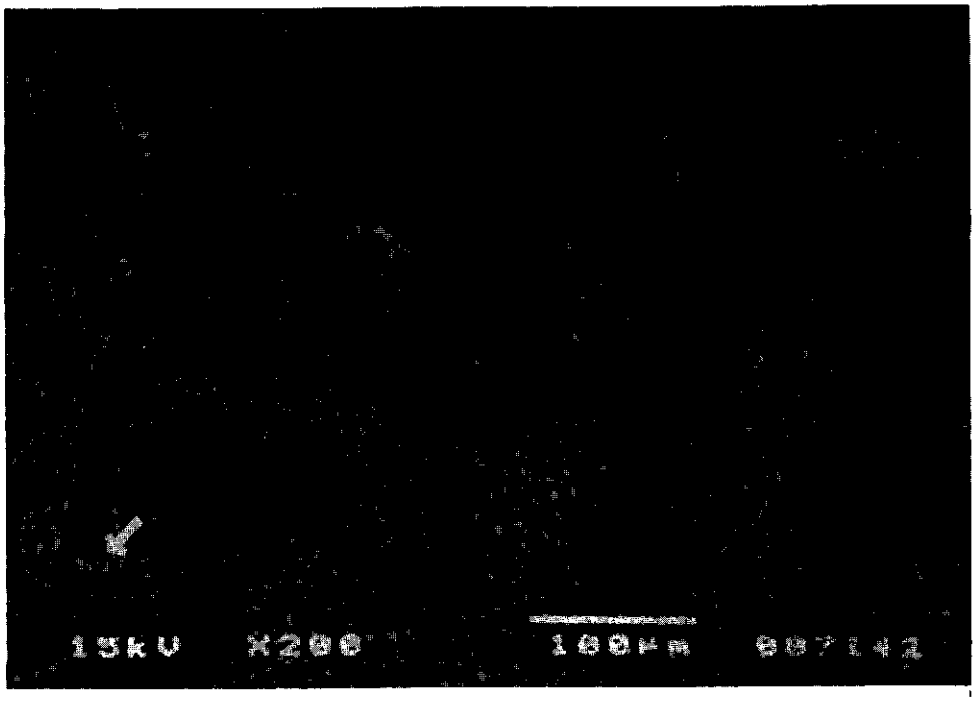

Fig. 2.5: The first signs of a secondary axillary bud (arrow) in a primary axillary bud.

extreme variability within single varieties is present (Popham and Chan, 1950).
In our research about 16 leaf primordia were developed inside a primary axillary bud at the moment 5 cm shoot above this bud was topped. According to Horridge and Cockshull (1979) axillary buds of *Chrysanthemum morifolium* cultivar 'Polaris' usually have initiated between seven and ten leaf primordia at the moment of topping. This large difference could be due to differences in cultivar but it is also possible that the length of the shoot above the topped axillary bud was less and therefore the bud was less developed.
On the bases of the present observations and the described literature, we decided to begin with the treatments for influencing the formation of a secondary axillary bud, at the time the fourth leaf primordium is initiated in the primary axillary bud. We can be pretty sure that at this time the formation has not yet begun.

3. Cuttings affected by age and position of the axillary buds

Abstract

In three experiments (two in-vivo, one in-vitro) an attempt was made to separate the possible effect of age and position of axillary buds of chrysanthemum on bud outgrowth and subsequent cutting quality.
In the in-vivo experiments, bud age and bud position did not seem to be important factors for bud outgrowth and subsequent cutting quality. Nevertheless most outgrowth parameters showed somewhat higher values for the lower positioned buds and, furthermore, the time needed to produce a cutting tended to decrease with the age of the axillary bud.
In the in-vitro experiment, the relationship between age and the various parameters showed an optimum.

Introduction

Chrysanthemum cuttings are usually harvested from stock plants. The cuttings originate from an axillary bud situated in the axil of a leaf. Axillary buds are able to develop into shoots after release from inhibition by some factor emanating from the growing shoot tip. When these developing shoots have reached a certain length, the top (the 'cutting') is taken. The axillary buds on the remaining part of these shoots are able to sprout to give the next generation of cuttings.
In the commercial production of chrysanthemum cuttings homogeneity of cuttings is a requisite since uniform, well grown cuttings offer uniformity and predictability in harvesting and flowering. This requirement of homogeneity is not always satisfied probably because axillary buds differ in age and in position along a shoot. These factors are linked and their relative importance for bud outgrowth and subsequent cutting quality is not easy to determine. In an experiment where chrysanthemum shoots were pruned above the basal four or eight nodes, the apical axillary bud produced the maximum number of cuttings (total of all generations) and the basal produced the minimum (Heins and Wilkins, 1979). Similarly, in *Nicotiana tabacum* it could be shown that the number of nodes produced by an axillary bud is a function of its position on the stem (McDaniel and Hsu, 1976). In an in-vitro culture study, explants of *Vitis rotundifolia* originating from the ten basal nodes of a shoot, having at least 25 nodes, gave better shoot proliferation than those originating from

the ten distal nodes (Sudarsono and Goldy, 1991). Cuttings from *Schefflera arboricola* from subapical positions rooted more slowly, produced fewer roots with a lower rooting percentage than cuttings from the more basal regions (Hansen, 1986). Comparison of the structure of axillary buds along a rose shoot showed several anatomical and morphological differences (Zamski, Oshri and Zieslin, 1985). Cockshull and Horridge (1977) suggested that the bud inhibition gradient along a shoot originates from differences in the anatomical structure laid down during the early stages of bud development.

In the present study of two in-vivo and one in-vitro experiment an attempt was made to separate the factors of age and position and to study their possible effect upon the outgrowth of an axillary bud and the subsequent cutting quality. The following quality parameters of the cuttings considered were: diameter, number of leaves, total leaf area and fresh- and dry weight.

Materials and methods

Experiment 1: This experiment is designed to assess whether position and age as such have any effect on bud outgrowth. Axillary buds on an intact shoot were therefore compared with isolated buds on isolated shoots.

At the end of September 1993, cuttings (5 cm in length) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev.) cv. Cassa (Fides, De Lier) were rooted in a mixture of sand and peat (1:1 by volume). Each cutting was pinched either at leaf five (6 Oct, experiment 1.1) or at leaf eight (11 Oct, experiment 1.2) counting from the soil surface, when there was 5 cm of shoot above the fifth or eighth bud, respectively. In this way buds 1-5 in experiment 1.1 and buds 4-8 in experiment 1.2 were of the same age, but were not in the same position. In both experiments buds 1-5 were in the same position but differed in age (Fig. 3.1A). Thereafter, from a number of pinched cuttings, five or eight internodes (including one bud and attached leaf) were severed at a few mm above the axillary buds and each separate segment was put into the rooting medium in a rooting tray covered by a glass lid (Fig. 3.1B). The other batch of cuttings was kept intact and after cutting just above the soil surface also put in the tray (Fig. 3.1C). The rooting trays were kept closed for two weeks. Four weeks after commencing the experiment the following parameters of the axillary sprouting buds or shoots were recorded: length, diameter, number of leaves (over 0.5 cm in length), total leaf area and fresh- and dry weight. The experiments were carried out in the greenhouse. In the first two weeks, average day/night temperature was approximately 24°/22°C respectively, relative humidity approximately 100% and mean irradiance 160 J cm^{-2} per day. In

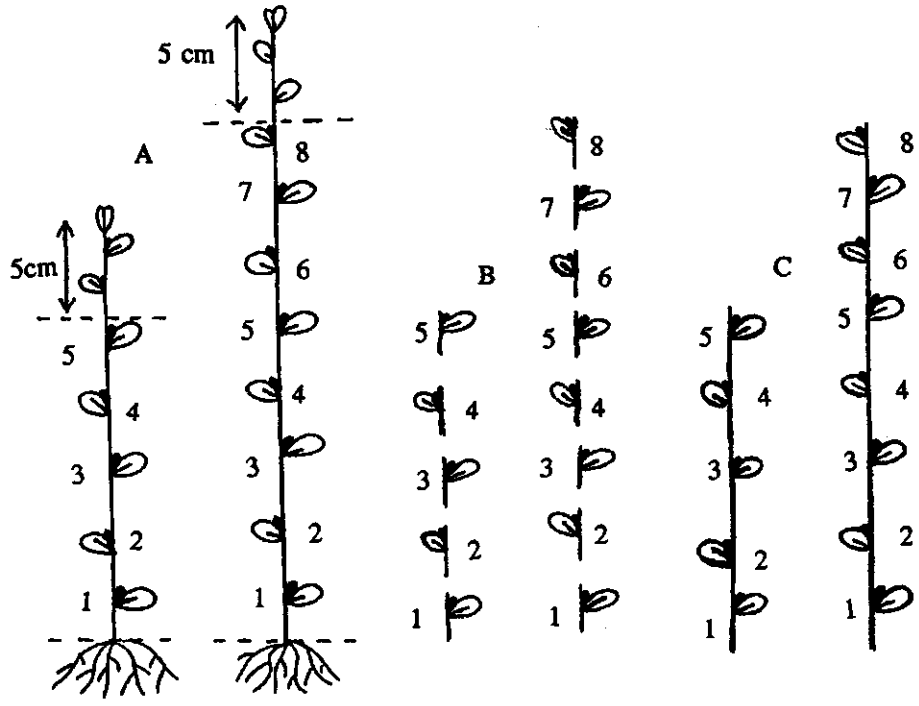

Fig. 3.1: Schematic representation of the plant material used in Experiment 1.

the last two weeks, average day/night temperature was approximately 23°/21°C respectively, relative humidity approximately 70% and mean irradiance 780 J cm^{-2} per day. If the global irradiation outside the rooting tray was below 30W m^{-2} (from 6.00 am - 12.00 pm), an additional illumination (Philips SON-T 400W) of 70W m^{-2} (PAR) at plant level was switched on, above 50W m^{-2} the lamps were switched off. In both experiments there were four replicated groups of four plants in each treatment, positioned at random.

Experiment 2: In this experiment bud position was the same but bud age varied.
In July 1991 chrysanthemum cuttings were rooted as in experiment 1 and after 14 days planted into plastic pots (14 cm) containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:25 by volume) (Lentse potgrond, No.4, Coöp Tuinbouwcentrum Lent). According to length, the cuttings were divided into three groups of about 12, 10 and 8 cm respectively. When the distance between the growing point and the sixth axillary bud (counting from the soil surface) was 1, 3, 5, 7 and 9 cm, from each length group four randomly chosen groups of four plants each were topped just above the sixth bud (Fig. 3.2). In this way the sixth axillary bud was of different age at the moment it was allowed to sprout. Throughout the experiment temperature was 21°C, relative humidity approximately 70% and irradiance (fluorescent tubes, Philips TLD50W/84HF) about 30 W m^{-2} (PAR); day

length was 16h. The plants were fed once every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5). Measurements were made when there was 5 cm stem (the cutting) above four leaves. Measured parameters were: diameter, number of leaves (over 0.5 cm in length), total leaf area and fresh- and dry weight.

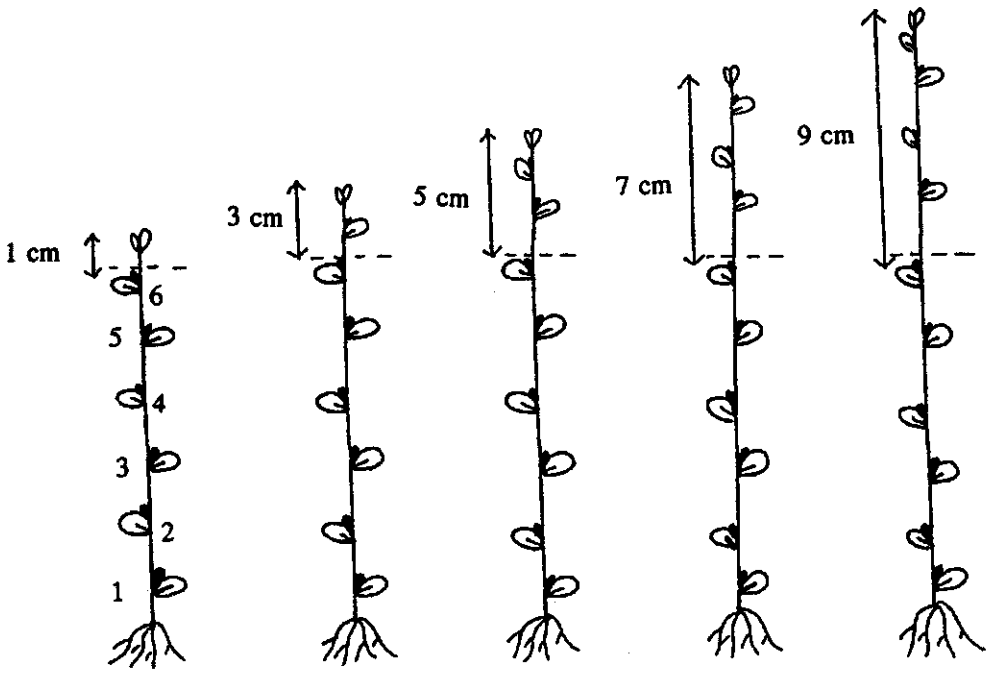

Fig. 3.2: Schematic representation of the plant material used in Experiment 2.

Experiment 3: Also in this experiment bud age was varied but unlike experiment 2 bud outgrowth occurred in-vitro isolated from the rest of the plant.
At the end of July and in the beginning of September cuttings were rooted as in experiment 1 and 14 days later planted into plastic pots as in experiment 2. Thereafter the plants were grown in a controlled-environment room at a day/night temperature of 18°C, a relative humidity of approximately 70% and an irradiance of 30 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD50W/84HF). Day length was 16 h. The plants were fed as in experiment 2. Starting when the distance between the growing point and the sixth axillary bud (counting from the soil surface) was about 4 cm, in four successive weeks (age 1 to 4, Fig. 3.3), the sixth axillary bud of 16 plants in four randomly chosen groups of four plants each was taken. In this way bud age increased with time of sampling. The buds were sterilised in 70% alcohol for a few seconds, followed by 15 minutes in 1% NaOCl to which a few

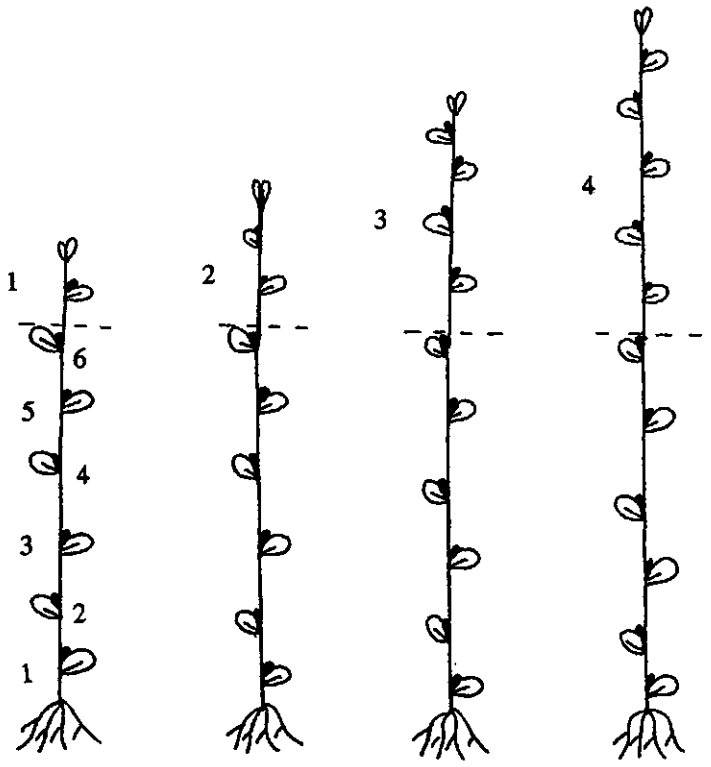

Fig. 3.3: Schematic representation of the plant material used in Experiment 3.

drops of Tween 20 was added. Explants were then washed three times with sterile water. The axillary buds were inoculated individually in Pyrex glass tubes (20 mm diameter) containing 10 ml of a culture medium, and after inoculation covered with a cotton plug and Vitafilm. The axillary buds were grown on a basic culture medium of macrosalts and microsalts at full strength according to Murashige and Skoog (1962) to which NaFeEDTA 37.5 mg l^{-1}, 4 % saccharose and 0.7% Daichin agar in distilled water was added. The pH of the medium was adjusted to 6.0 (before addition of agar). The tubes with the axillary buds were incubated in a growth chamber at 23°C at an irradiance of 6 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD36W/84). The day length was 14 h. Four weeks after the buds were put in-vitro, the following measurements were made: shoot length, diameter, number of leaves (visible to the naked eye) and fresh- and dry weight.

In all experiments results were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

Results

Experiment 1: Although some difference in outgrowth could be observed when axillary buds were separated, these were mostly slight compared to those occurring when axillary buds grew out on the shoot.
The length and diameter of the shoots growing out from five or eight isolated axillary buds showed no clear pattern. The number of leaves over 0.5 cm, however, increased from position 5 or 8 to position 1, and most of the other parameters also tended to be slightly higher for the lower positioned buds (Tables 3.1 and 3.2). Outgrowth parameters (not statistically tested) of buds of the same age (buds 1-5) in the two experiments showed little difference, if any. Similarly, there was no effect of position as shown by the comparison of bud outgrowth of buds 1-5 in experiment 1.1 with 4-8 in experiment 1.2. However, it should be kept in mind that the experimental conditions were not identical for the two experiments.
When the axillary buds grew out on the shoot, the values for all parameters, except diameter, decreased with increasing distance from the top. Measurements were only taken from the topmost three axillary buds, because lower positioned buds hardly grew out (Tables 3.1 and 3.2).

Experiment 2: Most outgrowth parameters tended to decrease somewhat with bud age, especially at the cutting length of 10 and 8 cm at the start of the experiment, but the differences usually were of no statistical significance (Table 3.3). Noteworthy is that in all three length classes the time needed to produce a 5 cm cutting declined with increasing bud age.

Experiment 3: In this experiment bud outgrowth occurred in-vitro, isolated from the rest of the plant. As Table 3.4 shows the values for the various growth parameters for 'age 2' exceeded those for 'age 1, 3 and 4'.

Discussion

The data from experiment 1 (Tables 3.1 and 3.2) do not supply any evidence for the view that bud age and bud position are important factors for bud outgrowth. Nevertheless, most outgrowth parameters showed somewhat higher values for the lower positioned buds. This outcome is in line with findings of Keppeler (1968),

Table 3.1: Effect of position and age of axillary buds (isolated or in situ) on bud outgrowth after four weeks.

Position Age	Shoot		Leaf		Weight	
	Length (cm)	Diameter (mm)	Number	Area (cm^2)	Fresh (g)	Dry (g)
isolated						
5	11.3	2.49	7.71	55.02	2.73	0.25
4	11.8	2.51	8.29	60.21	3.05	0.25
3	11.3	2.50	8.57	56.58	2.86	0.26
2	12.0	2.63	9.19	61.04	3.16	0.29
1	12.2	2.37	9.06	61.08	3.12	0.26
Tukey (P=0.05)	0.6	0.32	0.68	2.43	0.58	0.02
in situ						
5	16.4	2.63	8.44	62.85	3.44	0.34
4	13.4	2.58	7.80	56.66	2.74	0.26
3	6.1	2.27	5.94	27.01	1.24	0.11
Tukey (P=0.05)	1.1	0.40	0.93	3.20	0.62	0.03

also for chrysanthemum. In contrast, Zieslin, Haaze and Halevy (1976) reported for rose that sprouting ability is highest in the apical axillary buds. The slightly better performance of basal buds in experiment 1 may be due to quicker rooting of lower positioned shoot sections. Basal leaf-bud cuttings of *Schefflera arboricola* rooted quicker and produced more roots than cuttings from the more apical positions (Hansen, 1986). Hansen and Kristensen (1990) found a relationship between the number of roots, the bud position and the height of the plant. Basal axillary buds rooted more quickly and produced longer shoots. Light conditions in the basal regions usually are less favourable and as found for a majority of plant species more roots are produced with decreasing irradiance (Biran and Halevy, 1973; Hansen and Eriksen, 1974; Poulsen and Andersen, 1980). Another factor explaining the slight gradient in outgrowth of buds along the shoot could be the quality of the sustaining leaves. The basal leaves are developed on the stock plant and the more apical leaves during rooting i.e. they were formed under different environmental conditions. In some way this could have affected leaf structure, rooting ability and bud outgrowth.

Table 3.2: Effect of position and age of axillary buds (isolated or in situ) on bud outgrowth after four weeks.

	Shoot		Leaf		Weight	
Position Age	Length (cm)	Diameter (mm)	Number	Area (cm^2)	Fresh (g)	Dry (g)
isolated						
8	11.2	2.61	7.06	49.88	2.49	0.21
7	11.5	2.40	7.25	51.39	2.49	0.22
6	11.1	2.35	7.25	51.39	2.48	0.21
5	10.5	2.38	7.38	46.72	2.26	0.20
4	10.7	2.33	7.75	51.41	2.44	0.21
3	10.6	2.59	8.06	56.03	2.69	0.23
2	11.6	2.36	8.86	63.27	3.07	0.24
1	11.0	2.34	8.94	58.84	2.85	0.24
Tukey (P=0.05)	0.9	0.34	0.52	2.62	0.44	0.02
in situ						
8	14.3	2.55	7.93	55.40	3.07	0.31
7	11.9	2.58	6.73	48.79	2.51	0.22
6	7.4	2.35	5.40	30.47	1.45	0.10
Tukey (P=0.05)	1.2	0.32	0.56	2.70	0.48	0.04

In experiment 2 removing different lengths of shoots above the same axillary bud did not markedly influence bud outgrowth (Table 3.3) again indicating that in young shoots bud age is not relevant for bud outgrowth. However, the data strongly suggest that the time needed to produce a cutting of a certain length decreased with bud age.

In the in-vitro experiment (Table 3.4), surprisingly, where similar axillary buds were forced to grow out at four successive weeks, 'age-2' buds performed better than the buds of age 1, 3 or 4. This deviation is not easy to explain. It should be realized that in this experiment just the bud is put in-vitro and its outgrowth must have been determined almost completely by its own potential. In experiment 2 and, although to a lesser degree, in experiment 1 the outgrowing bud forms part of an intact plant, which certainly will affect bud behaviour to a high degree and that may

Table 3.3: Effect of length of cut-off shoot above the sixth axillary bud (age of the sixth axillary bud) on the outgrowing 5 cm cutting.

	Length (cm) of cut-off shoot					Tukey
	1	3	5	7	9	(P=0.05)
Length at start: 12 cm.						
Diameter (mm)	3.3	3.1	3.5	3.3	3.4	0.3
N. leaves	5.4	5.4	5.7	5.4	5.4	0.4
Leaf area (cm^2)	45.4	41.0	50.8	43.3	44.5	7.6
Fresh weight (g)	1.7	1.5	1.9	1.7	1.8	0.3
Dry weight (g)	0.20	0.19	0.22	0.18	0.21	0.03
Days	24	25	22	21	21	2
Length at start: 10 cm.						
Diameter (mm)	3.2	3.2	3.1	3.3	3.0	0.2
N. leaves	5.6	5.6	5.4	5.4	5.1	0.5
Leaf area (cm^2)	44.7	45.9	40.9	44.0	35.8	8.6
Fresh weight (g)	1.7	1.7	1.6	1.7	1.4	0.3
Dry weight (g)	0.20	0.19	0.18	0.18	0.16	0.03
Days	24	23	23	20	20	2
Length at start: 8 cm						
Diameter (mm)	3.3	3.3	3.2	3.1	3.2	0.2
N. leaves	5.8	5.3	5.4	4.9	4.8	0.5
Leaf area (cm^2)	48.4	41.7	40.0	37.0	36.2	7.0
Fresh weight (g)	1.7	1.6	1.5	1.5	1.5	0.2
Dry weight (g)	0.20	0.19	0.17	0.17	0.18	0.03
Days	26	27	24	23	22	2

level the own potential of the bud. The importance of the own potential for growing out was shown for rose by Zieslin and Halevy (1978). They found that upper buds were inhibited when budded on the basal part of the stem but that basal buds retained part of their inhibition when inserted in the upper part. Furthermore, the age range in experiment 3 was markedly larger than in experiment 2. Growing conditions being the same, the distance between the sixth axillary bud of age 3 and the apical meristem was about 21 cm in experiment 3, against only 9 cm in experiment 2.

Table 3.4: Effect of age of the sixth axillary bud on bud outgrowth in-vitro after four weeks (results of two identical experiments).

Age at	Length (cm)	Diameter (mm)	Number of leaves	Weight	
				Fresh (g)	Dry (g)
Week 1	4.1	1.48	10.0	0.53	0.05
Week 2	6.1	1.54	11.7	0.73	0.07
Week 3	4.4	1.41	10.5	0.55	0.05
Week 4	4.3	1.51	10.3	0.67	0.06
Tukey(P=0.05)	0.8	0.18	0.8	0.16	0.01

The pattern found for 'intact' shoots in experiment 1, a strong decrease in outgrowth of axillary buds from position 5 or 8 to position 1 (Tables 3.1 and 3.2), is in accordance with the 'hormonal' theory of apical dominance assuming that the most apical growing point is the source of some correlative signal of hormonal nature, probably auxin, which restricts development of lower meristems (Martin, 1987; Cline, 1994). In addition, a role is also attributed to cytokinins due to their ability to stimulate outgrowth of axillary buds. According to this theory it is not surprising that when isolated from each other, every bud along the shoot sprouts readily. The second important concept to explain the mechanism of apical dominance is the 'nutritive' theory which assigns a prominent role to the internal competition for nutrients and carbohydrates between the growing points along the shoot. However, it is unlikely that lack of carbohydrates has restricted growth of the axillary buds in the 'intact' shoot. Otherwise, total dry weight of the shoots produced by the five or eight isolated buds would not have greatly exceeded that of the intact shoot bearing the same number of buds (Table 3.1 and 3.2). More plausible is that the explanation of the difference of dry matter production between the 'intact' shoot and the isolated shoots sections should be sought in the supply of nutrients or cytokinins coming from the roots. It should be realized that in the isolated shoot sections each section has its own root system whereas in the intact shoot one single root system has to serve the whole shoot.

4. Axillary bud outgrowth

4.1 Effect of stock plant management on cutting quality

Abstract

During commercial cutting production of chrysanthemums, cutting quality in later generations declines as the stock plants age. Three stock plant management systems were investigated for their effect on cutting quality, by varying the number of axillary buds that could grow out and the number of leaves that remained on the plant. Quality parameters of the cuttings were: fresh- and dry weight, dry weight %, number and area of the leaves, leaf area per leaf, leaf area ratio and diameter. In the control stock plants, where every leaf was associated with an axillary bud, cutting quality declined with stock plant age. However, that decline was less marked when by bud removal not every leaf was associated with an axillary bud.

Introduction

Chrysanthemums are vegetatively propagated by means of cuttings taken from stock plants. In the commercial production of chrysanthemum cuttings for the cut flower and pot plant industry, homogeneity of cuttings is required as uniform, well-grown cuttings offer uniformity and predictability in harvesting and flowering (De Greef, 1989; Van der Hoeven, 1989; Van Vliet, 1990). When flowering is synchronised, it is possible to harvest at one time and if the branching is also uniform, grading is simple. As stock plants become older, cuttings of chrysanthemums usually, show a decrease in quality. This can be detected as for instance, a decrease in fresh weight and in thinner stems (Agustsson and Canham, 1981; Anderson and Carpenter, 1974; Eng et al., 1985). Röber (1978b) found a decrease in fresh weight per cutting in the second generation but, thereafter, weight remained the same. Cuttings also become more fibrous when the stock plants become older. 'Hard' cuttings, presumably with a woody type of growth, produced flowering plants with fewer flowers than 'soft' cuttings (Chan, 1955).

The scope of this work was to investigate whether cutting quality could be improved by manipulating the stock plants by varying the number of leaves and axillary buds. Three experiments were carried out: one under completely controlled conditions and

two in the greenhouse. Since the same general pattern was visible in all three experiments and for reasons of space, only the results of the experiment under controlled conditions will be discussed here.

Materials and methods

Cuttings (5 cm long) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev.) cv. Cassa (Fides, De Lier) were rooted in a mixture of sand and peat (1:1 by volume) and, after 14 days, planted into 14 cm-square plastic pots containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:25 by volume) (Lentse potgrond, number 4). The plants were fed once every two weeks with a solution containing alternately : N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5). When the distance from growing point to the fourth leaf from the base was 5 cm, the 5 cm stem (cutting) was picked. Axillary buds grew out into shoots and these in turn were treated in the same way. That procedure was done four times in total (four "generations"). The first cutting taken from the main shoot of the original cutting ("generation 0") did not take part in the experiment. By varying the number of axillary buds that could grow out and the number of the leaves remaining on the plant, three different kinds of stock plants were made as follows (Fig. 4.1.1).

A: Control: In each generation all four axillary buds were allowed to produce new shoots and all leaves were retained. This treatment approximates commercial stock plant production.

B: One shoot, all leaves: In each generation only the shoot from the topmost axillary bud was allowed to develop. All other buds were removed as soon as possible without damaging the plant. All leaves were retained.

C: One shoot, four newest leaves retained: As B, but only the four leaves of the latest generation were retained.

With each stock plant type, data were only recorded from the topmost cutting of each generation.

Measured parameters of quality were: fresh- and dry weight, total leaf number (> 0.5 cm in length), total leaf area using a LiCor 3100 (Leica, Rijswijk) and the diameter at the basis of the cutting. Because cutting development of treatment A

lagged behind that in the other treatments by up to four weeks, these measurements were not made at the same time. Finally, stem thickness at the different branching levels (generations) of the stock plant was measured at the same time in all treatments, at the end of the experiment.

Fig. 4.1.1: The three different types of stock plants (A: Control, normal branching and harvest; B: Topmost axial bud allowed to develop, all other buds removed all leaves retained; C: As B, only the newest four leaves retained).

The experiment was carried out in 3 controlled environment rooms from 21-8-1991 to 21-1-1992. Temperature was 18°C, relative humidity 70%, irradiance 21.3 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD50W/84HF) and day length 16 hours per day. Each room contained three groups of four plants of each treatment positioned at random. Results were statistically analyzed by Analysis of Variance followed by mean separation according to Tukey's HSD-test.

Results

Both fresh- and dry weights of the cuttings were influenced by the different types of stock plants (Table 4.1.1). In the first generation there was no significant difference but later, treatment A differed significantly from B and C. In the first generation

stock plants B and C showed the highest dry weight % but thereafter, except for generation 4, A exceeded that of B and C (Table 4.1.1).

The number of leaves per cutting decreased in control stock plants A from the first generation onwards, but remained almost constant in treatments B and C. It decreased slightly in generation 4. Total leaf area per cutting decreased for all treatments, eventually, by 40%, but the pattern of decrease differed from that of the other parameters. For the control stock plants, the decrease occurred mainly from generation 1 to 2. For the other treatments, it occurred later (Table 4.1.2). From generation 2 onwards, the leaf area ratio for stock plants B was lowest (Table 4.1.3). The area per leaf in generation 2, 3 and 4 of stock plants B was significantly lower than that of C. Cutting diameter in the control treatment (Fig. 4.1.2) showed a steady decrease with generation, resulting in a reduction of 63% by the fourth generation. In treatments B and C it tended to increase from generation 1 to 2 and thereafter it tended to decrease. In generations 2, 3 and 4 significant differences occurred, up to 43% in generation 4, between treatments.

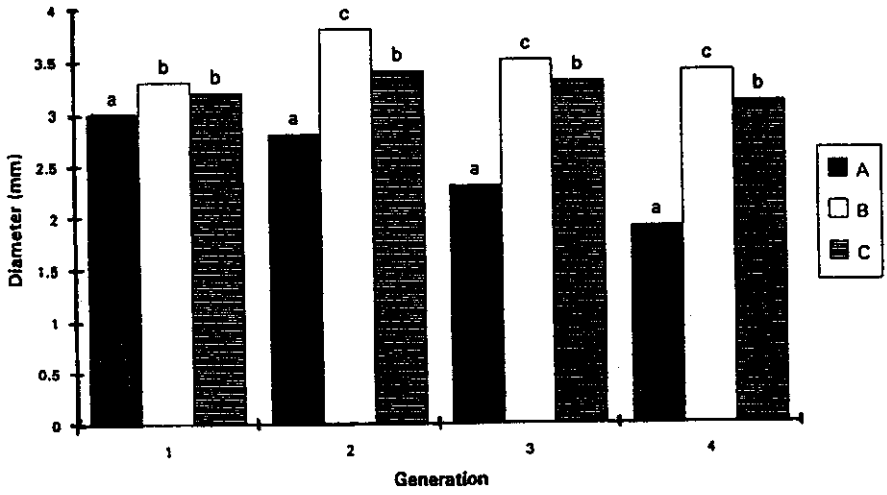

Fig. 4.1.2: Diameter (mm) of 5 cm cuttings from three different kinds of stock plants (values within generations followed by different letters differ significantly at the 5% level).

Table 4.1.1: Fresh weight(FW) (g), dry weight(DW) (g) and dry weight %(DW%) of 5 cm cuttings from three different kinds of stock plants.

	generation											
	1			2			3			4		
	FW	DW	DW%	FW	DW	DW%	FW	DW	DW%	FW	DW	DW%
stock plant												
A	2.1a	0.20a	9.5a	1.3a	0.14a	10.8b	1.1a	0.15a	13.6b	1.0a	0.14a	14.0b
B	2.1a	0.22a	10.5b	1.9b	0.20b	10.5b	1.8b	0.21b	11.7a	1.6b	0.23c	14.4b
C	2.0a	0.22a	11.0b	2.1c	0.20b	9.5a	1.7b	0.19b	11.2a	1.5b	0.19b	12.7a

Values within columns followed by different letters differ significantly at the 5% level.

Table 4.1.2: Number(N) and area (cm^2) of leaves(A) > 0.5 cm in length of 5 cm cuttings from three different kinds of stock plants.

	generation							
	1		2		3		4	
	N	A	N	A	N	A	N	A
stock plant								
A	8.2a	48.6a	7.6a	29.1a	6.7a	24.1a	6.9a	28.9a
B	8.7b	46.7a	8.7b	35.6b	8.7b	31.3b	8.2c	27.9a
C	8.4ab	43.6a	8.6b	43.2c	8.5b	33.9b	7.8b	29.9a

Values within columns followed by different letters differ significantly at the 5% level.

Table 4.1.3: Leaf area ratio(LAR) ($cm^2\ mg^{-1}$) and area per leaf(AL) (cm^2) of 5 cm cuttings from three different kinds of stock plants.

	generation							
	1		2		3		4	
	LAR	AL	LAR	AL	LAR	AL	LAR	AL
stock plant								
A	0.24b	5.9b	0.21b	3.8a	0.16ab	3.6a	0.21c	4.2c
B	0.21a	5.4a	0.18a	4.1a	0.15a	3.6a	0.12a	3.4a
C	0.20a	5.2a	0.22b	5.0b	0.18b	4.0b	0.16b	3.8b

Values within columns followed by different letters differ significantly at the 5% level.

Stem diameter at the different branching levels, showed differences among the three treatments. Control A showed a steady decrease from 5.5 to 3.4 mm. Treatments B and C increased from generation 0 to 2; thereafter B and C tended to decrease slightly. As a consequence, at the topmost branching level the differences between the treatments were most pronounced (Fig. 4.1.3).

Discussion

In chrysanthemums, during normal commercial cutting production, cutting quality declines as stock plants age. If the outgrowth of axillary buds was dependent only on factors such as age of the stock plant or distance between growing shoot tips and roots, quality would decline in all treatments. However since the behaviour of the stock plants of treatments B and C clearly differed from those of stock plants A, it

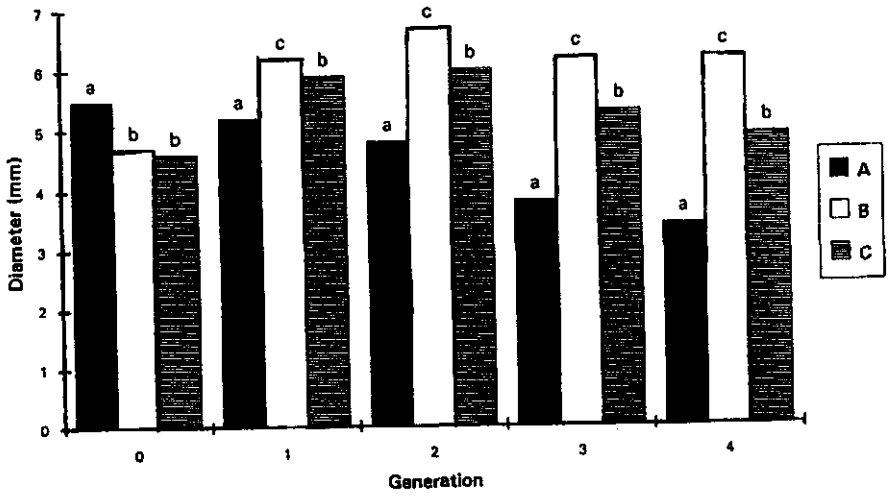

Fig. 4.1.3: Diameter (mm) of the stem at the different levels of branching at the end of the experiment from three different kinds of stock plants (values within generations followed by different letters differ significantly at the 5% level).

can be concluded that other factors must be involved as well, such as, for instance, the amount of assimilates per axillary bud as determined by leaf area, i.e. the number of available leaves. Tomato trusses (Slack and Calvert, 1977) and tomato and cucumber fruits (Marcelis and Heuvelink, 1990) increased in weight when there were more leaves available per truss or fruit. In the present experiment, the ratio number of axillary buds/number of leaves was in A: 1/1, in B: 1/a multiple of four and in C: 1/4. The amount of roots also differed but precise observations were not made. It also can not be ruled out that effects of hormone nature are involved.

Tables 4.1.1, 4.1.2 and 4.1.3 and Figs. 4.1.2 and 4.1.3 show that the more leaves were available per cutting the better the quality. Although stock plants B had more leaves than stock plants C, differences between B and C for the various parameters were not found in all instances. It could be that not all the leaves were functioning because the leaf was browning and becoming harder when it was ageing. It is also possible that the upper, younger leaves shaded the lower, older leaves. (Aikin and Hanan, 1975; Bozarth et al., 1982; Lieth and Pasian., 1991). Fresh weight decreased strongly from generation 1 to 2 for stock plants A and Röber (1978), also found a decrease only from generation 1 to 2 for chrysanthemum.

The significant higher leaf area of the cuttings from stock plants C as compared with B in generation 2 is remarkable. The reason could be that the fewer leaves remain, the larger they get because the plant may tend to compensate for the missing leaves. In generations 2, 3 and 4, the leaf area ratio and area per leaf are also higher for stock plants C compared with B. For young tomato, cucumber and sweet pepper plants, a higher mean daily light integral resulted in a decrease in LAR (Bruggink and Heuvelink, 1987). It is a reasonable assumption that plants are also able to compensate for missing leaves.

The reduced stem diameter of the control cuttings in later generations may be the result of competition from other growing shoots. This view would fit in with observations of Kool et al. (1991) who showed that the thickness of a bottom break in rose plants was negatively related to the total amount of bottom breaks per plant.

Our data show that axillary buds in later generations were able to develop in a better way than during commercial cutting production if sufficient leaves for their outgrowths were available. The reason that there was still a small decrease in quality, as reflected in for instance fresh weight and diameter, may be that total tissue mass that had to be maintained, increased during the experiment. However, alternatively the age of the stock plant might play a role. The older it gets, the less the quality of the axillary buds.

On the whole, cutting quality could be maintained longer if there were more leaves available per axillary bud. However the number of cuttings harvested from the

control stock plants was about five times higher than from the two other treatments. An economic optimum should be found between the number and the quality of the cuttings per stock plant per square meter.

4.2 Effect of number of leaves and position of axillary buds on cutting quality

Abstract

Under completely controlled conditions, the effect of assimilate supply (as determined by leaf area, i.e. the number of available leaves) on quality of chrysanthemum cuttings reflected in weight, cutting diameter and growth rate, was evaluated. Cutting quality increased when the ratio between the number of axillary shoots and the number of leaves decreased from 4/16 to 1/16. Even when the ratio was 4/16, cutting quality was high and differences between the four cuttings were small if present. The number of pith cells at the largest diameter in transverse sections of an axillary bud was lower than at the base of the cutting it produces. The number of pith cells at the cutting base increased when the ratio between the number of axillary shoots and the number of leaves decreased. The data are discussed in terms of effects of assimilate level on apical dominance and on sink-source relationships.

Introduction

Since cuttings form the basis of each chrysanthemum plant, control of cutting quality is important. However, there is no unanimity how to express quality. Cutting fresh weight and stem diameter were taken as quality criteria by Agustsson and Canham, (1981), Anderson and Carpenter (1974) and Eng *et al.* (1985), dry weight was used by Wott and Tukey (1969), the number of roots after propagation by Röber (1976) and hardness of the cutting by Chan (1955).

Cuttings of chrysanthemum are taken from stock plants. Stock plant behaviour, and as a consequence cutting quality, can be influenced by varying the environmental conditions (Eng *et al.*, 1983; Fisher and Hansen, 1977; Molitor and Von Hentig, 1987) and by nutrition (Röber, 1976; Krause, 1981). De Ruiter (1993) recently could increase cutting quality by reducing the number of axillary buds (with subtending leaves) that were allowed to sprout. The pith forms an important part of the total stem diameter. In rose pith diameter and shoot diameter are found to be correlated (Marcelis-van Acker, 1994a). Pith consists of cells which can vary in number and size. Given a certain potential cell size, the more cells the thicker the cutting may become.

The aim of the present study was to investigate how far cutting quality of chrysanthemum is determined by the number and the rank order of cuttings that develop simultaneously on the same shoot under conditions of a similar supply of assimilates. Apart from fresh- and dry weight, and cutting diameter - probably the most important factors for expressing cutting quality - a number of other parameters as leaf number and leaf area were recorded as well. In addition, pith cell countings were done in transverse sections of buds and cuttings. The experiment was carried out twice, under completely controlled conditions.

Materials and methods

For each of the two, identical, experiments carried out in 1992 and 1993, 144 cuttings of *Dendranthema grandiflora* Tzvelev (*Chrysanthemum morifolium* Ramat) 'Cassa' (Fides, De Lier) were rooted in a mixture of sand and peat (1:1 by volume). Thereafter they were potted into 14-cm square plastic pots containing a mixture of peat, river clay, Swedish peat moss and peat dust (Lentse potgrond, number 4) (40:15:20:25 by volume) and kept in a climate chamber for three months (from the middle of May until the middle of August for experiment 1 and from the middle of October until the middle of January for experiment 2) under constant environmental conditions of temperature (18°C), relative humidity (approximately 70%), day length (16 h) and irradiance (30 W m^{-2} (PAR) at plant level given by fluorescent tubes (Philips TLD50W/84HF)). The plants were fed every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5) and further watered when needed.

Each plant was decapitated above leaf four (counted from the plant base) when the distance between the growing point and that leaf was 5 cm (cutting "generation 0"). Thereafter only the topmost axillary bud was allowed to grow out (giving cutting "generation 1"); all other buds were removed as soon as possible without damaging the plant. All leaves were retained. This procedure was repeated two times ("generations 2 and 3"). So far the manipulated stock plant was the same as used by De Ruiter (1993). However, in the fourth generation the number of axillary buds that was allowed to grow out was one, two or four i.e. the first axillary bud, counting from the top (4.1, treatment 1), the first and second axillary buds (4.1 and 4.2, treatment 2) and all four (4.1, 4.2, 4.3 and 4.4, treatment 3) (Fig. 4.2.1). As a consequence the ratio between the number of axillary buds and the number of leaves was 1/16, 2/16 and 4/16 respectively.

Measurements were made at the moment 5 cm shoot (the cutting) was standing above the fourth leaf (counting from the base of the new formed shoot). So, the

Fig. 4.2.1: Classification of the axillary buds at the three different kinds of manipulated plants.

cuttings were harvested at different times. Measured parameters of the harvested cuttings were: fresh- and dry weight, total leaf number (> 0.5 cm in length), total leaf area using a LiCor 3100 (Leica, Rijswijk), days until harvest, the diameter at the base of the cutting and the number of pith cells. For determining the number of pith cells, transverse sections from the base of the harvested cuttings were made by hand. The pith cells were counted on the largest diameter between two vascular bundles. The same procedure was executed for the axillary buds.
There were six blocks at right angles to the air stream. Each block contained three (treatments) x two (replicates) = six experimental units of four plants each. The three treatments were randomized over each block. Results were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

Results

In both experiments fresh weight per cutting decreased when the number of sprouting axillary buds, i.e. the number of cuttings, increased. One 'extra' sprouting

axillary bud decreased individual cutting weight by 20-30 % and three extra sprouting buds even by about 35%. There were no real differences in rank order when two or four axillary buds grew out (Table 4.2.1). Roughly speaking the same

Table 4.2.1: Fresh weight (g), dry weight (g) and dry weight % per cutting as affected by the number of cuttings and position in two experiments.

	number of cuttings						
	1	2		4			
position	4.1	4.1	4.2	4.1	4.2	4.3	4.4
fresh 1	1.81a	1.42b	1.52b	1.17cd	1.24c	1.20cd	1.07d
weight2	2.07a	1.48b	1.47b	1.30c	1.37bc	1.34bc	1.23c
dry 1	0.24a	0.19cd	0.21b	0.18d	0.20bc	0.19cd	0.16e
weight2	0.26a	0.19b	0.19b	0.17cd	0.18bc	0.17cd	0.16d
% dry 1	13.3a	13.4a	13.8a	15.4b	16.1b	15.8b	15.0b
weight2	12.6a	12.8a	12.9a	13.1a	13.1a	12.7a	13.0a

Values within lines followed by different letters differ significantly at the 5% level.

pattern was found for dry weight. However, as can be calculated easily from Table 4.2.1, total produced weight strongly increased with the number of buds that were allowed to develop. Dry weight percentages hardly varied in experiment 2 but had increased somewhat in experiment 1, when four sprouting axillary buds were present (Table 4.2.1). The number of leaves per cutting also decreased with an increase in number of sprouting buds. There was no consistent relationship between the number or position of cuttings and their leaf area. In both experiments, leaf area per leaf was unaffected by treatments and by cutting position (Table 4.2.2).

In experiment 1 cutting diameter decreased when the number of sprouting axillary buds increased but in experiment 2 no difference could be seen between two and four sprouting buds (Table 4.2.3). The number of days necessary to develop a 5 cm cutting varied little in the various situations; the data suggest a somewhat slower growth when four buds were permitted to sprout, except for position 4.4.

Calculating the dry weight production per day shows a decrease with increasing number of sprouting axillary buds.

Table 4.2.2: Leaf number, total leaf area (cm^2) and area per leaf (cm^2) per cutting as affected by the number of cuttings and position in two experiments.

		number of cuttings						
		1	2		4			
position		4.1	4.1	4.2	4.1	4.2	4.3	4.4
leaf number	1	9.21a	8.06c	8.66b	7.61d	8.08c	7.86c	7.15e
	2	8.61a	7.67bc	7.63bc	7.44c	7.70b	7.43c	7.17d
tot. leaf area	1	26.8a	22.2cd	25.3ab	21.2cd	23.6bc	22.9bc	19.9d
	2	32.9a	27.6b	27.4b	26.5b	28.6b	28.6b	27.0b
area per leaf	1	2.9a	2.8a	2.9a	2.8a	2.9a	2.9a	2.8a
	2	3.8a	3.6a	3.6a	3.6a	3.7a	3.8a	3.8a

Values within lines followed by different letters differ significantly at the 5% level.

The number of pith cells at the largest diameter in a transverse section of a cutting followed the usual pattern: the more developing cuttings, the lower the number of pith cells (Table 4.2.3). Independent of number and position of the buds the number of pith cells at the largest diameter in an axillary bud was 17.6 $\pm$ 0.8, i.e. clearly lower than was found in the respective cuttings.
Finally it should be remarked that if only one axillary bud was allowed to develop, axillary buds from that developing shoot grew out prematurely, and, in addition, shoots emerged from the basal region of the plants below compost level. This phenomenon also occurred in the other treatments but decreased in importance as the number of sprouting buds increased.

Discussion

Taking a high fresh- and dry weight, and a large stem diameter as criteria for high cutting quality, the best cutting was obtained when only one bud was allowed to develop. As soon as two or more buds were left on the plant, cutting quality

decreased, and, in addition, more time was needed to reach the 'harvest' cutting length of 5 cm (Table 4.2.3). In view of uniformity requirements in cutting production, it is further noteworthy that the difference in quality between cuttings 4.1 and 4.2 (two buds left on the plant) and between 4.1-4.4 (four buds left) was

Table 4.2.3: Diameter (mm), number of pith cells, number of days needed to reach the 5 cm stage and dry weight production (dw) per day (g per day) per cutting as affected by the number of cuttings and position in two experiments.

		number of cuttings					
	1	2		4			
position	4.1	4.1	4.2	4.1	4.2	4.3	4.4
diameter	1 3.80a	3.34b	3.41b	2.88c	2.90c	2.88c	2.82c
	2 4.00a	3.11b	2.95bc	2.70de	2.88cd	2.84cd	2.65e
number	1 24.6a	23.1a	23.5a	21.1bc	21.4b	20.3bc	19.7c
of cells	2 26.2a	23.7b	23.1b	21.3c	21.4c	20.6c	20.3c
days	1 22.7bc	22.6bc	23.8b	25.9a	27.7a	25.9a	21.6bc
needed	2 21.6b	22.4b	20.6b	23.4ab	24.6a	21.6b	21.5b
dw per	1 0.011a	0.008b	0.009b	0.007b	0.007b	0.007b	0.007b
day	2 0.012a	0.009b	0.009b	0.007b	0.007b	0.008b	0.007b

Values within lines followed by different letters differ significantly at the 5% level.

little pronounced. In the few cases where statistical significance between treatments was reached a consistent pattern did not occur. Only, when comparing 4.2 with 4.1 it could be seen that almost all parameters were higher for 4.2. Bud position might be an important factor, as has been found for rose (Zieslin and Halevy, 1978) and for apple (Mullins and Rogers, 1971).

In the concept of apical dominance it would be expected that when two or four buds were allowed to grow out shoot weight of individual shoots would have been decreased and the time required to attain a 5 cm cutting would have been increased

going from top to base. Similarly, Keppeler (1986) and Schwabe (1979) for chrysanthemums and a number of plant species, respectively, showed that axillary buds closer to the apex were less inhibited than the lower positioned basal buds. However, in the present experiment a suchlike effect did not occur or, as seen for 4.3 and 4.4 (Table 4.2.3, the number of days needed to reach the 5 cm stage) the reverse was actually found. The explanation of these conflicting data, probably must be sought in the fact that in the present experiment all but (maximal) four buds were removed whereas total leaf area was kept intact. As a consequence, the amount of assimilates available for the outgrowth of the (maximal) four buds may have been so large that the growth pattern imposed by apical dominance is completely outstripped. As a result there is no or little difference in growth behaviour between the two or four buds left on the shoot.

Although individual cutting dry weight decreased as more buds were allowed to grow out, total dry weight invested in the cuttings as a whole increased considerably. This outcome is not surprising if it is realised that two sink centres, i.e. the root system and one or more shoot tips compete for assimilates produced by a fixed number (16) of full-grown leaves. If it is further assumed that root 'sink' demand and assimilate 'source' production were little affected by treatments, increasing sink strength at the top by multiplying sink number at the top by two or four will certainly attract a greater part of the available assimilates but not in proportion to the number of 'extra' growth centres. When only one bud is allowed to develop assimilate availability may exceed demand resulting on the one hand in premature development of axillary buds on that only shoot and on the other hand in the emergence of shoots in the root collar region. Zieslin *et al.* (1976) found a similar effect after removal of lateral buds in roses; in this case structural shoots developed at the bottom of the plant. It is easily to see why as more buds are allowed to grow out the tendency to form 'extra' shoots decreases. It should be noticed that the foregoing reasoning mainly holds for the beginning of shoot development. The effect will become weaker when the developing shoots start to export assimilates. Most likely is that in chrysanthemum just as in rose, the leaves must have reached a certain age to be fully active (Aikin and Hanan, 1975). Also, the harvesting period is somewhat extended when there are multiple shoots and this in turn increased assimilate supply.

In both experiments it is shown that the number of pith cells at the largest diameter in a transverse section of a cutting may be affected by stock plant management. Because of the lower number of pith cells at the largest diameter of an axillary bud, in comparison with the cutting growing out from that bud, cell division in the pith must have continued during shoot development at a rate dependent on the amount of

available assimilates. Popham (1958) for chrysanthemum and Kassner (1884) for a few woody perennials, however found hardly any transverse cell divisions in the pith of a growing shoot. Sachs *et al.* (1959, 1960) could influence longitudinal cell division in pith tissue by applying gibberellins or Amo-1618. Sachs and Kofranek (1963) also found that transverse divisions could be influenced by Amo-1618, CCC and Phosfon.

Cutting quality is higher when there are more leaves available per axillary bud. However, as a consequence, the number of cuttings harvested is less. For commercial practice an economic optimum should be found between the number and quality of the cuttings.

4.3 Effect of temperature on cutting quality

Abstract

The effect of temperature on the quality and production of chrysanthemum cuttings in two stock plant management systems was investigated. In the control stock plants, where every leaf was associated with an axillary bud, cutting quality declined with stock plant age. Removing axillary buds, so that more leaves were available per bud, increased cutting quality. As a consequence however, the number of cuttings harvested from these manipulated stock plants was less. High temperatures increased cutting production but decreased cutting quality. However, even at high temperatures, the quality of the cuttings reflected in fresh weight and stem diameter of manipulated stock plants was still high. An important part of the stem diameter is the pith. For all temperatures tested, the pith diameter was about 70-75% of the shoot diameter. A higher pith- and subsequently shoot diameter were mainly due to an increase in the number of pith cells and not to an increase in cell size.

Introduction

Chrysanthemum cuttings are picked from vegetatively grown stock plants. As stock plants age, the quality of the cuttings usually declines, reflected in for instance a decrease in fresh weight and stem diameter (Anderson and Carpenter, 1974; Agustsson and Canham, 1981; Eng et al., 1985). As De Ruiter (1993) showed, cutting quality may be improved by stock plant management, i.e. removing axillary buds so that more leaves are available per bud. This improvement however, occurred at the expense of the number of cuttings harvested. According to De Lint and Heij (1987) temperature has a speeding up effect on vegetative growth in chrysanthemum and De Jong and Smeets (1982) showed that the rate of leaf production increased with temperature. Similarly in asparagus (Blumenfield et al., 1961), apples (Tromp, 1993) and rose (Marcelis-van Acker, 1994b) increasing temperature favoured shoot growth.

The pith forms an important part of the total stem diameter, which is a parameter for cutting quality. The cells of the pith can vary in number and size. The more or the larger the cells, the wider the pith could be and the thicker the cutting. Shoot- and pith diameter in rose are found to be correlated (Marcelis-van Acker, 1994a). Eames and MacDaniels (1925) reported that in old stems the pith is present in size,

shape and structure exactly as it was in the young twig.
The present study was undertaken to see if it is possible to compensate for the lower cutting production of manipulated stock plants by increasing temperature. Attention is also paid to the number of pith cells on the largest diameter of a cross section, the diameter of the pith and the thickness of the remaining tissues (xylem, phloem and cortex) of a cutting in relation to cutting quality.

Materials and methods

Rooted cuttings of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev.) cultivar 'Cassa' (Fides, De Lier) were planted into 14 cm square plastic pots containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:25 by volume) (Lentse potgrond, No. 4, Coöp Tuinbouwcentrum, Lent).
The plants were watered when necessary and fed once every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5). The rooted cuttings were topped at the moment the distance between the growing point and the fourth leaf (counting from the soil surface) was 5 cm. Axillary buds grew out into shoots and these in turn were treated in the same way. This procedure was repeated three or four times giving cuttings of three or four generations.
Two different kinds of stock plants were made as follows:
A. Control stock plant. In each generation (total of 3) all four axillary buds were allowed to grow out. This treatment approximates commercial stock plant production.
B. Manipulated stock plant. In each generation (total of 4) only the topmost axillary bud was allowed to grow out. The other three buds were removed as soon as possible.
In each generation, measurements were only taken from the topmost cutting. Several parameters were measured: fresh- and dry weight, total leaf number (over 0.5 cm in length), total leaf area using a LiCor 3100 (Leica, Rijswijk) and the diameter at the basis of the cutting. On the largest diameter of a cross section at the basis of the cutting the number of pith cells was counted and the diameter of the pith and thickness of the remaining tissue, i.e. xylem, phloem and cortex were measured (using an ocular micrometer). Of the lowest axillary buds of the picked cuttings the number of pith cells was counted (in the same way as in the cuttings). These axillary buds were just standing above the axillary buds which grew out into shoots and in which also the number of pith cells was counted. Previous research showed (data not shown) that there were hardly any differences in number of pith cells between the

axillary buds of these two positions.
The experiment was carried out from 30 June until 2 November 1993 in four controlled environment rooms kept at 15, 18, 21 and 24°C at day and during the night. Relative humidity was about 70% which means that the vapour pressure deficit of the air was different at the various temperatures. Day length was 16 hours and irradiance 21.3 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD50W/84HF). Each room contained ten blocks of four plants each positioned at random. Results were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

Results

Fresh weight, dry weight and dry weight percentage decreased with higher temperatures for both the manipulated stock plants and the control stock plants. Fresh- and dry weights were higher in the manipulated stock plants but dry weight percentages were higher in the control. The fresh weight of the cuttings of the manipulated stock plants in generation 4 was still higher than the fresh weight of the cuttings of the control stock plants in generation 1 (Table 4.3.1).
The number and area of the leaves also decreased for both types of stock plants with higher temperatures and were higher for the manipulated stock plants (Table 4.3.2).
The diameter, the number of pith cells in cross section in the cutting and the number of pith cells in cross section in an axillary bud decreased with higher temperatures (Table 4.3.3). The number of pith cells in cross section in an axillary bud was less than in a cutting.
Also the diameter of the pith and the thickness of the remaining tissue, decreased with higher temperatures (Table 4.3.4). For both kinds of stock plants and for all temperatures, pith diameter was about 70-75% of the total shoot diameter (Table 4.3.5).
High temperatures (up to 24°C) had a speeding-up effect on shoot growth for both types of stock plants. Differences in time needed to produce a 5 cm cutting already occurred in generation 1. Differences between the two types of stock plants increased with generation (Table 4.3.5).

Discussion

In accordance with Porter and Delecolle (1988), stating that rates of development are linear with increasing temperatures, less days were needed to produce a 5 cm cutting when using higher temperatures. Between the manipulated- and control stock plants

large differences in number of days necessary to produce a 5 cm cutting were present. These differences increased with generation. In our experiment the control stock plant approximates the commercial stock plant. These commercial plants are usually grown at 18°C, according to our experiments it takes about 41.2 days to produce a new cutting in generation 3 on such plants. A manipulated stock plant grown at 24°C only needs 17.6 days. Measured parameters of the latter cuttings were still higher than those of the control stock plants. Taking diameter and fresh weight as quality parameters, it is possible to produce a better cutting in a shorter time.

The fact that a control stock plant still produces more cuttings can probably be solved since a manipulated stock plant needs less space, so more plants per square meter can be grown, which produces more cuttings (Röber, 1977). However, we must keep in mind that it could also be possible to improve the cutting quality of control stock plants by placing less stock plants per square meter.

For the four examined temperatures and the two kinds of stock plants, pith diameter (representing primary thickness) was about 70-75% of the total diameter of a cutting. A decrease in temperature let increase both the diameter of the pith and the thickness of the remaining tissues (xylem, phloem and cortex), thus keeping the percentages of pith constant. In rose it is also found that the pith diameter is correlated with the shoot diameter (Marcelis-van Acker, 1994a) and in Pinus (Ladell, 1963) a correlation between the diameter of the pith and the vascular tissue was reported. According to Kassner (1884) and Popham (1958) there are hardly any divisions in radial and tangential direction in the pith of a growing chrysanthemum shoot. However, this research showed that with higher temperatures the number of pith cells on the largest diameter of a cross section of a cutting decreased. Differences in final pith diameter of the cutting due to different temperatures were largely due to an increase in pith cell number and, not as in rose (Marcelis-van Acker, 1994b), to cell extension (Table 4.3.3, D/PC). In conclusion: the more pith cells on the largest diameter of a cross section of a cutting, the larger the pith diameter, the larger the cutting diameter and the better the cutting quality.

The shape of the pith cells in the axillary buds and in the shoots was isodiametric, in the cuttings the cells near the vascular bundles were smaller than the cells in the middle of the pith. There were no small vital cells forming a network throughout the pith as was found in roses (Marcelis-van Acker, 1994a) and in other woody species (Kassner, 1884).

Table 4.3.1: Fresh weight (g) and dry weight (g) of 5 cm cuttings from control and manipulated stock plants grown at four different temperatures (15, 18, 21 and 24°C).

	Generation							
	1		2		3		4	
	weight		weight		weight		weight	
Temp.	fresh	dry	fresh	dry	fresh	dry	fresh	dry
Control								
15°C	2.1a	0.33a	2.0a	0.32a	1.5a	0.39a		
18°C	2.1a	0.26ab	1.8a	0.25ab	1.5a	0.30ab		
21°C	1.8b	0.24ab	1.3b	0.19bc	1.2b	0.27bc		
24°C	1.5c	0.19b	1.2b	0.15c	1.0b	0.20c		
Manipulated								
15°C	3.4a	0.47a	4.4a	0.57a	3.4a	0.56a	2.9a	0.57a
18°C	2.9b	0.33b	3.2b	0.40b	3.2ab	0.42b	2.4b	0.39b
21°C	2.6c	0.31b	2.4c	0.30c	3.0b	0.38b	2.3b	0.37b
24°C	2.2d	0.26b	2.5c	0.30c	2.7c	0.33b	2.3b	0.32b

Values within columns and within treatments followed by different letters differ significantly at the 5% level.

Table 4.3.2: Number of leaves and leaf area (cm^2) of 5 cm cuttings from control and manipulated stock plants grown at four different temperatures (15, 18, 21 and 24°C).

	Generation							
	1		2		3		4	
	leaf		leaf		leaf		leaf	
Temp.	number	area	number	area	number	area	number	area
Control								
15°C	9.7a	37.3ab	9.8a	39.3a	9.5a	28.6b		
18°C	9.4a	41.1a	8.8b	36.6a	9.0b	34.3a		
21°C	8.7b	38.6ab	7.4c	29.3b	7.7c	27.7b		
24°C	7.6c	35.8b	6.5d	25.9b	6.8d	25.3b		
Manipulated								
15°C	10.6a	52.0a	12.6a	65.5a	11.4a	54.4a	11.3a	42.8a
18°C	10.0b	50.7a	10.3b	54.0b	11.1a	52.3ab	10.6b	41.1a
21°C	9.7bc	49.8a	9.5c	43.8c	10.3b	49.0b	9.7c	40.1ab
24°C	9.3c	43.3b	8.2d	28.1d	10.0b	40.2c	9.5c	36.0b

Values within columns and within treatments followed by different letters differ significantly at the 5% level.

Table 4.3.3: Diameter (mm) and number of pith cells of 5 cm cuttings and the number of pith cells in the belonging axillary buds from control and manipulated stock plants grown at four different temperatures (15, 18, 21 and 24°C).

	Generation											
	1			2			3			4		
		pith cells			pith cells			pith cells			pith cells	
Temp.	diameter	cutting	bud	diameter	cutting	bud	diameter	cutting	bud	diameter	cutting	bud
Control												
15°C	4.3a	28.8a	17.9a	3.3a	25.2a	15.3ab	2.7a	21.4a	15.3ab			
18°C	3.7b	27.6a	16.4ab	3.3a	22.9b	15.5a	2.6ab	20.8a	16.0a			
21°C	3.5b	25.6b	15.6b	2.9b	19.7c	14.6ab	2.4bc	20.1a	15.1ab			
24°C	3.1c	21.6c	13.3c	2.8b	20.2c	13.5b	2.3c	19.9a	13.6b			
Manipulated												
15°C	5.7a	35.4a	18.7a	6.5a	34.7a	22.6a	5.2a	28.3b	19.0a	4.8a	28.4a	18.3a
18°C	4.5b	31.1b	17.4ab	4.9b	28.1b	19.6b	5.1a	33.7a	19.2a	4.3b	25.9b	16.9ab
21°C	4.4b	28.4c	16.1b	4.4c	24.8c	16.1c	5.0a	31.9a	19.3a	4.3b	25.8b	16.2bc
24°C	4.1c	23.9d	15.5b	3.6d	23.1c	14.8c	4.7b	29.4b	17.4a	4.5b	20.7c	14.3c

Values within columns and within treatments followed by different letters differ significantly at the 5% level.

Table 4.3.4: Pith diameter (mm) and total thickness of the remaining tissue (of both sides of the pith) (mm) from control and manipulated stock plants grown at four different temperatures (15, 18, 21 and 24°C).

	Generation							
	1		2		3		4	
Temp.	pith diameter	remaining tissue	pith diameter	remaining tissue	pith diameter	remaining tissue	pith diameter	remaining tissue
Control								
15°C	3.2a	1.1a	2.4a	0.9a	1.9a	0.8a		
18°C	2.6b	1.1a	2.3ab	1.0a	1.8ab	0.8a		
21°C	2.5bc	1.0a	2.0c	0.9a	1.7b	0.7ab		
24°C	2.3c	0.8b	2.1bc	0.7b	1.7b	0.6b		
Manipulated								
15°C	4.3a	1.4a	4.7a	1.8a	3.7a	1.5a	3.4a	1.4ab
18°C	3.2b	1.3ab	3.4bc	1.5b	3.7a	1.4a	2.9b	1.4ab
21°C	3.2b	1.2bc	3.2c	1.2c	3.5ab	1.5a	3.0b	1.3b
24°C	3.0b	1.1c	2.6d	1.0d	3.3b	1.4a	3.0b	1.5a

Values within columns and within treatments followed by different letters differ significantly at the 5% level.

Table 4.3.5: Number of days necessary to produce a 5 cm cutting and the percentage pith from the total diameter from control and manipulated stock plants grown at four different temperatures (15, 18, 21 and 24°C).

	Generation							
	1		2		3		4	
Temp.	days	percentage	days	percentage	days	percentage	days	percentage
Control								
15°C	29.3a	73.2a	44.4a	72.3a	56.7a	71.0a		
18°C	22.2b	70.2a	31.1b	69.3a	41.2b	70.0a		
21°C	18.7c	71.1a	36.8a	69.4a	42.1b	70.1a		
24°C	12.7d	73.1a	30.9c	73.6a	35.2c	73.5a		
Manipulated								
15°C	26.3a	75.4a	31.6a	72.3a	30.7a	71.3a	38.1a	70.3a
18°C	21.7b	71.0a	27.1a	69.3a	22.7b	72.4a	24.7b	67.1a
21°C	15.0c	73.3a	22.8b	71.9a	19.7c	70.5a	22.4c	70.7a
24°C	12.0d	73.8a	21.7c	72.6a	17.6d	70.3a	19.3d	67.5a

Values within columns and within treatments followed by different letters differ significantly at the 5% level.

The vapour pressure deficit differed between the treatments because the air humidity was kept constant. It cannot be ruled out that this may have affected the results, as reported by Hoffman (1979). However, humidities between 1.0 and 0.2 kPa vapour pressure deficit have little effect on the physiology and development of horticultural crops (Grange and Hand, 1987). The vapour pressure deficits at the temperatures in our experiment fell within this range.

4.4 Effect of number and position of leaves on cutting quality

Abstract

The influence of number and position of leaves on cutting quality in chrysanthemum was studied. Differences in the ratio: number of leaves/axillary bud were imposed by differential defoliation. The more leaves per axillary bud, the better the cutting quality, as expressed in shoot diameter and weight. Varying the position of the remaining leaves did not have an influence on the quality of the cutting, indicating that the distance between the axillary bud and the leaves was not important. Pith diameter was found to be correlated with the shoot diameter and was about 70% of the latter. A larger diameter (shoot and pith) was due to the increase in cell number. The total number of pith cells on the largest diameter of a cross section did not vary with place in the stem, nor with time.

Introduction

In each leaf axil of chrysanthemum, one axillary bud is present. Axillary buds are able to develop into cuttings, after release from apical dominance. Homogeneity of cuttings is important, since cuttings should offer uniformity and predictability in harvesting and flowering (De Greef, 1989; Van der Hoeven, 1989; Van Vliet, 1990). To get a better insight in the factors controlling axillary bud outgrowth, the influence of the number of leaves per axillary bud is studied and the influence of the position, i.e. the distance between the axillary bud and the leaves.

Cutting quality, as reflected in for instance fresh weight and diameter (Anderson and Carpenter, 1974; Agustsson and Canham, 1981; Eng et al., 1985), can be improved by removing axillary buds so that more leaves are available per remaining bud (De Ruiter, 1993). Keppeler (1968) found for chrysanthemum that there are basic differences between top and bottom leaves. Top leaves are younger and more efficient in photosynthesis.

The pith forms an important part of the total stem diameter and stem diameter is a parameter of stem quality. In rose (Marcelis-van Acker, 1994a) pith and stem diameter are found to be correlated. An increase in assimilate supply during axillary bud outgrowth of rose had a positive influence on the pith diameter, mainly due to cell enlargement, and therefore on the total stem diameter (Marcelis-van Acker, 1994b).

In experiment 1 attention is paid to the influence of the number and position of leaves on the outgrowth of axillary buds. Morphological parameters, the diameter of the pith and the total number of pith cells at the largest diameter of a cross section are noted. The development of the pith cells in time and at different places in the shoot is examined in experiment 2 to test the relation between quality of the cutting and number of pith cells.

Materials and methods

Rooted cuttings (5 cm in length) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev.) cultivar 'Cassa' (Fides, De Lier) were potted in 14 cm square plastic pots, containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:25 by volume) (Lentse potgrond, No. 4, Coöp Tuinbouwcentrum, Lent). The plants were fed once every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5).
Experiment 1: the influence of the leaves on the quality of the cuttings is studied. The rooted cuttings were topped above the sixth leaf (counting from the basis) when the distance between the growing point and this sixth leaf was about 5 cm. All axillary buds were removed except the top one. Leaves were partially removed so that zero, two, four or six leaves remained on the cutting in eight different combinations (Fig. 4.4.1).
Experiment 1.1: Start 21 April 1992, the average day/night temperature was 22°C/18°C, the relative humidity about 70% and the mean irradiance 1220 J cm^{-2} per day. Average total number of days necessary to produce 5 cm stem (the cutting) above four leaves was 20 days.
Experiment 1.2: Starting 15 June 1992, the average day/night temperature was 28°C/22°C, the relative humidity about 80% and the mean irradiance 2004 J cm^{-2} per day. Average total number of days necessary to produce 5 cm stem (the cutting) above four leaves was 23 days.
Experiment 1.3: Starting 31 August 1992, the average day/night temperature was 23°C/19°C, the relative humidity about 60% and the mean irradiance 1103 J cm^{-2} per day. Average total number of days necessary to produce 5 cm stem (the cutting) above four leaves was 25 days.
Measured parameters of the 5 cm cuttings were: fresh- and dry weight, dry weight percentage, leaf number and total leaf area. Pith diameter and the number of pith cells on the largest diameter in a cross section at the base of the cutting were only measured in experiments 1.2 and 1.3. The plants in experiment 1.1 were placed in four replicated groups of four plants of each treatment positioned at random. The

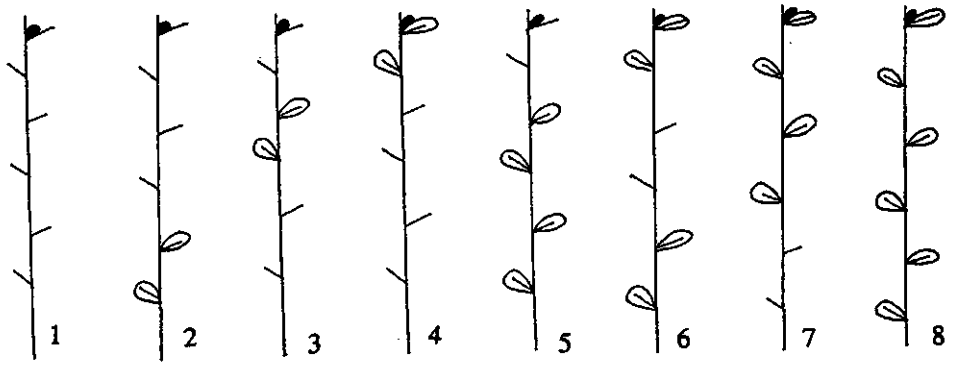

Fig. 4.4.1: The number (zero, two, four or six) and position of the leaves in relation to the topmost axillary bud.

plants in experiment 1.2 and 1.3 were placed in three replicated groups of six plants of each treatment positioned at random.

Experiment 2: the relation between cutting quality and number of pith cells is studied. The cuttings were topped above the sixth leaf (counting from the basis) at the end of May 1992. Because the plants were grown in a glasshouse and we did not want the axillary buds to grow out at different circumstances, different lengths of shoot were topped above the sixth leaf.

Experiment 2.1: The average topped length was 5.1 cm, only the topmost axillary bud (1) was allowed to develop.

Experiment 2.2: the average topped length was 3.7 cm, the two topmost axillary buds (1 and 2) were allowed to grow out.

Experiment 2.3: the average length was also 3.7 cm, no axillary buds (1 to 6) were removed (Fig. 4.4.2).

The axillary buds grew out and cross sections were made at the moment the length of the shoots was 5, 10, 15 or 20 cm, respectively between the first leaf and the axil (place a), between the second and third leaf (place b), between the fourth and fifth leaf (place c) and between the six and seventh leaf (place d). To examine the number

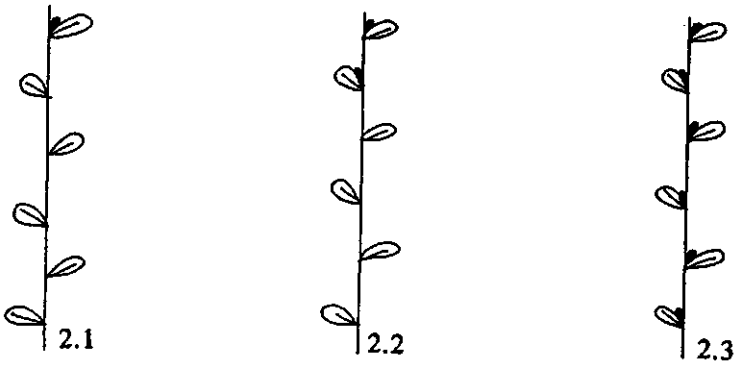

Fig. 4.4.2: The number (one, two or six) and position of the axillary buds.

of pith cells in time, cross sections at place b were made at all shoot lengths (5, 10, 15 and 20 cm). The number of pith cells on the largest diameter of these cross sections was counted. The average day temperature during the experiments was 27°C and average night temperature 22°C, the average relative humidity was about 90% and the mean irradiance 1827 J cm^{-2} per day.
The plants in experiment 2.1 were placed in three replicated groups of four plants each per treatment positioned at random and the plants in experiments 2.2 and 2.3 in four replicated groups of three plants each per treatment positioned at random.
Results of both experiments were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

Results

Experiment 1: The number of leaves (zero, two, four or six) did affect the outgrowth of the topmost axillary bud. In general, the values of the various parameters showed an increase with increasing leaf number. Comparing the fresh weights in the three experiments for zero, two, four or six leaves, no clear pattern

Table 4.4.1: Fresh weight (g) and dry weight (g) of 5 cm cuttings grown with different number of leaves (zero, two, four or six) in eight different combinations (Fig. 4.4.1) in three experiments.

combination number	number of leaves	Experiment 1.1	1.2	1.3
Fresh weight				
1	0	1.91a	2.06b	1.49a
2	2	2.43c	1.77a	1.54ab
3		2.12ab		
4		2.20bc		
5	4	2.15ab	1.79a	1.71bc
6		2.26bc		
7		2.11ab		
8	6	2.19abc	1.78a	1.87c
Dry weight				
1	0	0.17a	0.19a	0.13a
2	2	0.24b	0.20a	0.16b
3		0.22ab		
4		0.23b		
5	4	0.24b	0.21a	0.18c
6		0.25b		
7		0.24b		
8	6	0.26b	0.23a	0.21d

Values within columns followed by different letters differ significantly at the 5% level.

could be seen. Dry weight and dry weight/fresh weight however increased with increasing number of leaves (Table 4.4.1). Leaf area also showed no clear pattern, whereas the total number of leaves per cutting and the diameter of the cutting increased with increasing number of leaves (Table 4.4.2). The growth rate of the shoot was higher if more leaves were available per axillary bud, with a maximum of four leaves (data not shown).

Leaf position, i.e. the distance between the leaves and the topmost axillary bud did not have any influence on the outgrowth. Measured parameters between combinations 2, 3 and 4 (two leaves present) did not differ much, nor were there

Table 4.4.2: Leaf area (cm^2), number of leaves and diameter (mm) of 5 cm cuttings grown with different number of leaves (zero, two, four or six) in eight different combinations (Fig. 4.4.1) in three experiments.

		Experiment		
combination number	number of leaves	1.1	1.2	1.3
L. area				
1	0	42.4a	50.2c	34.8a
2	2	49.0a	41.4b	33.7a
3		41.9a		
4		44.8a		
5	4	40.6a	38.5ab	34.7a
6		43.6a		
7		39.7a		
8	6	39.4a	34.3a	36.1a
Number				
1	0	7.27a	7.29a	7.06a
2	2	8.13b	7.24a	7.38abc
3		7.88b		
4		8.31b		
5	4	8.25b	7.58a	7.71bc
6		8.31b		
7		8.00b		
8	6	8.38b	7.94b	7.83c
Diameter				
1	0	3.91a	3.64a	3.24a
2	2	4.67b	3.90b	3.85b
3		4.51b		
4		4.59b		
5	4	4.61b	4.20c	4.10c
6		4.76b		
7		4.63b		
8	6	4.63b	4.32c	4.40d

Values within columns followed by different letters differ significantly at the 5% level.

Table 4.4.3: Diameter of the pith (mm), number of pith cells at the largest diameter of a cross section and percentage pith of 5 cm cuttings grown with different number of leaves (zero, two, four or six) in two experiments.

	Experiment	
number of leaves	1.2	1.3
Diameter pith		
0	2.66a	2.24a
2	2.84ab	2.80b
4	3.02b	2.98c
6	3.01b	3.19d
Number pith cells		
0	20.1a	17.8a
2	23.4b	23.7b
4	25.6c	25.7c
6	27.7d	29.4d
Pith%		
0	72a	69a
2	72a	70a
4	72a	70a
6	71a	70a

Values within columns followed by different letters differ significantly at the 5% level.

any clear differences between combinations 5, 6 and 7 (a total of four leaves present) (Tables 4.4.1 and 4.4.2). There were also no differences in growth rate of the shoots due to position of the leaves (data not shown).

The diameter of the pith and the number of pith cells on the largest diameter of a cross section were significant higher if more leaves were available per axillary bud. The part of the pith versus the total stem diameter expressed in percentage was the same (70%) irrespective of the number of leaves present (Table 4.4.3).

Experiment 2: The number of pith cells at the largest diameter of a cross section in a shoot appeared to be the same at the different places. However, it was reduced slightly (not statistically tested) when more shoots were allowed to develop (Table 4.4.4). The number of pith cells did not increase in the course of time (Table 4.4.5).

Table 4.4.4: The average number of pith cells at the largest diameter of a cross section in a shoot at four different heights (a,b,c or d) with one, two or six developing shoots per six leaves.

exp.	position ax. bud	number of shoots	height a	b	c	d
2.1	1	1	24a	26a	26a	26a
2.2	1	2	25a	25a	23a	24a
	2		25a	24a	23a	23a
2.3	1	6	22a	22a	21a	22a
	2		22a	21a	21a	21a
	3		22a	20a	21a	21a
	4		21a	20a	20a	19a
	5		21a	22a	21a	19a
	6		22a	22a		

Values within rows followed by different letters differ significantly at the 5% level.

Discussion

An increase in the number of leaves (assimilates) per developing cutting increased the final cutting quality. Also tomato trusses (Slack and Calvert, 1977) and tomato and cucumber fruits (Marcelis and Heuvelink, 1990), increased in weight with increasing number of leaves per truss or fruit. A higher amount of available assimilates per axillary bud decreased the shoot growth period. This is in accordance with Marcelis-van Acker (1994b) who found for rose that an increase in assimilate supply shortened the subsequent growth period of the shoots.

In our experiments there was no effect of the position of the leaves on cutting quality. The two lower leaves gave a topcutting of the same quality as the two upper (closer) leaves. This is in contrast with findings of Keppeler (1968), who did experiments with a total of ten leaves. He concluded that there are differences between top and bottom leaves of chrysanthemum and that top leaves are more efficient in photosynthesis. For rose is found that leaf age has an influence on

Table 4.4.5: The average number of pith cells at the largest diameter of a cross section in the top shoot at position b when the total length is about 5, 10, 15 or 20 cm.

exp.	total length top shoot	Number of pith cells
2.1	5	24a
	10	26a
	15	26a
	20	26a
2.2	5	24a
	10	25a
	15	23a
	20	24a
2.3	5	22a
	10	22a
	15	21a
	20	22a

Values within columns followed by different letters differ significantly at the 5% level.

photosynthesis (Aikin and Hanan, 1975 and Bozarth et al., 1982). The older the leaf, the lesser the photosynthesis.

It could be that partial defoliation results in an enhancement of photosynthetic rates in the remaining leaves (Hardwick et al., 1968). Other studies on partial defoliation of tobacco and sunflower have shown rejuvenation of photosynthetic rates in lower, shaded leaves when exposed to higher irradiance, indicating that the photosynthetic capacity is not reduced (Rawson and Hackett, 1976). However, the differences in leaf position might have been too small to have any effect on the outgrowth of the topmost axillary bud.

The number of pith cells on the largest diameter of a cross section of a cutting increased with the number of leaves per axillary bud (experiment 1). The larger shoot- and pith diameter is expressed in an increase in number of pith cells at the largest diameter of a cross section and not in an increase in cell size (i.e. extension). The diameter of a chrysanthemum pith cell even tended to decrease with increasing number of leaves per bud. The diameters of pith and shoot were found to be closely related, which is also reported for rose by Marcelis-van Acker (1994a).

In experiment 2, the total number of pith cells, counted on the largest diameter of a cross section of a shoot, remained equal in time. This number was also independent of the place in the shoot (Tables 4.4.4 and 4.4.5). Similar results were found for rose (Marcelis-van Acker, 1994b). Since the number of pith cells did not differ with time or place for one, two or six shoots and the number of pith cells in all the axillary buds at the beginning of the experiments was the same, differences must have been set soon after sprouting (Table 4.4.4). The findings of experiment 2 are in accordance with experiment 1: the more leaves available per developing axillary bud, the more pith cells at the largest diameter of a cross section.

5. Axillary bud formation

5.1 Effect of number of leaves on cutting quality

Abstract

The effect of assimilate supply on formation of axillary buds of chrysanthemum was studied. Three stock plant management systems were made by varying the number of leaves (assimilates) available per axillary bud and time of topping. Axillary bud development was divided into axillary bud formation, i.e. before topping and axillary bud outgrowth, i.e. after topping. To study the effect of bud formation and outgrowth separately, the axillary buds grew out both in-vitro (formation) and on the stock plants (formation and outgrowth).
These experiments show that assimilates influence both axillary bud formation as axillary bud outgrowth. A higher amount of assimilates accelerated axillary bud development before topping and more developed axillary buds resulted in plantlets with a higher quality (reflected in diameter and fresh weight in-vitro).
Pith diameter was found to be correlated with the shoot diameter and was about 70-75%.

Introduction

Every chrysanthemum cutting originates from an axillary bud. Axillary bud development can be divided into axillary bud formation (before topping) and axillary bud outgrowth (after topping). In the commercial cutting production it is impossible to tell which part of the variation between cuttings is caused by differences during axillary bud formation and which part during the axillary bud outgrowth because these two are always related. In the commercial production for the cut flower and pot plant industry, homogeneity of cuttings is required, as uniform cuttings offer uniformity and predictability in harvesting and flowering (De Greef, 1989; Van der Hoeven, 1989; Van Vliet, 1990).
Inside a mature axillary bud, other axillary buds might be initiated. These two generations of buds are referred to as 'primary' and 'secondary', the former term being applied to the mature bud, the latter applied to the buds that are developing in the axils of the leaves of the larger bud (Majumdar and Datta, 1946; Garrison,

1949a; Garrison, 1949b; Marcelis-van Acker, 1994a). According to Berg (1970), the first signs of a secondary axillary bud in a primary axillary bud of chrysanthemum are visible at the moment the fourth leaf primordium (P4) of the primary axillary bud is initiated (Fig. 5.1.1).

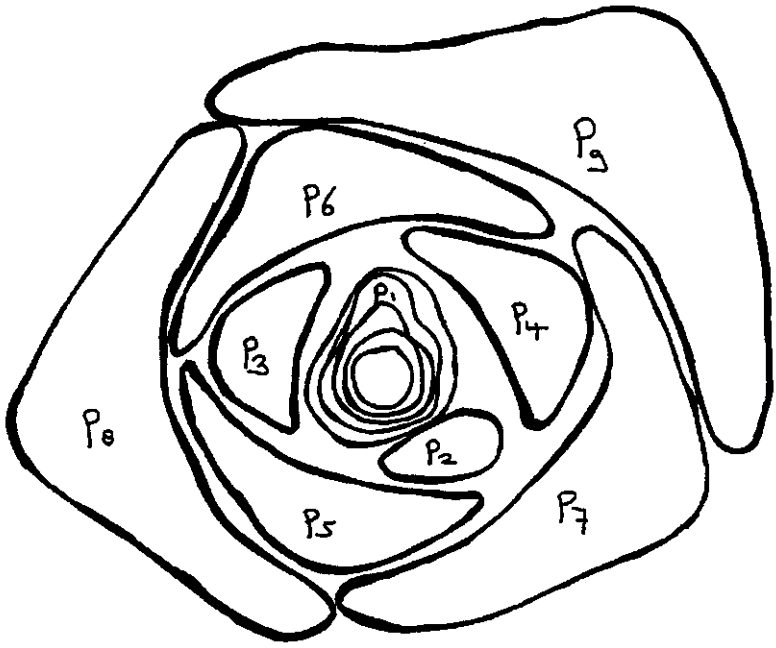

Fig. 5.1.1: A chrysanthemum shoot apex, P9 is the oldest leaf primordium and P1 the youngest (Berg and Cutter, 1969).

By the time the leaf primordium becomes P9, a hump of cells (representing the axillary bud) was very evident. In our research with the chrysanthemum cultivar Cassa, secondary axillary buds started their initiation between P5 and P6.
It is possible to influence the outgrowth and formation of an axillary bud. An axillary bud grows out after release from apical dominance by removal of the top part (the cutting) of the shoot. The outgrowth of the buds is at least partially dependent on environmental conditions (Eng et al., 1983; Fisher and Hansen, 1977; Molitor and Von Hentig, 1987) and nutrition (Röber, 1976; Krause, 1981), but it can also be regulated by manipulation of the stock plant; the more assimilates available per axillary bud the better the cutting quality. (De Ruiter, 1993; De Ruiter and Tromp, in press). In rose the number of pith cells (representing the initial width) in the bud was influenced by different assimilate supplies (Marcelis-van Acker, 1994c).

The aim of the present study was to examine if differences between cuttings, caused by different assimilate supplies, were already set during axillary bud formation. Differences in assimilate supply were realized by removing axillary buds so that more leaves were available per bud (De Ruiter, 1993). Trying to compensate for differences in the developmental stage between the axillary buds of those stock plants, stock plants were topped at different times. Outgrowth of the axillary buds took place both in-vitro and on the stock plants. Axillary buds growing out in-vitro were only influenced during their formation, differences in outgrowth must therefore have been developed during formation. Axillary buds on the stock plants were influenced both during their formation as during outgrowth.

Materials and methods

Experiment 1: Cuttings of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev) cultivar 'Cassa' were rooted and thereafter potted in 14 cm square plastic pots, containing a commercial potting compost (Lentse potgrond, No. 4, Coöp Tuinbouwcentrum, Lent). The experiment was carried out in a controlled environment room from 2 May 1992 until 10 November 1992. Day/night temperature was 18°C, relative humidity about 70% and irradiance 21.3 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD50W/84HF). Day length was 16 h. The plants were fed once every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5).

At the time there was 5 cm shoot standing above the fifth axillary bud (counting from the soil surface), the cuttings were topped above the fourth axillary bud. The removed fifth axillary bud was put in-vitro culture (Generation 0), the fourth axillary bud (remaining on the plant) developed into a shoot. The fourth and fifth axillary buds were almost in the same developmental stage and were assumed to be similar. In this way it was possible to separate the two stages of development, i.e. formation and outgrowth.

Three kinds of stock plants were made (Fig. 5.1.2).

A5: all four axillary buds were allowed to develop into shoots, the topmost shoot (developed from the fourth axillary bud, counting from the soil surface) was topped above the fourth axillary bud when 5 cm shoot was standing above the fifth axillary bud. The fifth axillary bud was put in-vitro. The other shoots were topped at the moment there was 5 cm shoot standing above the fourth axillary bud. Axillary buds grew out into shoots and these in turn were treated in the same way. This was done four times.

B5: Only the topmost axillary bud (the fourth axillary bud, counting from the soil

surface) was allowed to develop, the other three axillary buds were removed. The topmost shoot was topped above the fourth axillary bud when 5 cm shoot was standing above the fifth axillary bud. The fifth axillary buds were put in-vitro and the fourth axillary bud grew out into a shoot and was treated in the same way. This procedure was repeated four times.

B3: As treatment B5, but the topmost shoot was topped above the fourth axillary bud when there was three cm shoot standing above the fifth axillary bud.

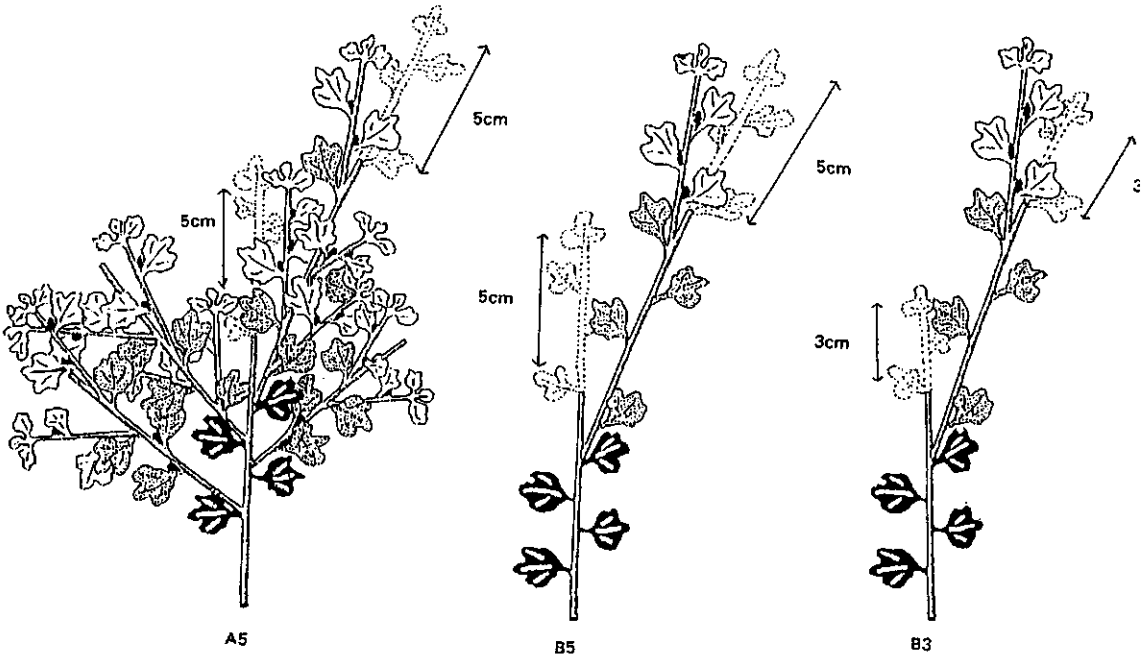

Fig. 5.1.2: Three kinds of stock plants: A5, B5 and B3.

The above stock plants approximate the stock plants used by De Ruiter (1993). The stock plant B3 was created because the axillary buds of the B5 stock plants were more developed than the axillary buds of A5 at the moment of topping. The axillary buds of B3 were probably in the same developmental stage as those of A5 (as control number of initiated leaves was counted).

In Generation 0, the axillary buds of A and B plants were the same therefore only one type of axillary bud was studied.

For in-vitro culture the axillary buds were sterilised in 70% alcohol for a few seconds, followed by 15 minutes in 1% NaOCl to which a few drops of Tween 20

was added. The axillary buds were then washed three times with sterile water. The axillary buds were grown individually in Pyrex glass tubes (20 mm diameter) containing 10 ml culture medium, and after inoculation covered with a cotton plug and Vitafilm. The axillary buds were grown on a basic culture medium containing: macrosalts and microsalts at full strength according to Murashige and Skoog (1962), NaFeEDTA 37.5 mg l^{-1}, 4% saccharose and 0.7% Daichin agar in distilled water. The pH of the medium was adjusted to 6.0. Incubation occurred in a growth chamber at 23°C, irradiance 6 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD36W/84). Day length was 14h.

Measurements of the plantlets in-vitro were: length, number of leaves (visible with the naked eye), fresh weight, diameter and the number of pith cells at the largest diameter of a cross section at the basis of the plantlets. Of the three and 5 cm shoot above the fifth axillary bud the number of pith cells at the largest diameter of a cross section at the basis of these shoots was counted.

There were six blocks of plants, orientated perpendicularly to the air stream of the controlled environment room. Each block contained six experimental units of four plants. The three treatments were randomized over each block. Results were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

Experiment 2: The experiment was carried out from 8 December 1992 until 2 April 1993. Only stock plants A5 and B5 were studied for four generations (0 till 3). Measurements made at the plants grown in-vitro were: length, fresh- and dry weight, diameter and number of leaves.

There were four blocks of plants, orientated perpendicularly to the air stream of the controlled environment room. Each block contained four experimental units of four plants. The two treatments were randomized over each block. Results were analyzed by analysis of variance and the significance of differences determined by student's t-test ($P=0.05$).

Experiment 3: This experiment was a replicate of experiment 2 and was carried out from 4 June 1993 until 6 November 1993.

Measurements were also made at the 5 cm shoots; the diameter of the shoot and of the pith was measured (using an ocular micrometer) and the number of pith cells on the largest diameter of a cross section at the basis of the shoots.

Results

Experiment 1: Study of A5, B5 and B3. Comparing A5 and B5 in Tables 5.1.1 and 5.1.2 almost all parameter values were higher for B5, i.e. when more assimilates

were available per axillary bud (not always statistically). Between A5 and B3 differences were not clear. The length, number of leaves, fresh weight and diameter of plantlets of B5 were mostly higher as of plantlets of B3. The number of pith cells on the largest diameter of a cross section in-vitro and in the shoot were almost the same for B5 and B3. Remarkable were the high parameter values in-vitro in Generation 0 for length, number of leaves, fresh weight and diameter (Table 5.1.1 and 5.1.2).

Table 5.1.1: Length (cm), number of leaves and fresh weight (g) of axillary buds grown in-vitro for four weeks taken from three kinds of stock plants (A5, B5 and B3) of five generations (0 to 4).

		Vitro		
Generation	Stock plant	Length	Number of leaves	Fresh weight
0	A5/B5	6.7	11.0	0.76
1	A5	3.0a	8.1a	0.55a
1	B5	3.4a	8.9b	0.52a
1	B3	2.9a	7.8a	0.46a
2	A5	2.3a	8.6a	0.33a
2	B5	4.1c	8.7a	0.57b
2	B3	3.1b	8.7a	0.38a
3	A5	3.6a	9.5b	0.45ab
3	B5	4.6b	9.2b	0.54b
3	B3	3.1a	8.4a	0.37a
4	A5	3.7a	10.0ab	0.47a
4	B5	7.6c	10.4b	0.80b
4	B3	4.8b	9.7a	0.57a

Values within rows (per generation) followed by different letters differ significantly at the 5% level.

Experiment 2: Study of A5 and B5. There were large differences in length, fresh weight, dry weight and number of leaves between plantlets of A5 and B5 (Table 5.1.3). The diameter of the plantlets however, hardly differed. Again almost all parameter values in Generation 0 were very high in comparison with the parameter values of later Generations.

Experiment 3: Almost as in experiment 2; all parameter values were higher for stock plants B5 (Table 5.1.4). Also the shoots grown on the stock plants had higher parameter values for B5, except for the percentage pith which remained constant (Table 5.1.5).

Table 5.1.2: Diameter (mm) and number of pith cells on the largest diameter of a cross section of axillary buds grown in-vitro for four weeks taken from three kinds of stock plants (A5, B5 and B3) of five generations (0 to 4), as well as the number of pith cells on the largest diameter of a cross section of 5 and 3 cm shoots taken from the stock plant.

Generation	Stock plant	Diameter vitro	Number pith cells vitro	Number pith cells shoot
0	A5/B5	1.7	15.9	23.0
1	A5	1.7a	15.8a	22.7a
1	B5	1.8a	16.3a	26.3b
1	B3	1.8a	15.4a	26.3b
2	A5	1.3a	14.2a	23.6a
2	B5	1.4a	15.9ab	29.9b
2	B3	1.3a	16.5b	29.9b
3	A5	1.4a	15.4a	22.5a
3	B5	1.5a	15.9a	29.3b
3	B3	1.4a	16.0a	29.5b
4	A5	1.3a	16.3a	22.7a
4	B5	1.6b	18.6b	28.7b
4	B3	1.5b	17.9a	28.4b

Values within rows (per generation) followed by different letters differ significantly at the 5% level.

Discussion

In all experiments in-vitro and in-vivo the outgrowth of axillary buds of stock plants A5 and B5 differed. The more leaves (assimilates) per axillary bud the higher the parameter values. The circumstances in-vitro during outgrowth were the same, so

'factors' responsible for the differences in axillary bud outgrowth must have been present during formation, i.e. before topping. Axillary buds of stock plants A5 and B5 differed in the developmental stage, whereas axillary buds of A5 and B3 were probably in the same stage. If only the developmental stage was responsible for the differences between A5 and B5, differences in parameter values between A5 and B3

Table 5.1.3: Length (cm), number of leaves, fresh weight (g), dry weight (g) and diameter of axillary buds grown in-vitro for four weeks taken from stock plants A5 and B5 of four generations (0 to 3).

Generation	Stock plant	Length	Number leaves	Vitro Fresh weight	Dry weight	Diameter
0	A5/B5	7.4	9.7	0.63	0.06	1.5
1	A5	3.2a	9.5a	0.30a	0.03a	1.3a
1	B5	4.2b	9.6a	0.43b	0.05b	1.4a
2	A5	2.5a	8.6a	0.33a	0.03a	1.4a
2	B5	5.0b	10.0b	0.52b	0.05b	1.4a
3	A5	1.8a	7.3a	0.31a	0.04a	1.4a
3	B5	4.7b	9.6b	0.55b	0.05a	1.5a

Values within rows (per generation) followed by different letters differ significantly at the 5% level.

would not exist. The results of A5 and B3 differed however, no clear pattern could be seen. More likely therefore is that small differences in the developmental stage between A5 and B3 were still present.

Because of the above results, it can be concluded that in the experiments of De Ruiter (1993) in which she made the same kind of stock plants, differences between axillary buds were present before the plants were topped. This confirms findings of other researchers: temperature could influence the morphology of chrysanthemum leaves during initiation (Schwabe, 1959). Also in other crops environmental and

developmental factors during formation could have an influence. Patrick (1988) found that the final size of leaves, fruits and grains is largely set during an early phase of development. The seed size, germination characteristics, subsequent growth rate and morphology of soybean and tomato plants were also found to be affected by the environment in which seeds had developed (Paul et al., 1984 and Caulfield and Bunce, 1991). Zamski et al. (1985) suggest that the growth potential of axillary buds

Table 5.1.4: Length (cm), number of leaves, fresh weight (g), dry weight (g) and diameter of axillary buds grown in-vitro for four weeks taken from stock plants A5 and B5 of four generations (0 to 3).

Generation	Stock plant	Length	Vitro Number leaves	Fresh weight	Dry weight	Diameter
0	A5/B5	7.6	11.7	0.73	0.07	1.5
1	A5	3.6a	9.4a	0.40a	0.04a	1.2a
1	B5	6.7b	11.5b	0.71b	0.07b	1.4b
2	A5	4.1a	10.2a	0.39a	0.04a	1.3a
2	B5	4.5a	10.6a	0.54b	0.06b	1.4a
3	A5	3.5a	9.6a	0.38a	0.03a	1.3a
3	B5	6.0b	10.7a	0.59b	0.06b	1.5b

Values within rows (per generation) followed by different letters differ significantly at the 5% level.

from rose may be influenced during axillary bud formation. Also temperature during axillary bud formation might have an effect on subsequent growth of rose (Moe, 1971). In an article about axillary buds Audus (1959) reports that there is no doubt that during the early developmental stages of bud growth the future course of development can be considerably altered by exogenous influences. Champagnat (1954) reports that an axillary bud of *Sambucus* can be 'conditioned' during the early developmental stages.

Comparing the number of pith cells at the largest diameter of a cross section (Table 5.1.2) and the diameters (Table 5.1.4 and 5.1.5) in-vitro and in-vivo, it can be concluded that differences in cutting quality due to different circumstances during the outgrowth, i.e. after topping of the axillary buds were proportionally larger than during the formation, i.e. before topping. Probably the high parameters in Generation 0 could be explained by the fact that these axillary buds were already developed on the stock plants of which the cuttings were taken used for the experiments.

Table 5.1.5: Diameter (mm), number of pith cells on the largest diameter of a cross section, diameter of the pith (mm) and pith percentage of 5 cm shoots taken from stock plants A5 and B5 of four generations (0 to 3).

Generation	Stock plant	Diameter shoot	Number pith cells shoot	Diameter pith	pith%
0	A5/B5	3.6	23.5	2.7	75
1	A5	3.7a	22.4a	2.7a	73a
1	B5	4.3b	25.3b	3.2b	74a
2	B5	3.1a	20.0a	2.3a	74a
2	A5	4.3b	27.1b	3.1b	72a
3	A5	2.7a	18.7a	2.0a	74a
3	B5	3.9b	26.4b	2.9b	74a

Values within rows (per generation) followed by different letters differ significantly at the 5% level.

The fact that the percentage pith, i.e. pith diameter of the shoot versus total diameter of the shoot, was almost the same for the two kinds of stock plants and for the different generations confirms the findings of Marcelis-van Acker (1994a).
In conclusion we can say that differences in cutting quality caused by differences in assimilate supply were set before and after topping. Differences initiated before topping were mainly due to differences in the developmental stage. The more developed the axillary bud the better the final quality of the plantlets after a certain time.

5.2 Effect of temperature on cutting quality

Abstract

The effect of temperature on axillary bud formation of chrysanthemum was studied. Stock plants of *Chrysanthemum morifolium* Ramat cultivar 'Cassa' were grown at different temperatures. Axillary buds of these plants grew out under uniform conditions in-vitro or were oculated on stock plants grown at the same temperature level. The aftereffect of temperature on the growth of the axillary buds varied for the different experiments. These contradicting results are discussed.

Introduction

Chrysanthemums are vegetatively propagated by means of cuttings taken from stock plants. Every cutting originates from an axillary bud situated in the axil of a leaf. For the commercial production of cuttings it is important that cuttings are uniform and of good quality, as determined by for instance diameter and weight (Anderson and Carpenter, 1994; Agustsson and Canham, 1981; Eng et al., 1985). It is possible to influence the outgrowth of an axillary bud by for instance manipulation of the stock plant (reducing the number of competing growth centres) (De Ruiter, 1993) and by nutrition (Röber, 1976; Krause, 1981). In addition, environmental conditions play an important role (Eng et al., 1983; Fisher and Hansen, 1977; Molitor and Von Hentig, 1987). In the range of temperatures of 10°C to 20°C chrysanthemum cutting production increased with temperature (Winkler, 1967). De Jong and Smeets (1982) and De Lint and Heij (1989) found a better outgrowth at higher temperatures in chrysanthemums but stem quality as expressed as g cm^{-1} was reduced (De Lint and Heij, 1987). The rate of leaf initiation of chrysanthemums increased with temperature (Cockshull, 1979).

Apart from the possibility to manipulate bud outgrowth, the preceding process of axillary bud formation can also be influenced. Audus (1959) studied axillary buds from different kinds of plants and concluded that during the early developmental stages of bud growth the future course of development can be considerably altered by external factors. Similarly, Champagnat (1954) showed that for *Sambucus nigra* L. the bud is already conditioned during the early developmental stages.

The aim of this research was to study the effect of temperature on axillary bud formation and subsequent shoot growth in chrysanthemum. To separate the effects of

temperature on the stock plant from those on the axillary bud, axillary buds were isolated and grown in-vitro or oculated on stock plants, that were grown at the 'standard' temperature of 21°C in all cases.

Materials and methods

Experiment 1: Cuttings (5 cm in length) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev) cultivar 'Cassa' (Fides, De Lier), were rooted and potted in 14 cm square plastic pots containing a commercial potting medium (Lentse potgrond, No. 4, Coöp Tuinbouwcentrum, Lent). Fourty plants were placed in controlled environment rooms at temperatures of 10°C and 30°C (day and night). Relative humidity was approximately 70%, irradiance 21.5 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD50W/84HF) and day length 16 h. The plants were fed once every two weeks with a solution containing alternately: N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5). When the distance from the growing point to the sixth leaf (counting from the soil surface) was 5 cm, the 5 cm top stem section (cutting) was picked. Axillary buds grew out and the fourth axillary bud (counting from the base of the new formed shoots) from the topmost shoot was put in-vitro culture (30°C: 20 March 1991, 10°C: 15 April 1991). For in-vitro culture the axillary buds were sterilised in 70% alcohol for a few seconds, followed by 15 minutes in 1% NaOCl to which a few drops of Tween 20 was added, and, finally, washed three times with sterile water. The axillary buds were grown individually in Pyrex glass tubes (20 mm diameter) containing 10 ml culture medium. After inoculation the tubes were closed by a cotton plug and Vitafilm. The axillary buds were grown on a basic culture medium of 0.7% Daichin agar in distilled water containing macrosalts and microsalts at full strength according to Murashige and Skoog (1962), NaFeEDTA 37.5 mg l^{-1} and 4% saccharose. The pH of the medium was adjusted to 6.0. Incubation occurred in a growth chamber at 23°C; irradiance was 6 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD36W/84). Day length was 14h. Length and number of leaves (visible to the naked eye) of every plantlet was measured at four, five and six weeks after inoculation.

Experiment 2: The experiment was similar as experiment 1 but, in addition, 40 axillary buds of plants grown at 20°C were put in-vitro culture (30°C: 12 september 1991, 20°C: 17 September 1991 and 10°C: 9 October 1991).

Experiment 3: The experiment was similar as experiment 1; axillary buds of plants grown at 15°C and 30°C were put in-vitro culture (30°C: 7 January 1992, 15°C: 29

January 1992).

Experiment 4: As described in more detail for experiment 1, cuttings were picked at 19 October 1993 and stored at 5°C. At three intervals a number of cuttings were rooted and 14 days later potted in 14 cm square plastic pots. The rooted cuttings were, at the same intervals, transferred to controlled environment rooms adjusted at 15°C, 21°C and 27°C (day and night). Relative humidity was approximately 70%, irradiance 21.5 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD 50W/84HF) and day length 16 hours. The plants were fed as in experiment 1. The intervals were chosen in such a way that at the start of the experiment (3 December 1993) the plants had the same developmental stage. Axillary buds, including the attached leaf, taken from the same position as used in experiment 1 of plants grown at the three temperatures were oculated on stock plants of the same batch grown at controlled conditions at 21°C. The axillary bud (including the attached leaf) was oculated between the second and third leaf (counting from the base of the topmost shoot) at the moment there was 5 cm stem removed above the fourth leaf of this shoot (Fig. 5.2.1). When after bud outgrowth the distance from the growing point to the fourth

Table 5.2.1: The effect of temperature on 5 cm cuttings grown out from axillary buds formed at 15, 21 or 27°C and oculated on stock plants grown at 21°C.

		weight		leaf		
Temperature (°C)	diameter (mm)	fresh (g)	dry (g)	number	area (cm^2)	days
15	2.36a	0.86a	0.11a	5.33a	19.38a	29.4a
21	2.42a	0.88a	0.12a	5.45a	22.43a	30.2a
27	2.42a	0.88a	0.11a	5.58a	22.26a	29.2a

Values within rows followed by different letters differ significantly at the 5% level.

leaf (counting from the base of the new formed shoots) was 5 cm, the 5 cm cutting was picked. Its diameter (mm), fresh- and dry weight (g), leaf area (cm^2) and number of leaves (> 1 cm^2) was determined. In addition, the time necessary to produce the cutting was recorded. There were five blocks, perpendicularly orientated to the air stream. Each block contained three (treatment) x two (replicates)= six experimental units of four plants. The six units were randomized over each block.

Results of the experiments were statistically analyzed by analysis of variance followed by mean separation according to Tukey's HSD-test.

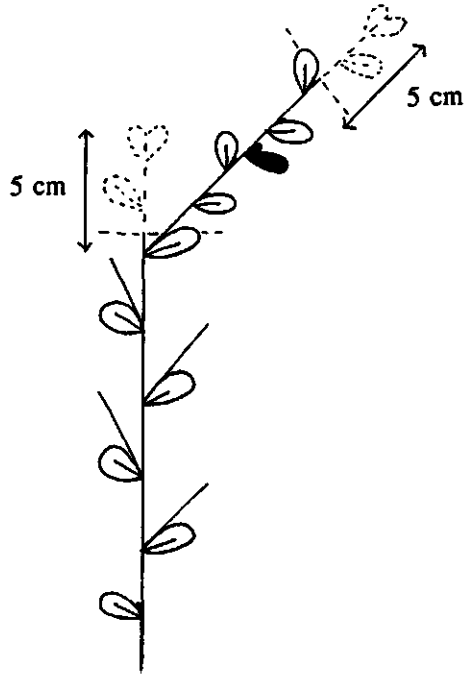

Fig. 5.2.1: The position of the oculated axillary bud (including the attached leaf).

Results

As Fig. 5.2.2 and 5.2.3 show for experiment 1, bud outgrowth at constant temperature was faster and number of leaves found was larger for buds laid down at 30°C than at 10°C. In contrast, in experiment 2, the initial low temperature of 10°C was reflected in the best outgrowth and, with respect to length, 20°C occupied an intermediate position (Fig. 5.2.4 and 5.2.5). Finally, in experiment 3 no effect of temperature during bud formation on bud outgrowth at constant temperature could be found (Fig. 5.2.6 and 5.2.7). When buds were oculated on stock plants (experiment 4) bud outgrowth did also not respond to the temperature during the bud formation process (Table 5.2.1).

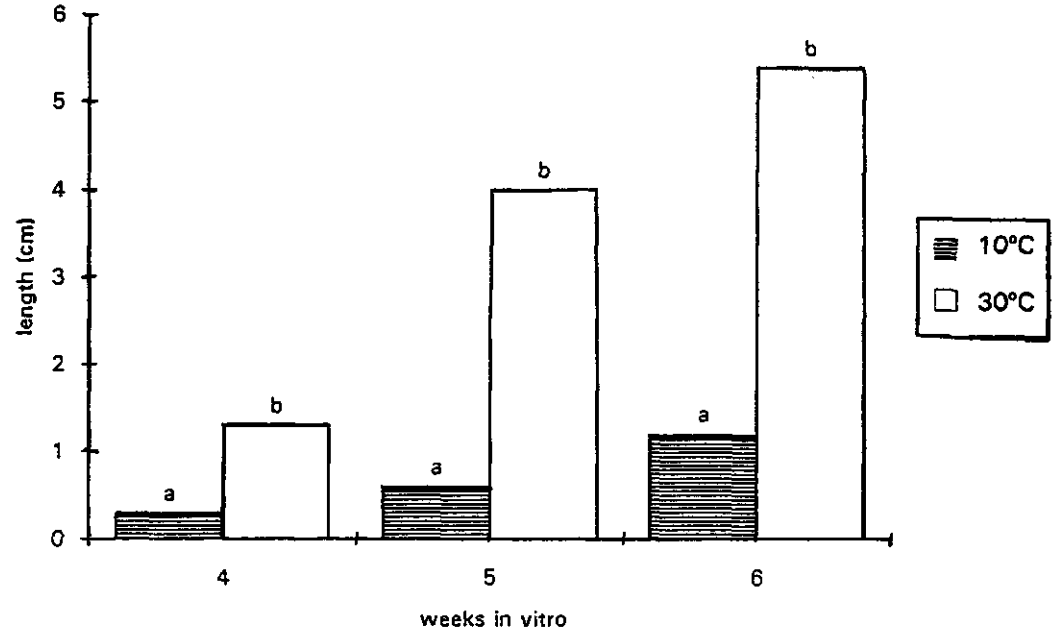

Fig. 5.2.2: Length of the plantlets (during different weeks) grown in-vitro and pre-treated at 10 and 30°C.

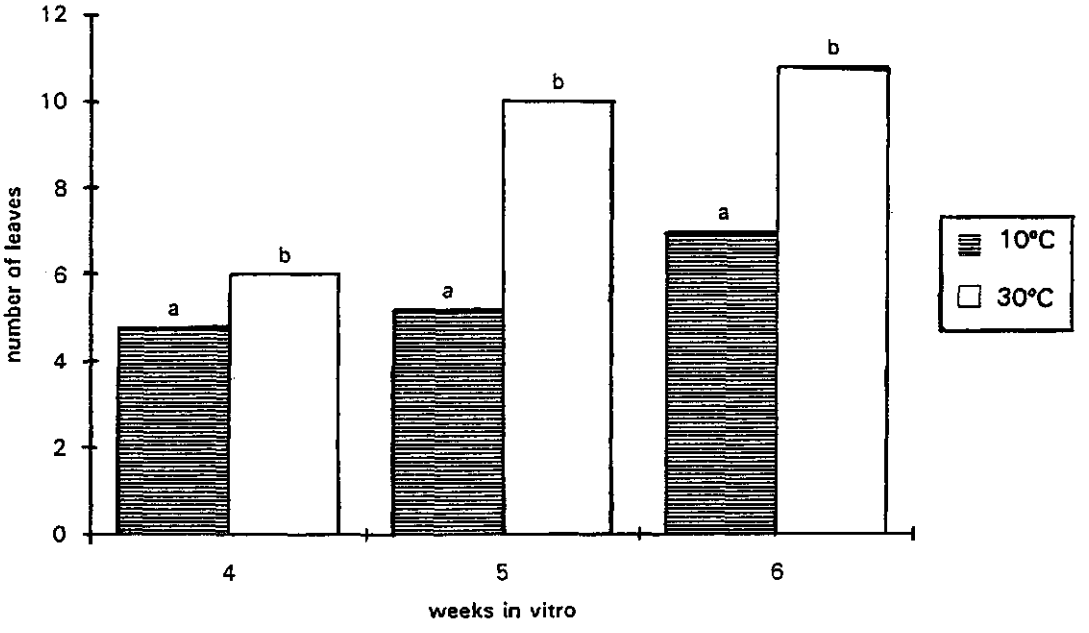

Fig. 5.2.3: Number of leaves of the plantlets (during different weeks) grown in-vitro and pre-treated at 10 and 30°C.

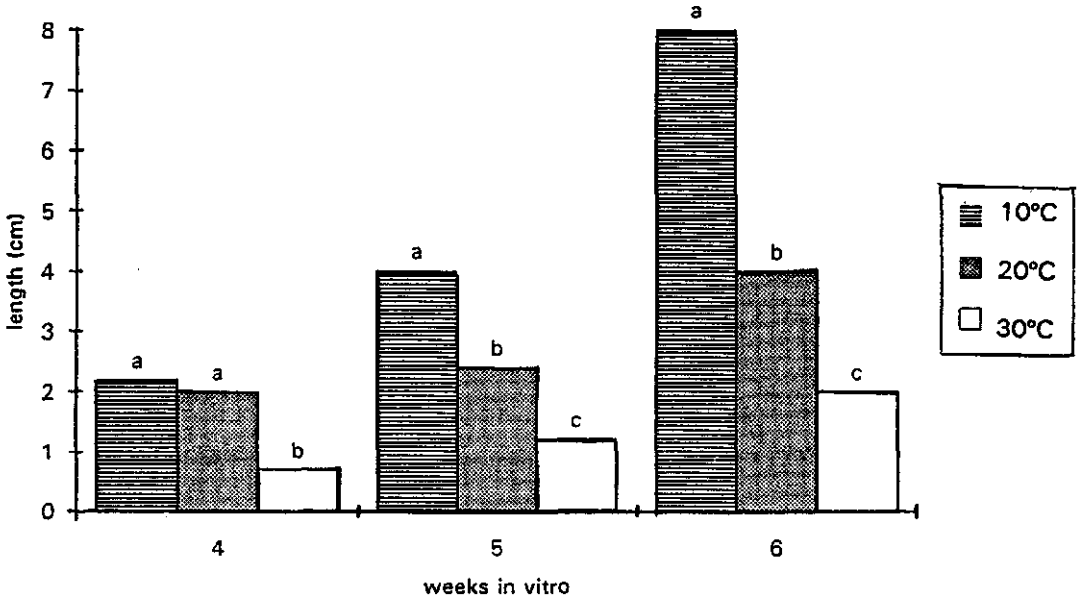

Fig. 5.2.4: Length of the plantlets (during different weeks) grown in-vitro and pre-treated at 10, 20 and 30°C.

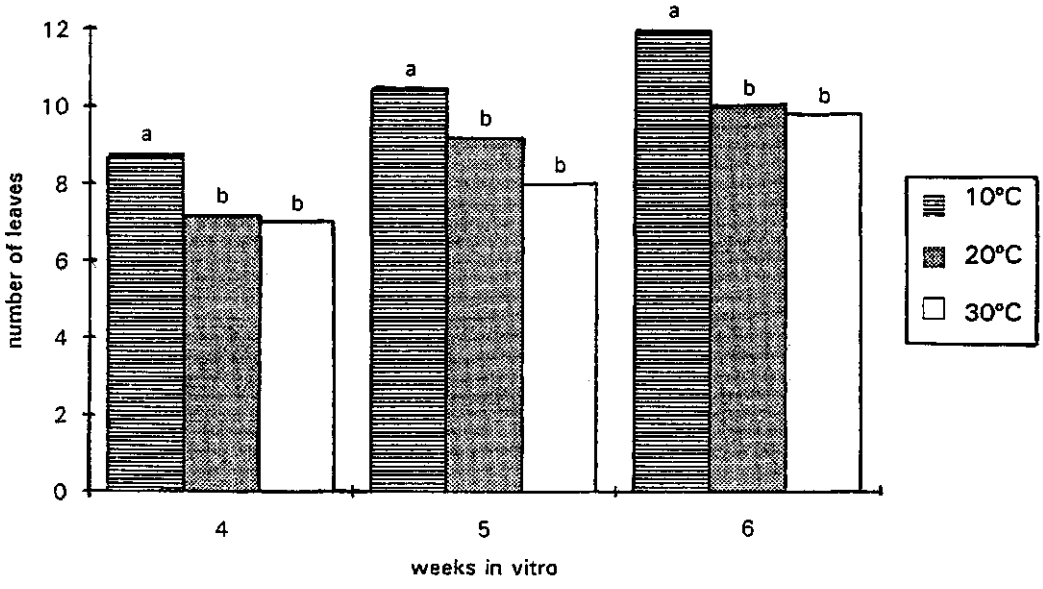

Fig. 5.2.5: Number of leaves of the plantlets (during different weeks) grown in-vitro and pre-treated at 10, 20 and 30°C.

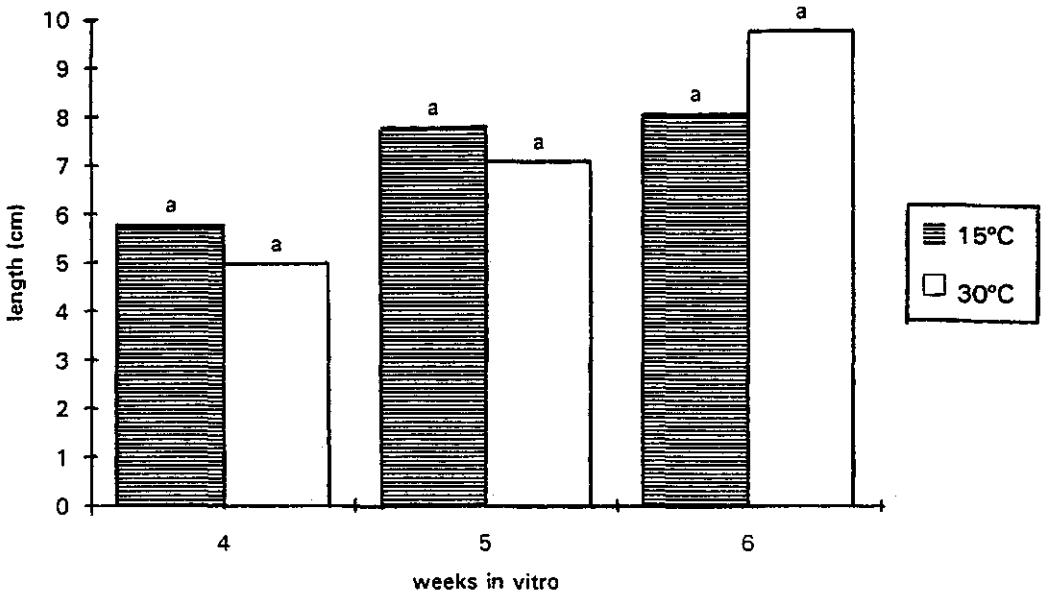

Fig. 5.2.6: Length of the plantlets (during different weeks) grown in-vitro and pre-treated at 15 and 30°C.

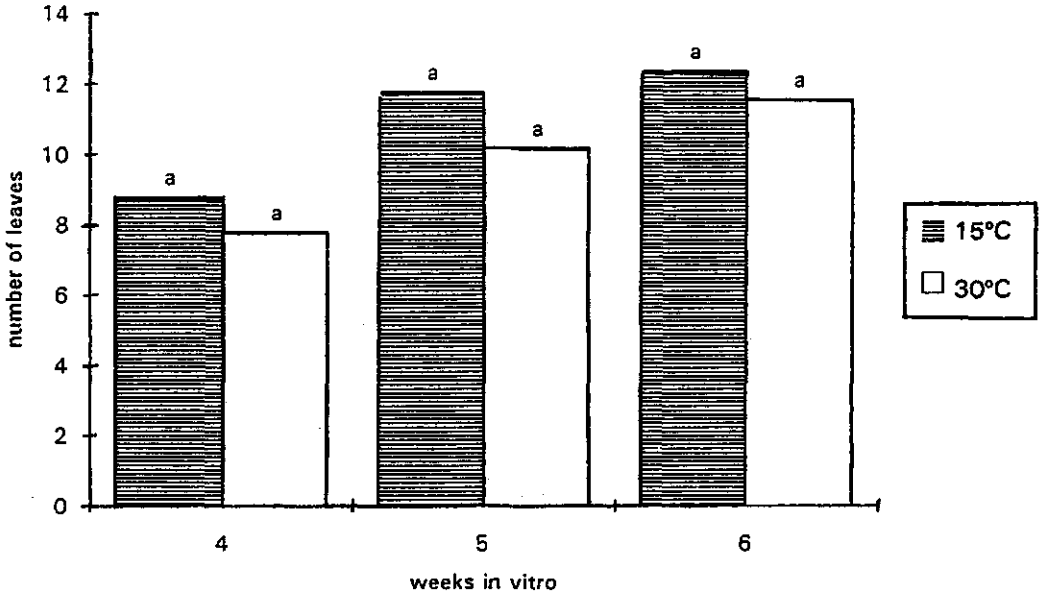

Fig. 5.2.7: Number of leaves of the plantlets (during different weeks) grown in-vitro and pre-treated at 15 and 30°C.

Discussion

In the present three experiments outgrowth of axillary buds at the same temperature, that were exposed to different temperatures during their formation, showed a quite divergent pattern. That difference in response may only become clear when it is assumed that the ability to grow out is controlled by two opposing factors, which relative importance is dependent on the specific experimental conditions of each experiment. In this idea the first factor would be inhibition of bud outgrowth imposed by lower temperatures (Terry et al., 1983) leading to a delay in outgrowth when the buds were exposed to the constant temperature of 23°C (in-vitro culture) or 21°C (controlled environment room). In an experiment on citrus, axillary buds produced under winter conditions, required more time until bud burst occurred than those formed at high summer temperatures (Halim et al., 1988).

The second factor may be the age of the buds when they were transferred to the in-vitro conditions. It should be realized that since bud development is accelerated at higher temperatures, at the start of the outgrowth period the buds produced at low temperatures were older than those formed at high temperatures. It is not unlikely that the time buds need until sprouting declines with age. Thus, for rose Marcelis-van Acker (1994b) found that axillary bud break was hastened at increasing pre-treatment temperatures. Similarly, raising the cultivation temperature of stock plants of *Nephrolepis exaltata* favoured the growth of daughter plants grown in-vitro (Hveslof-Eide, 1991).

It is not illogical to assume that the supposed balance between two factors influencing bud outgrowth in opposite direction is dependent on time of season. There is little information available, but Cathey (1954) found for chrysanthemum cuttings harvested from stock plants grown at different temperatures, that the after-effect depended on the time of year in which propagation took place (Cathey, 1954).

Finally, it should be kept in mind that the air humidity was kept constant at the various temperatures, and thus the vapour pressure deficit of the air was different, which may have affected the results, as discussed by Hoffman (1979). However, Grange and Hand (1987) reported that humidities between 1.0 and 0.2 kPa vapour pressure deficit have little effect on the physiology and development of horticultural crops. The vapour pressure deficits occurring in our experiments fell within this range.

6. General discussion

Introduction

In the commercial production of chrysanthemum cuttings for the cut flower and pot plant industry, homogeneity of cuttings is required as uniform, well grown cuttings offer uniformity and predictability in harvesting and flowering (De Greef, 1989; Van der Hoeven, 1989; Van Vliet, 1990).
In order to get a better insight in factors influencing axillary bud development and subsequent cutting quality the effect of assimilate supply, position and age of the bud, and temperature on axillary buds attached to as well as separated from the plant were studied. In the preceding Chapters a distinction was made between axillary bud formation and axillary bud outgrowth. Results show that the examined factors can have a small influence on axillary bud formation (Table 6.1) but not enough to influence subsequent cutting quality. However, the effect of external factors on axillary bud outgrowth (Table 6.1) is strong enough to affect cutting quality. Therefore in this present Chapter no further attention will be paid to the distinction between formation and outgrowth.
Vegetative axillary buds were studied until they produce a cutting of just 5 cm length and fresh- and dry weight, diameter, number and area of the leaves, number of pith cells and diameter of the pith of only that cutting were recorded. No attention was paid to the other part of the plant, and the further development of the cutting into a flower.

Axillary buds attached to the plant

Assimilates

In chrysanthemums, during normal commercial cutting production, cutting quality declines as stock plants age. However, axillary buds in later generations are able to develop in a better way if, by varying the number of leaves as assimilate producers and the axillary buds as target organs on a stock plant, more leaves become available per axillary bud (Chapters 4.1 and 4.2). This is confirmed for tomato trusses (Slack and Calvert, 1977) and tomato and cucumber fruits (Marcelis and Heuvelink, 1990) which increased in weight when more leaves were available per truss or fruit. In addition, for chrysanthemum less time is needed to reach the

Table 6.1: The effect of several factors on axillary bud development, i.e. formation and outgrowth.

				weight		leaves		pith		
	length	growth period	diameter	fresh	dry	number	area	cell nr	diameter	%
Formation										
temperature	?	0	0	0	0	0	0	-	?	?
assimilates	+	?	+	+	+	+	?	-+	?	?
Outgrowth										
temperature	?	-	-	-	-	-	-	-	-	0
assimilates	?	-	+	+	+	+	+	+	+	0
age/position	0	-	0	-+	-+	-+	-+	?	?	?

\+ = higher at increased level of factor
\- = lower at increased level of factor
-+ = little higher at increased level of factor
0 = no effect
? = not investigated factor

'harvest' cutting length of 5 cm if less axillary buds are left on the stock plant. Obviously, the available assimilates have an influence on the growth rate of the shoot as discussed in Chapter 5.1. Marcelis-van Acker (1994b) also found for rose that an increase in assimilate supply shortened the subsequent growth period of the shoots.

Besides the increase in cutting quality and the decrease in time needed to produce a high quality cutting, the removal of axillary buds from a stock plant also has a negative effect. For the number of good quality cuttings harvested from a commercially grown stock plant is higher than the number of cuttings harvested from a stock plant where a number of axillary buds have been removed. Other side effects of increasing the number of leaves per axillary bud are the emergence of shoots in the root collar region and the premature development of axillary buds on the outgrowing shoots. This latter effect demonstrates that assimilates will be used also by other parts of the plant besides the outgrowing shoot.

In Chapters 4.2 and 4.4 is shown that the number of pith cells as indirect quality parameter (representing primary thickness) at the largest diameter in a transverse section of a cutting is dependent on the amount of available assimilates; the more assimilates the more pith cells. The percentage pith, i.e. pith diameter with regard to total shoot diameter, is not influenced. Hardly any transverse cell divisions in the pith of a growing shoot occur as found in chrysanthemums by Popham (1958) and in a few woody perennials by Kassner (1884). However, Sachs et al. (1959, 1960) could increase and decrease longitudinal cell division in pith tissue by applying respectively GA (gibberellin) or Amo ([4-hydroxy-5 isopropyl-2 methylphenyl] trimethylammonium chloride, 1-piperidine carboxylate). Sachs and Kofranek (1963) found that transverse divisions could also be influenced by Amo, CCC (2-chloroethyl trimethylammonium chloride) and Phosfon (2,4-dichlorobenzyl tributylphosphonium chloride). A larger shoot diameter means an enlarged pith diameter due to an increase in number of pith cells in a cross section and not due to an increase in pith cell size. The diameter of a chrysanthemum pith cell even tended to decrease with increasing number of leaves per bud (Chapter 4.4). The total number of pith cells counted in a cross section of a shoot, remains equal in time and is independent of the place in the shoot (Chapter 4.4). Similar results were found for rose (Marcelis-van Acker, 1994b). Therefore differences in number of pith cells due to assimilate supply (Chapters 4.2 and 4.4) must have been set soon after sprouting because extra assimilate supply stimulates cell division in the pith of a young sprouting axillary bud.

Position and age

In 'intact' shoots, there is a strong decrease in outgrowth of axillary buds going from the top to the basis (Chapter 3). This fits in with the 'hormonal' theory of apical dominance assuming that the most apical growing point is the source of some correlative signal of hormonal nature, probably auxin, which restricts development of lower meristems (Martin, 1987; Cline, 1994).

However, varying the ratio between number of leaves and axillary buds on a stock plant (as described above) influences the pattern in outgrowth of axillary buds from the top to the basis. In the concept of apical dominance it would be expected that when more axillary buds were allowed to grow out, shoot weight of individual shoots would have been decreased and the time required to attain a 5 cm cutting would have been increased going from the top to the basis, as found in topped intact cuttings in Chapter 3. Similarly, Keppeler (1986) and Schwabe (1979) for chrysanthemums and a number of plant species, respectively, showed that axillary buds closer to the apex were less inhibited than the lower positioned basal buds. In stock plants with two or four axillary buds per 16 leaves (Chapter 4.2) there were almost no differences in quality of the cuttings and the time required. The explanation of these conflicting data, probably must be sought in the fact that in the present experiment all but (maximal) four buds were removed whereas total leaf area was kept intact. As a consequence, the amount of assimilates available for the outgrowth of the (maximal) four buds may have been so abundant that the growth pattern imposed by apical dominance is completely outstripped. As a result there is no or little difference in growth behaviour between the two or four buds left on the shoot.

For the outgrowth of an axillary bud on a shoot the influence of leaf position is not important (Chapter 4.4). This is in contrast with findings for chrysanthemum of Keppeler (1968). He concluded that there are differences between top and bottom leaves in this respect and that top leaves are more efficient in photosynthesis. Partial defoliation may result in an enhancement of photosynthetic rates in the remaining leaves (Hardwick et al., 1968). Other studies on partial defoliation in tobacco and sunflower have shown recovery of photosynthetic rates in lower, originally shaded leaves when exposed to higher irradiance, indicating that the photosynthetic capacity is not reduced (Rawson and Hackett, 1976). However, the differences in position in our chrysanthemum experiment were not very large. So when using a larger shoot (with more leaves), differences between top and bottom leaves might have been expressed.

Temperature

As described in the foregoing it is possible to produce cuttings of higher quality if more leaves are available per axillary bud. However, the number of cuttings harvested from a commercially grown stock plant is higher than from a stock plant in which axillary buds are removed (manipulated stock plant). Although the time needed to produce a cutting is less in a manipulated stock plant, commercially grown stock plants still produce more cuttings in a given time. In accordance with Porter and Delecolle (1988), stating that rates of development of plants are linear with increasing temperature, less days were needed to produce a 5 cm cutting when applying higher temperatures. Cutting quality was still higher in manipulated stock plants as in commercially grown stock plants, so higher temperatures partially solves the problem of less cuttings (Chapter 4.3). Temperature however has an influence on the shape of the leaves. Chrysanthemum leaves initiated at relatively high temperatures become very much less dissected than those formed at a lower temperature (Schwabe, 1959). In *Nephrolepis* a carry-over effect of temperature during growth of the parent plant on the subsequent growth in-vitro was found (Hvoslef-Eide, 1991). Because of the effect of temperature on the morphology of a cutting it must not been ruled out that cuttings produced at higher temperatures results in flowering plants of a lesser quality than those grown at lower temperatures.

The percentage pith, i.e. pith diameter of the shoot with regards to total diameter of the shoot, is not influenced by temperature (Chapter 4.3). Similarly in rose pith diameter was found to be correlated with shoot diameter (Marcelis-van Acker, 1994b) and also in Pinus (Ladell, 1963) a correlation between the diameter of the pith and the vascular tissue was reported. The total number of pith cells, counted on the largest diameter of a cross section of a shoot, remained equal in time and was independent of the place in the shoot (Chapter 4.4). Therefore, differences in number of pith cells due to temperature (the lower the temperature, the higher the number of pith cells counted in a cross section, Chapter 4.3) must have been set soon after sprouting as was also discussed for the effect assimilate supply. As described in Chapter 4.3, part of the difference in number of pith cells was already set during axillary bud formation. The lower the temperature, the higher the number of pith cells. In Chapter 5.2, axillary buds formed at different temperatures and oculated on plants grown at the same temperature showed no differences in subsequent cutting quality. As possible differences between the axillary buds were not large enough to have any effect on cutting quality.

In summary, removing axillary buds of a stock plant, so that more leaves (assimilates) are available per bud, favours the quality of the cuttings and shortens the time needed to produce a cutting. However, the number of cuttings harvested is reduced when axillary buds are removed. Temperature has a positive effect on growth rate of the cuttings and cutting quality is still higher than in control stock plants grown at normal temperatures. Thus, applying higher temperatures may partially solve the problem of too few cuttings formed in a given time. However, we must keep in mind that temperature can affect leaf shape which effect may carry over into the mature plant. An alternative for producing more good quality cuttings could be growing more stock plants per square meter, because manipulated stock plants need less space than control plants. However, placing less control plants per square meter also may have a positive influence on cutting quality. The side effects of more assimilates per axillary bud (the outgrowth of shoots in the root collar region, the premature development of axillary buds on outgrowing shoots and the influence on apical dominance) are supposed to have no effect on the cutting and the subsequent flowering shoot. The pith of a cutting (expressed in the number of pith cells) is also influenced by assimilate supply and temperature. The more assimilates or the lower the temperature, the higher the number of pith cells in a cross section of a shoot and the thicker the cutting. Temperature also affects the number of pith cells. But these differences in cell number have no consequence for the quality of the subsequent cutting.

Axillary buds separated from the plant

Assimilates

Assimilates affect the rate of development of an axillary bud. An increase in assimilate supply accelerates bud development before topping. Further developed buds grow out somewhat better in-vitro culture than less developed buds (Chapter 5.1). Zamski et al. (1985) suggest that the growth potential of axillary buds from rose is influenced during axillary bud formation. Audus (1959) also states that there is no doubt that early in bud development the future course of development can be altered considerably by exogenous factors. Champagnat (1954) showed that an axillary bud of *Sambucus* can be 'conditioned' during the early developmental stages. By applying the in-vitro culture technique the developmental stage of an axillary bud can easily be checked because in this way the outgrowth of buds that differ in developmental stage is synchronized. Differences in quality clearly come to the fore.

Position and age

In isolated shoot sections (axillary bud with attached leaf) age and position of axillary buds are not important factors for axillary bud outgrowth (Chapter 3). Nevertheless, the time needed to produce a cutting of a certain length decreases with bud age and most outgrowth parameters show somewhat higher values for the lower positioned buds. The first fact is in accordance with earlier findings that in-vitro culture more developed axillary buds give longer shoots in a certain time (Chapters 5.1 and 5.2). The second fact is in line with findings of Keppeler (1968), also for chrysanthemum. In contrast, Zieslin et al. (1976) reported for rose that sprouting ability is highest in the apical axillary buds. The slightly better performance of basal buds may be due to quicker rooting of lower positioned shoot sections as reported by Hansen (1986) and Hansen and Kristensen (1990) for *Schefflera arboricola*. Light conditions in the basal regions usually are less favourable and as found for many plant species more roots are produced at decreasing irradiance (Biran and Halevy, 1973; Hansen and Eriksen, 1974; Poulsen and Andersen, 1980).
When comparing the influence of position of the axillary bud in intact shoots and in isolated shoot segments, it can be seen that in isolated shoots axillary bud position is not important whereas in intact shoots it is (Chapter 3).
In an in-vitro experiment where axillary buds were forced to grow out at four successive weeks, 'age-2' buds performed better than the buds of age 1, 3 and 4 (Chapter 3). Probably the degree of inhibition of the axillary buds might play a role.

Temperature

Because at lower temperatures topping is delayed, at the time the cutting is 'ready' to be picked the axillary buds are further developed at lower than at higher temperatures. The rate of outgrowth however, at constant temperatures, was not the same in all experiments, as described in Chapter 5.2. Probably two factors are involved, being an increase in the stage of development of an axillary bud, which affects axillary bud outgrowth positively and an increase in the degree of inhibition of axillary buds, having a negative effect. In *Citrus*, buds produced under winter conditions required considerably more time before bud burst than those produced under summer conditions (Halim et al., 1988). Marcelis-van Acker (1994) found for rose that axillary bud break was hastened by increasing pre-treatment temperatures. High temperatures accelerate leaf initiation rate in axillary buds (Terry et al., 1983). Temperatures around 10°C are probably critical for the degree of inhibition.

In summary the influence of assimilates and temperature on axillary bud formation is small. There are some differences in outgrowth in-vitro but these are mainly due to differences in developmental stage of the axillary buds and in degree of inhibition of the buds. Age and position of the axillary buds also are not important factors for bud outgrowth when separated from the plant.

Practical consequences

The best way to improve cutting quality is increasing the amount of assimilates, i.e. number of leaves per outgrowing axillary bud on the plant and/or decreasing the temperature. Unfortunately, increasing cutting quality in these ways reduces the number of produced cuttings. In the commercial cutting production both factors are important. An economic optimum should be found between the ratio leaves/axillary bud, number of stock plants per square meter, temperature and quality of the cutting.

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Summary

Chrysanthemums are vegetatively propagated by means of cuttings taken from stock plants. These stock plants are made by removing the uppermost 5 cm shoot from above the sixth leaf (counting from the soil surface) of a rooted cutting. The axillary buds in the axils of the remaining sixth leaves are able to grow out into shoots. Due to apical dominance, the upper buds sprout more rapidly than the lower buds. At the moment there is 5 cm shoot standing above the fourth leaf (counting from the base of the new formed shoots) this 5 cm shoot (the cutting) is picked. This procedure is repeated until the stock plants are about three months old, whereafter the stock plants are discarded.

Every cutting develops from an axillary bud. To get a better insight in factors influencing cutting quality, the effect of some of these factors on axillary bud development was studied. The process of axillary bud development can be divided in axillary bud formation and axillary bud outgrowth. Used quality parameters were: fresh- and dry weight, diameter, number of leaves, leaf area, length, number of pith cells on the largest diameter of a cross section and the diameter of the pith.

In Chapter 1 a summary is given of research done on factors influencing axillary bud formation and axillary bud outgrowth.

The early development of an axillary bud was followed using a scanning electron microscope (SEM). Already during the formation of an axillary bud (primary bud), the next generation of axillary buds (secondary buds) started their development. At the moment the primary axillary bud had developed five or sixth leaves, the first signs of a secondary axillary bud could be seen inside the axil of the first leaf of the primary bud (Chapter 2).

In Chapter 3, on the basis of two in-vivo experiments and one in-vitro experiment, the influence of age and position of axillary buds on subsequent cutting quality was discussed. The two in-vivo experiments showed that the outgrowth of axillary buds and the quality of the subsequent cuttings was not much affected by age and position of the bud. Nevertheless, the values for the various quality parameters of the lower axillary buds were somewhat higher than those of the higher axillary buds. The time needed to produce a cutting also seemed somewhat shorter for the older buds than for the younger buds. The results of the in-vitro experiment showed that there was an optimum age for axillary buds to grow out.

In Chapter 4 the attention was focused on a few factors influencing axillary bud outgrowth. In the normal chrysanthemum cultivation quality of cuttings declines as stock plants age. Three different kind of stock plants were made by varying the

number of axillary buds and leaves on the plant (Chapter 4.1). Cutting quality in later generations was improved when there were more leaves available per axillary bud. This type of stock plant management was further elaborated in Chapter 4.2. However, it should be kept in mind that the number of cuttings produced in a certain time was lower when axillary buds were removed and that the increase in cutting quality that occurred was not in proportion with the increase in number of leaves. If leaf area is not reduced up to a certain number of axillary buds that are allowed to develop, almost no differences in quality between these cuttings occur. Against expectance (according to the apical dominance theory) also hardly any differences in growth rate between these cuttings were present. The pith is also considered as a quality parameter of a cutting. An important part of the stem is formed by the pith (representing primary thickness). In the pith the number of pith cells and the diameter of pith cells are not constant. The more pith cells and the larger the cells, the thicker the cutting will be and the better its quality. An increase in the number of leaves per axillary bud goes together with an increase in the number of pith cells in a cross section of a shoot.

The influence of temperature on the outgrowth of axillary buds is described in Chapter 4.3. When temperature was increased axillary buds grew out faster and, therefore, more cuttings could be picked in a certain time. Quality however, declined but was still higher than of normally cultivated cuttings. With respect to the pith the ratio between pith- and shoot diameter appeared to be the same at the various temperatures. The greater pith and, consequently, the greater shoot diameter at higher temperatures is mainly due to an increase in the number of pith cells.

The effect of leaf position on bud outgrowth was studied by removing leaves of different positions and removing all axillary buds except the top one (Chapter 4.4). The outgrowth of that top bud appeared to be independent of the position of the leaves.

The effect of assimilate supply and temperature on axillary bud outgrowth is analyzed in Chapter 5. The experiment described in Chapter 5.1 was carried out to examine whether the number of leaves per axillary bud during the formation was relevant for the later cutting quality. In that experiment bud outgrowth occurred in in-vitro culture. Results showed that the more assimilates were available during axillary bud formation, the more developed the axillary bud was at the time of topping. As a result cuttings of a better quality were produced. In experiments done in-vitro and in-vivo on the influence of temperature on axillary bud formation no consistent results could be obtained (Chapter 5.2)

Relationships between the results described in the previous Chapters were analyzed in Chapter 6. It was concluded that in the scope of the present study the process of

axillary bud formation was much less important than that of axillary bud outgrowth, because the quality of the subsequent cuttings could hardly be influenced during formation. Manipulation of cutting quality was only possible during bud outgrowth. Increase in assimilate supply as well as decrease in temperature improved cutting quality, but, as a consequence, the number of produced cuttings was reduced. In the commercial cutting production quality and number of cuttings are important. Therefore, an economic optimum should be found for each of the interrelated factors: number of leaves and buds on the plant, number of plants per square meter, temperature and cutting quality.

Samenvatting

Chrysanten worden vegetatief vermeerderd door middel van stekken afkomstig van moerplanten. Deze moerplanten worden gevormd door van een bewortelde stek de bovenste 5 cm te verwijderen, zodat een stengel met zes bladeren met bijbehorende okselknoppen overblijft. De okselknoppen in de oksels van deze bladeren kunnen uitlopen. Als gevolg van de apicale dominantie lopen de bovenste okselknoppen meestal sneller uit dan de onderste okselknoppen. Op het moment dat 5 cm scheut (de eerste stek) gevormd is in de zone boven vier bladeren (geteld vanaf het blad waar de scheut staat ingeplant), wordt deze 5 cm stek geplukt. De okselknoppen in de oksels van de overblijvende vier bladeren kunnen dan uitlopen en vormen zo de nieuwe generatie stekken. Dit proces herhaalt zich gedurende drie maanden. Na drie maanden is de kwaliteit van de geplukte stekken niet meer voldoende en de kans dat de stekken voortijdig generatief worden, wordt te groot. De moerplanten worden dan afgedankt.

Elke stek ontstaat dus uit een okselknop. Om meer inzicht te krijgen in de factoren die van invloed zijn op de uiteindelijke stekkwaliteit is de okselknopontwikkeling bestudeerd. In het proces van ontwikkeling kan okselknopaanleg onderscheiden worden van okselknopuitgroei. Als kwaliteitsparameters van de stek zijn de volgende factoren gebruikt: vers- en drooggewicht, diameter, aantal bladeren, bladoppervlak, lengte, aantal mergcellen op de grootste diameter van de stek en de diameter van het merg.

In Hoofdstuk 1 wordt een samenvatting gegeven van eerder onderzoek naar factoren die van invloed kunnen zijn op de okselknopaanleg en -uitgroei.

Alvorens met het eigenlijke onderzoek te beginnen is met behulp van een scanning electronen microscoop (SEM) bestudeerd op welk moment een okselknop wordt aangelegd. Het bleek dat tijdens de aanleg van een (primaire) okselknop reeds een volgende generatie (secundaire) okselknoppen gevormd wordt. Op het moment dat de primaire okselknop zijn vijfde of zesde blad afsplitst zijn de eerste tekenen van een secundaire okselknop in de oksel van het eerste blad waarneembaar (Hoofdstuk 2).

In Hoofdstuk 3 wordt aan de hand van twee in-vivo experimenten en één in-vitro experiment besproken in hoeverre de leeftijd en de positie van okselknoppen van invloed zijn op de kwaliteit van de uiteindelijke stekken. De twee experimenten in-vivo tonen aan dat voor de uitgroei van okselknoppen en de kwaliteit van de uiteindelijke stekken zowel de leeftijd als de positie van de okselknoppen van weinig belang zijn. Niettemin waren de meetwaarden voor de kwaliteitsparameters van de

lager gelegen okselknoppen iets hoger dan die van de hoger gelegen okselknoppen. Eveneens leek het erop, dat oudere okselknoppen wat minder tijd nodig hebben voor het produceren van een stek dan jongere okselknoppen. De resultaten van het in-vitro experiment laten zien, dat er een optimale okselknopleeftijd is voor de uitgroei in-vitro.

Enkele factoren die van invloed zijn op de okselknopuitgroei zijn bestudeerd in Hoofdstuk 4. In de normale chrysantenteelt neemt de kwaliteit van de stekken af naarmate de moerplanten ouder worden. Er werden drie verschillende typen moerplanten gecreëerd door het aantal okselknoppen en bladeren aan de moerplanten te variëren (Hoofdstuk 4.1). Het bleek dat indien meer bladeren beschikbaar zijn per okselknop de kwaliteit van de uiteindelijke stek in oudere moerplanten beter is. In Hoofdstuk 4.2 is hierop verder ingegaan. Echter, het aantal geproduceerde stekken per plant is als gevolg van het verwijderen van okselknoppen lager en de stijging in kwaliteit die optreedt is niet evenredig met de toename in het aantal bladeren per knop. Opmerkelijk is dat indien veel bladeren per okselknop beschikbaar zijn en er meer okselknoppen per moerplant uit mogen lopen de verschillen in kwaliteit tussen deze stekken van één moerplant uitermate gering zijn. In tegenspraak met wat er volgens de apicale-dominantietheorie verwacht zou mogen worden is er geen verschil in uitgroeisnelheid tussen de okselknoppen van verschillende posities aan één plant. Een aparte kwaliteitsfactor die bestudeerd werd, is het merg. Het merg vormt een belangrijk gedeelte van de scheut (primaire dikte). Zowel het aantal cellen op de grootste dwarsdoorsnede als de grootte van de afzonderlijke cellen kan variëren. Hoe meer cellen op een dwarsdoorsnede en hoe groter de cellen, des te dikker de scheut en des te beter de uiteindelijke kwaliteit daarvan. Met een toename van het aantal bladeren per okselknop blijkt het aantal mergcellen op de grootste diameter van de scheut toe te nemen. In Hoofdstuk 4.3 is vervolgens de invloed van de temperatuur op de uitgroei van de okselknoppen bestudeerd. Een verhoging van de temperatuur deed de okselknoppen sneller uitlopen zodat meer stekken in een bepaalde tijd geproduceerd kunnen worden. De kwaliteit van de stekken neemt bij hogere temperaturen weliswaar af, maar zij is nog steeds hoger dan indien geen okselknoppen verwijderd worden. Ook in dit Hoofdstuk is het merg bestudeerd. Het bleek dat de verhouding tussen merg- en scheutdiameter constant is, ongeacht de temperatuur. Een grote mergdiameter en dientengevolge een grote scheutdiameter wordt voornamelijk veroorzaakt door een toename in het aantal mergcellen. In Hoofdstuk 4.4. is het effect van de bladpositie op de uitgroei van de okselknop beschreven aan de hand van een proef waarin bladeren van diverse posities werden verwijderd en de bovenste okselknop werd gehandhaafd. Het bleek dat de uitgroei van een okselknop onafhankelijk is van de positie van het blad. Eveneens werd

aangetoond dat het aantal mergcellen op de grootste diameter onafhankelijk is van de positie aan de scheut en van de leeftijd van de scheut.
Het effect van de hoeveelheid assimilaten en de temperatuur op de okselknopaanleg is geanalyseerd in Hoofdstuk 5. In het in Hoofdstuk 5.1 beschreven onderzoek groeiden geïsoleerde okselknoppen uit in-vitro om na te gaan of het aantal bladeren dat beschikbaar is tijdens de aanleg van de okselknop van invloed is op de uiteindelijke uitgroei van die knop. De resultaten geven aan dat de hoeveelheid assimilaten die beschikbaar zijn tijdens de aanleg van een okselknop een positieve invloed heeft op de snelheid van ontwikkeling van zo'n knop. Hoe verder de okselknoppen ontwikkeld zijn op het moment dat ze in-vitro worden gezet des te beter de kwaliteit van de gevormde scheuten. In enkele in-vitro en in-vivo uitgevoerde proeven bleek dat de invloed van de temperatuur op de okselknopaanleg nogal varieerde (Hoofdstuk 5.2).
In Hoofdstuk 6 tenslotte worden de resultaten uit de voorgaande Hoofdstukken geïntegreerd. Okselknopontwikkeling is niet langer onderverdeeld in okselknopaanleg en okselknopuitgroei, omdat bleek dat de kwaliteit van de stekken nauwelijks te beïnvloeden is tijdens de aanleg van de knop. Tijdens de uitgroei van de knop is het wel mogelijk de kwaliteit te beïnvloeden. Door een toename van de hoeveelheid assimilaten tijdens de uitgroei van de knop en/of een verlaging van de temperatuur ontstaan kwalitatief betere stekken. Nadeel is echter, dat op deze manier minder stekken geproduceerd kunnen worden. In de commerciële teelt is zowel de kwaliteit van de stek als het aantal geproduceerde stekken belangrijk. Daarom moet een economisch optimum gevonden worden tussen het aantal bladeren en het aantal okselknoppen aan een plant, het aantal planten per vierkante meter, de temperatuur en de kwaliteit van de uiteindelijke stek.

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Curriculum vitae

Henrica Alberta de Ruiter werd op 25 juli 1967 geboren in Delft. In 1985 behaalde zij haar VWO-diploma aan de Christelijke Scholengemeenschap te Emmen. In datzelfde jaar begon zij de studie Plantenveredeling aan de toenmalige Landbouwhogeschool te Wageningen. In de doctoraalfase werd een gecombineerd afstudeervak gedaan op de vakgroepen Plantencytologie en -morfologie en Tuinbouwplantenteelt. Aansluitend deed zij als Erasmusstudent drie maanden onderzoek op de universiteit van Siena in Italië en liep zij stage op het chrysantenveredelingsbedrijf 'Fides' in De Lier. In maart 1990 studeerde zij af aan de Landbouwuniversiteit.

Van februari 1990 tot augustus 1990 was zij als toegevoegd onderzoeker werkzaam op de vakgroepen Plantencytologie en -morfologie en Tuinbouwplantenteelt. Van augustus 1990 tot maart 1995 deed zij op bovengenoemde vakgroepen, als assistent in opleiding (AIO), onderzoek aan de okselknopontwikkeling van chrysant, een project dat gefinancierd werd door de stichting 'Chryso'. Het onderzoek, zoals beschreven in dit proefschrift, is uitgevoerd onder leiding van Prof. dr. J. Tromp, Prof. dr. M.T.M. Willemse, Dr. ir. C.J. Keijzer en Dr. ir. P.A. van de Pol.

Vanaf juli 1994 is zij werkzaam als beleidsmedewerker op de griffie van de Landbouwuniversiteit.