Effects of Growth Conditions on External Quality of Cut Chrysanthemum: analysis and simulation

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Key words: *Chrysanthemum morifolium*, *Dendranthema grandiflorum*, modelling, simulation, external quality, visual quality, stem length, internode length, flower number, flower size, flower position, flower colour, temperature, DIF, light, CO_2 concentration, plant density, photoperiod.

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Propositions (Stellingen)

1. Although the DIF concept (difference between day and night temperature) has no biological meaning it is still a good tool to manipulate plant height in chrysanthemum.
   This thesis

2. When a chrysanthemum has liberty to choose, it will produce more flowers rather than larger flowers.
   This thesis

3. Models are very useful at present times since we are drowning in information, but starving for knowledge.

4. It is not because things are difficult that we do not dare. It is because we do not dare that they are difficult. (Seneca)

5. Because words are actions they make things happen. Once they are out you cannot put them back. (Hanif Kureishi)

6. Just like the plants if we loose our roots we cannot grow.

7. Dutch find a 'broodje kaas' always 'heel lekker en gezond' as long as there is no 'gratis' warm meal around.

Stellingen bij het proefschrift:
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Susana Maria Pinto de Carvalho
Wageningen, 28 May 2003.

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General Introduction

Year-round production of cut chrysanthemum

Chrysanthemum is an important greenhouse crop worldwide, both as cut flower and as pot plant (Machin, 1996). In The Netherlands, cut chrysanthemum is since 1969 the second most important cut flower, after the rose (Spaargaren, 2002). There are few cut flowers that have developed as rapidly as year-round chrysanthemum (Van der Hoeven, 1987). Breeding, research and new cultivation methods have contributed considerably to this fast development (Bakker *et al.*, 1990; Van der Hoeven, 1987). Growers can choose from a vast range of chrysanthemum cultivars differing in form, size, colour, reaction time, etc. (Trip *et al.*, 2000). Cultivars are commonly divided in three groups: 'spray', 'santini' and 'standard' type. The most important commercial flower group is the 'spray' type, where the axillary shoots are allowed to develop and only the terminal flower bud is removed to obtain a cluster of similar flowers. In contrast with the 'spray' cultivars, in the 'standard' type (monoflower) all the axillary shoots are removed to allow the terminal flower to develop only. The 'santini' cultivars are a special kind of the 'spray' type, which has more flowers of a smaller diameter (< 4 cm) (Spaargaren, 2002). Chrysanthemum cultivars are also classified according to their response group, i.e. number of weeks from beginning of short-day (SD) period till harvest (Kofranek, 1992).

Thanks to a strong productivity increase, improved energy utilisation and labour saving measures, the cost price per unit of product has hardly changed in the last years in The Netherlands, despite the steady increase in the total production costs per m^2 (Van der Hoeven, 1987; Spaargaren, 2002). From 1990 to 2000, glasshouse production of chrysanthemum increased from 165 to 207 plants $m^{-2}year^{-1}$ and the total production costs raised from € 38 to 46 $m^{-2}year^{-1}$. The build-up of the production costs was, however, roughly the same: 30 % materials, 20 % labour, 20 % capital, 15 % energy and 15 % others. In 2000, cut chrysanthemum production in Dutch glasshouses covered 774 ha, which represented 21 % of the total surface of cut flower cultivation, with a return of € 319 million (Spaargaren, 2002). In the same year, 83 % of the cut chrysanthemum cultivars sold at the Dutch auctions belonged to the 'spray' type group, followed by the 'santini' (12 %) and the 'standard' (5 %) cultivars. Five out of the top ten 'spray' cultivars were 'Reagan', which represented 50 % from the total share of this group. The main reasons for their dominance are related with the fast development (7-7.5 weeks, response group), high uniformity, strong stem, no brown leaves, diversity of colours and good keeping quality of these cultivars (Spaargaren, 2002).

Chrysanthemum is one of the oldest cultivated flowers (Salinger, 1985). Most of the current cultivars (*Chrysanthemum Indicum* group, syn. *Chrysanthemum morifolium* Ramat. and *Dendranthema* × *grandiflorum* (Ramat.) Kitam.) are complex hybrids of species that are native to China and Japan (Spaargaren, 2002). The genus *Chrysanthemum* belongs to the family *Asteraceae* (former *Compositae*) and its inflorescences are known as capitulum. The single inflorescences (daisy-like) are formed of ray florets (outside row) and disc florets (central fertile florets). These florets open from the periphery to the centre and are supported by a flat or convex receptacle, which is surrounded by an involucre of bracts (Salinger, 1985; Kofranek, 1992).

Chrysanthemum is a qualitative short-day (long-night) plant, which means that it will not produce flowers when day-length is above a critical value that is cultivar dependent (Furuta, 1954). Hence, natural flowering occurs in autumn in the Northern Hemisphere (Kofranek, 1992). Actually, in plants grown under long-day (LD) conditions after a certain number of leaves has been initiated (cultivar dependent), the terminal meristem becomes generative, but the flower bud aborts and vegetative growth continues with formation of side shoots (Cockshull and Kofranek, 1985). Under SD conditions, chrysanthemum has a determinate growth pattern (Pearson *et al.*, 1995), with a basipetal progression of flower initiation (Langton, 1992). Therefore, year-round production is only possible by an effective day-length control. In general, cut chrysanthemum cultivation period varies from 9 to 13 weeks, depending on the cultivar and on the season. In the first 10 to 25 days, depending on the season, plants are submitted to a LD period (Fig. 1A). This aims at promoting the vegetative development (extension growth). When plants have reached the desired height, a SD treatment is given till harvest, i.e. during 7 to 9 weeks depending on the cultivar's response group, to stimulate flower induction and development (Kofranek, 1992). The chrysanthemum crop can be programmed accurately using supplementary light to extend the natural photoperiod, to promote LD conditions in autumn and winter. Blackout screens are used during the SD treatment in spring and summer crops (Bakker *et al.*, 1990). As the production is continuous, this can allow up to five cultivations per year, in the same area (Salinger, 1985). Besides photoperiod, also temperature, light intensity, CO_2 concentration and relative humidity are commonly controlled in the glasshouse cultivation. For these reasons, chrysanthemum cultivation is considered one of the most intensive and controlled crop production systems in horticulture (Machin, 1996).

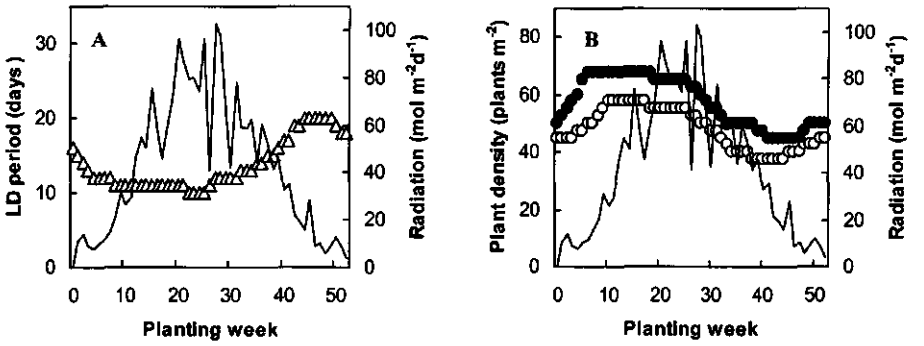

FIG. 1. Duration of the long-day (LD) period (A, △) and plant density (B, ○ 5 years old glasshouse without assimilation light, ● new glasshouse with assimilation light) as a function of the planting week in cut chrysanthemum production (After: Spaargaren, 2002; Roelofs, DLV consultancy group, personal comm.). Solid line repents outside global radiation for Dutch reference year (Breuer and Van de Braak, 1989). Based on assimilation light levels of 4000 lux (49 μmol $m^{-2}d^{-1}$) (A) and 6000 lux (73 μmol $m^{-2}d^{-1}$) (B).

External quality in cut chrysanthemum

Commercial flower production is an example of an agricultural sector where product differentiation has always been important (Trip *et al.*, 2000). Nevertheless, for many years the main line in floricultural research was quantity rather than quality (Vonk Noordegraaf, 1994). Nowadays, since the prices are often determined on the basis of the visual quality aspects, morphogenesis is receiving more attention mainly to improve these aspects (Vonk Noordegraaf, 1994; Vonk Noordegraaf and Welles, 1995). Furthermore, the majority of the chrysanthemum production in The Netherlands is sold at the auctions under the producer's name, which is an incentive for growers to outperform their competitors (Trip *et al.*, 2000).

In general, quality can be defined as to what extent the product meets the consumers' expectations. Some quality aspects are not visible (internal quality) and others are (external quality) (Vonk Noordegraaf and Welles, 1995). For instance, internal quality of cut chrysanthemum refers to its vase life (Nijsse, 2001), whereas external quality can be evaluated in terms of stem and leaf morphology and flower characteristics (Fig. 2). Because cut chrysanthemum is frequently divided into weight categories with a different selling price, plant fresh mass can be also considered as a quality aspect. The priority within these external quality aspects depends on the

market (Vonk Noordegraaf, 1994). Nowadays, growers have been commercially pressured to supply a constant product quality throughout the year (Langton *et al.*, 1999). Several growth conditions have an impact on the quality and price of the final product (Trip *et al.*, 2000). A strong seasonal variation in the outside daily light integral leads to a seasonal variation in the production and product quality. In Northern Europe, daily light integral can vary by a factor ten, when comparing different weeks of the year (Fig. 1). When integrating the complete chrysanthemum cultivation period, and taking into account the screening out of light during the SD period in spring and summer crops, still the seasonal light variation can be around a factor four to five (Fig. 1). To face this huge variation in the outside global light intensity, growers commonly adjust the duration of the LD period and plant density to the planting week, depending on the use of supplementary assimilation light and age of the greenhouse (e.g. Fig. 1). Nevertheless the impact of their choices on the external quality has not been quantified.

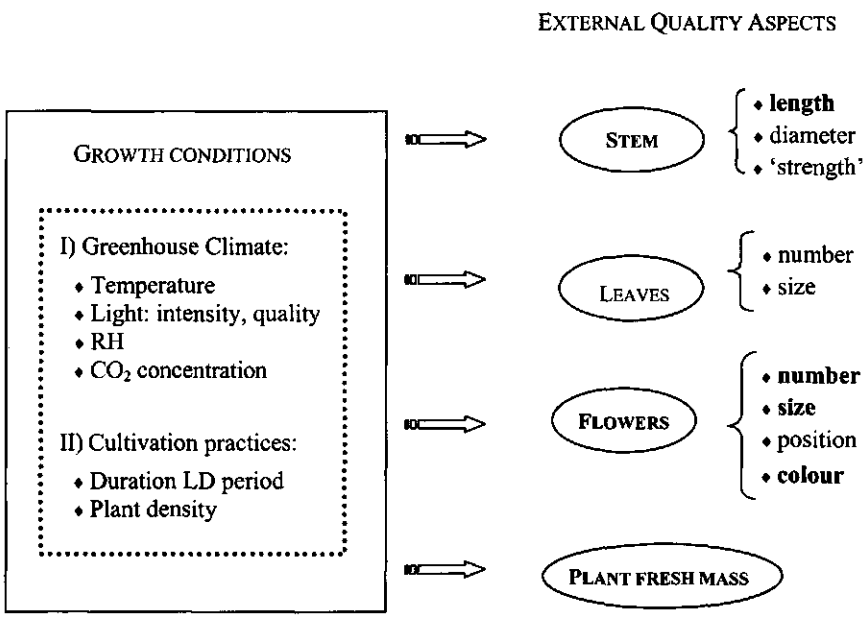

FIG. 2. Growth conditions affecting different external quality aspects of cut chrysanthemum. Leaf aspects refer to the ones on the main stem. Main external quality attributes presented in bold (Adapted from: Carvalho and Heuvelink, 2001). Abbreviations: LD = long-day; RH = relative humidity.

To produce year-round high quality chrysanthemum, it is necessary to know how the growth conditions influence plant quality, in order to choose the optimal strategy adapted to the growing season. Thus, the effects of the individual factors involved in chrysanthemum external quality should be analysed and combined in such a way that chrysanthemum achieves its maximum ornamental value and high uniformity year-round, whilst maintaining a low energy input and high productivity.

Crop growth models

When dealing with environmental regulation of plant morphogenesis the manipulation of a climatic factor may affect several processes at the same time. Furthermore, in greenhouse production most actuators (e.g. heating system, ventilators) have a simultaneous effect on a number of climatic factors (e.g. temperature, humidity and CO_2 concentration) (Challa, 1990). As a consequence experiments are often difficult to interpret and hence to generalise (Challa, 1997). For such complex systems, a common way to generalise knowledge and obtain quantitative information is to design a model, preferably an explanatory model (Challa, 1997). Two main categories of crop models can be distinguished: descriptive models, also called regression, statistical, empirical or 'black-box' models; and the explanatory models, also known as mechanistic or dynamic models (Challa, 1985; Marcelis *et al.*, 1998). The first type of models reflects little or none of the mechanisms behind the behaviour of the studied system, and often the parameters have no biological meaning (Marcelis *et al.*, 1998). Despite the advantage of the simplicity of the descriptive models, and their high predictive value under the conditions where they were developed, extrapolation to other species or locations is often impossible (Challa, 1985). Adding new input factors to such a model means building a new model based on an extended data set (Marcelis *et al.*, 1998). In contrast, the explanatory models enable the integration of knowledge at the level of the underlying processes that belong to the system (Challa, 1985; Marcelis *et al.*, 1998). Due to its modular character, an explanatory model can be divided into subunits ('modules'), each dealing with related groups of processes (Acock and Reynolds, 1989). This structure has the advantage of being easier to validate, by examining individual modules. It is also easier to transfer to other cultivars or crops, as only some modules may need to be adapted (Acock and Reynolds, 1989). However, subunits used to describe the behaviour of a process, mainly at the lowest hierarchical level, are often descriptive models (Challa, 1985;

Marcelis *et al.*, 1998).

Since chrysanthemum cut flower cultivation in greenhouses is intensively controlled, management of such a complex system would highly benefit from the use of simulation models. Furthermore, consumers' preferences may change rapidly over time (Trip *et al.*, 2000) and therefore a model can be an useful tool for decision support, to adapt the growth conditions to the new quality requirements. The introduction of climate computers, as well as the increased restrictions on the use of chemical growth retardants, have stimulated the interest in new greenhouse environmental control strategies, based on plant growth models (Karlsson *et al.*, 1983; Hendriks *et al.*, 1992). Although greenhouse culture offers far more potentials for application (Challa, 1985), most of the crop growth models currently available are for agricultural crops (Marcelis *et al.*, 1998). Recently, a photosynthesis-driven crop growth model for predicting yield in cut chrysanthemum has been developed (Lee, 2002).

Aim and outline of the thesis

This thesis aims at quantifying and understanding the effects of the aboveground growth conditions on the chrysanthemum external quality at harvest. The focus is, therefore, on the morphological responses. The ultimate goal of the study is to integrate this information into a model to predict the main external quality aspects of cut chrysanthemum. The model should, therefore, be able to predict the effects of temperature, light intensity, CO_2 concentration, duration of the LD period and plant density on plant height (stem length), number of flowers and flower size. Plant height is the same as stem length plus approximately the length of the uppermost flower peduncle, at harvest stage. Therefore, the first represents better the external quality of the plant.

Factors that influence external quality were limited to the main controls the grower has. Thus, the root environment is excluded from the studied factors as plants are grown in soil. Relative humidity was not studied, because when extreme levels are avoided plants grow well within a large interval (70 to 90 %) (Machin, 1996). Light quality was only varied via plant density, due to its effect on the phytochrome photoequilibrium (Heins and Wilkins, 1979).

Experiments were conducted always under glasshouse conditions, except when temperature needed to be strictly controlled and a constant light level was desirable to avoid possible interactions among these two factors. This was the case in Chapter 3. A

'Reagan' cultivar was chosen as a model cultivar, because of its above-referred importance. Besides 'Reagan Improved' two 'santini' type cultivars, 'Goldy' and 'Lupo', were included in this study because of their special flower characteristics (more flowers of a smaller size). In the present work the term 'flower' has been used throughout when referring to the complete inflorescence (after Cockshull and Hughes, 1972).

The general aim of this study is addressed in four main chapters. In **Chapter 2** a literature review is presented. This chapter gives an overview of the growth conditions, involved in the different external quality aspects, and identifies the gaps in literature. A synthesis of the available models, which have been built to predict some external quality attributes of chrysanthemum, is also presented.

The influence of temperature on stem length and flower characteristics is given in Chapter 3. **Chapter 3.1** aims at clarifying conflicting results in literature, concerning the effects of day and night temperature on internode and stem length. The validity of the DIF concept (difference between day and night temperature) is analysed. In contrast with the influence of temperature on stem length, only limited information is available for the flower characteristics. In **Chapter 3.2** an in depth study is presented on this topic. An attempt is made to separate the effects of temperature applied at different phases of the cultivation period on several flower characteristics.

Except for the temperature, all the other studied growth conditions (light intensity, CO_2 concentration, duration of the LD period and plant density) are known to primarily affect assimilate availability. The role of the assimilate availability on the flower characteristics and plant height is addressed in **Chapter 4.1**. In this chapter the results from a series of greenhouse experiments, performed in different seasons, are integrated. Functional relationships for predicting number of flowers and flower size are developed, to be further incorporated as 'modules' of an explanatory model. The influence of assimilate availability can also be tested by manipulating sink-source ratio, which is particularly interesting for studying flower size. This is done in **Chapter 4.2**, by removing flower buds, axillary shoots and varying light intensity. To understand flower size formation it is also important to consider the flower position within the stem. Therefore, the apical terminal flower, the apical lateral flowers (from first order axillary shoots) and the first flower located in a second order axillary shoot are compared in this chapter. In order to test how general the results are, both in Chapter 4.1 and 4.2 the analysis of the effects of assimilate availability on the flower characteristics is extended to the two 'santini' cultivars.

The data obtained in the previous chapters are further explored to model some external quality attributes (Chapter 5). In **Chapter 5.1** a process-based model is

developed, to describe internode elongation as a function of temperature. In **Chapter 5.2** a case study is presented on the interactive effects of duration of the LD period and plant density on external quality. Here, a photosynthesis-driven crop growth model for cut chrysanthemum (Lee *et al.*, 2002a) is validated and further used to simulate total dry mass on different growing seasons, using different combinations of these two factors. The possible trade-off between them is analysed, while aiming at certain quality attributes. The 'modules' developed in Chapter 4.1, concerning both flower number and size, are validated using an independent data set.

The main achievements, limitations and practical applications of this study are discussed in **Chapter 6**. Suggestions for future research are presented.

Effects of growth conditions on external quality aspects

Stem morphology

Chrysanthemum has to meet strict quality specifications for height, which makes stem elongation control of utmost importance (Karlsson and Heins, 1994; Langton, 1998). Total stem length results from the number of internodes (equal to leaf number) times the average of the internode length (Pearson *et al.*, 1995). In chrysanthemum, new internodes are formed until flower initiation starts. After that stage, stem length increase depends on internode elongation only. Chemical growth retardants are frequently used to achieve more compact and better-shaped plants. However, based on environmental considerations and also from the viewpoint of cost savings, there has recently been an effort to minimise their use (Myster and Moe, 1995; Langton, 1998). Therefore, it is a priority to find effective environmental friendly alternatives of regulating plant height (Bertram and Karlsen, 1995; Erwin and Heins, 1995; Pearson *et al.*, 1995; Khattak and Pearson, 1997). Efforts have been concentrated on temperature control, which could result in well-shaped plants without affecting time to flowering (Myster and Moe, 1995). Besides temperature control, other techniques are also presented in this chapter.

Temperature: Control of stem elongation by temperature manipulation is widely used in practice for chrysanthemum (Langton and Cockshull, 1997b; Langton, 1998). It is generally accepted that higher average day temperature (ADT) increases chrysanthemum stem elongation rate (SER) resulting in taller plants. This stem length increase is due to both higher internode number and longer internodes. Below the optimum temperature for flowering, the positive effect of increased ADT on node initiation rate is, however, counteracted by a reduced node initiation period, as a consequence of earlier flower initiation (Cockshull *et al.*, 1995; Pearson *et al.*, 1995). Therefore, only a small increase in the stem length is observed. Above the optimum temperature for flowering, an increase in temperature delays flower initiation and development (see flower characteristics section), which allows, respectively, more and longer internodes to be formed (Cockshull *et al.*, 1995), resulting in a stronger increase of final stem length.

The sensitivity of chrysanthemum stem elongation to temperature is not the same during 24 h, which can be used to control stem length. To obtain a maximum effect on elongation control a shift in temperature should be given when the sensitivity of the stem elongation to temperature is highest (Erwin and Heins, 1995; Hansen *et al.*,

1996). In chrysanthemum, a distinct response of stem elongation to day temperature (DT) and night temperature (NT) is observed (Mortensen and Moe, 1987; Pearson *et al.*, 1995; Langton and Cockshull, 1997a, 1997b). Changes in DT have a much larger effect on the internode length than the corresponding changes in NT (Fig. 1). Thus, the effect of average 24 h temperature mainly results from DT, the main environmental factor controlling height in chrysanthemum (Cockshull *et al.*, 1981; LePage *et al.*, 1984). Moreover, the beginning of the day is considered a moment with especially high sensitivity of elongation rate to temperature (Langton, 1998). This is likely to be triggered by a 'light-on' signal, as the light perception is assumed to be an important component of the sensitivity response (Langton, 1998).

In commercial practice, reduced stem elongation is obtained by higher NT than DT (negative DIF, DIFference between DT and NT; e.g. Moe and Heins, 1990; Jacobsen and Amsen, 1992; Bertram and Karlsen, 1994; Cockshull *et al.*, 1995) at the same average temperature. A strong positive correlation between internode length and DIF has been observed for a wide range of species (e.g. Heuvelink, 1989; Erwin and Heins, 1995; Myster and Moe, 1995). However, Langton and Cockshull (1997a) have clearly shown that internode length does not respond directly to DIF, but rather results from the higher sensitivity of elongation to DT than NT, as mentioned above.

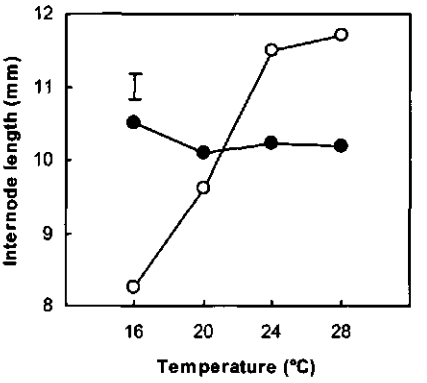

FIG. 1. Effects of DT (○) and NT (●) on the average length of chrysanthemum internode (≤ 1 mm at the start) after 10 days of growth. A total of 16 temperature combinations was studied. DT values were calculated by averaging over NT, and NT values by averaging over DT. Vertical bar represents s.e.d. for comparison of DT means and NT means (0.36 mm). (Reprinted from *Scientia Horticulturae*, **69**, Langton and Cockshull, page 234, copyright (1997), with permission from Elsevier Science).

These conflicting results are investigated in Chapter 3.1. The shift in temperature of a standard negative DIF treatment is commonly applied at daybreak (e.g. Bertram, 1992; Cockshull *et al.*, 1995; Langton and Cockshull, 1997a). However, it can also be done 2 h before sunrise, without reducing the effectiveness of negative DIF in decreasing internode length of chrysanthemum (Jacobsen and Amsen, 1992). This might be explained by the fact that in such a treatment the first hours of the day still have a low temperature.

A short-term temperature reduction with only a brief duration of temperature drop (DROP treatment; e.g. Bertram 1992; Sach, 1995; Cockshull *et al.*, 1995; Hansen *et al.*, 1996), usually during the early part of the light period, is also applied to obtain shorter stems in chrysanthemum (Table 1).The effectiveness of DROP treatments in reducing stem length is related with its magnitude, duration and timing (Table 1). In general it increases consistently with the magnitude (2, 4 and 8 °C DROP) or duration (3 and 6 h) (Cockshull *et al.*, 1995). There is also a strong influence of the moment that a shift in temperature occurs (Bertram, 1992; Cockshull *et al.*, 1995). In agreement with the above mentioned strong temperature sensitivity of stem elongation in the early morning hours, Bertram (1992) found that 8 °C temperature DROP for 2 h before sunrise did not affect chrysanthemum stem elongation. However, Cockshull *et al.* (1995) observed a stronger effect of 3 h drop treatment when applied 3 h after sunrise compared to directly following sunrise.

TABLE 1. Effectiveness of different DROP strategies (magnitude, duration and timing) in reducing internode length.

Magnitude (°C)	Duration (h)	Timing	Photoperiod	Effectiveness	Authors
8	6	from sunrise	SD	+	Sach (1995)
2, 4 or 8	3 or 6	from sunrise and 3h after sunrise	SD	+	Cockshull *et al.* (1995)
12	2	from sunrise	SD	+	Hansen *et al.* (1996)
12	2	different times of the day	LD	_	Hansen *et al.* (1996)
8	2	before sunrise	SD	_	Bertram (1992)

Many authors report that the efficiency of DROP treatment, in reducing stem elongation, is often lower than the DIF treatment (Bertram, 1992; Cuijpers and Vogelezang, 1992; Hendriks and Ueber, 1995). The use of different chrysanthemum cultivars (Hansen *et al.*, 1996) and an interaction between growth factors (Myster and Moe, 1995) are also possible reasons for differences in the effect of the temperature treatments. For instance, there is a higher sensitivity of length growth to temperature during the SD period, compared with LD (Myster and Moe, 1995). Several authors have shown this photoperiod influence on the efficiency of negative DIF (Cuijpers and Vogelezang, 1992) and DROP treatment (Vogelezang *et al.*, 1992; Hansen *et al.*, 1996) in reducing internode length (Table 1). However, the effect of both DIF and DROP treatments on shoot length is not persistent after transfer to a common environment. Hence, these treatments need to be continued for the most of the cultivation period of a chrysanthemum crop, to ensure that height reductions are visible at the marketing stage (Cockshull *et al.*, 1995).

Much attention has been given to the effects of temperature treatments on stem elongation of chrysanthemum but the physiological principles which underlie the effect of temperature regime on extension growth are not fully understood (Langton, 1998). Analyses of the kinetics of stem elongation, under different temperature regimes (Bertram and Karlsen, 1994; Tutty *et al.*, 1994), show that the suppressing effects of negative DIF and DROP regimes on chrysanthemum elongation are not satisfactorily explained by resultant SER patterns (Tutty *et al.*, 1994). Strong evidence suggests the involvement of gibberellin (GA) metabolism in the regulation of stem extension by temperature (Langton, 1998). Nevertheless, other factors such as phytochrome photoequilibrium appear to interact with DIF to affect stem elongation (Erwin and Heins, 1995).

Light: A number of studies have examined whether the manipulation of light intensity or spectral quality has potential for controlling chrysanthemum stem elongation. Higher irradiance during the day led to higher SER during the following night, which resulted in longer stems (Bertram and Karlsen, 1994). Therefore, the use of supplementary light during daytime significantly increases both stem length (Hughes and Cockshull, 1972; Parups, 1978; Eng *et al.*, 1985) and stem diameter (Eng *et al.*, 1985), but only at low natural irradiance levels. These findings suggest that higher levels of photoassimilates are used to sustain stem growth (Bertram and Karlsen, 1994). Moreover, the light intensity effect on promoting stem growth can also be partly linked to a consequently higher plant temperature.

Plant height is also regulated by the action of phytochrome and blue light level.

Increasing either level of blue light (Khattak and Pearson, 1997; Oyaert *et al.*, 1999) or level of phytochrome photoequilibrium (ϕ = Pfr/Ptotal ratio: ratio of Pfr to total phytochrome) decreases chrysanthemum height (Heins and Wilkins, 1979). Thus, the use of red light (R-light) inhibits elongation and far-red light (Fr-light) increases elongation through an induction response of phytochrome.

Many attempts have been made to remove the elongation stimulus of Fr-light from the natural spectrum, using different selective screening materials (Hendriks and Ueber, 1995). The 'fluid roof system' filter, with copper sulphate solutions ($CuSO_4$ filters: high ϕ and high blue light transmission), was very efficient in reducing plant height in trials with chrysanthemum (e.g. Mortensen and Strømme, 1987; McMahon *et al.*, 1991; Rajapakse and Kelly, 1992; McMahon and Kelly, 1999). Chrysanthemum grown under $CuSO_4$ filters presented a lower SER (Rajapakse and Kelly, 1992) resulting in shorter internodes (Rajapakse and Kelly, 1992; McMahon and Kelly, 1999). The effectiveness of these filters depends on the cultivar (Rajapakse *et al.*, 1993) and on the growing season (larger effect in spring and autumn compared with summer) (Rajapakse and Kelly, 1995). The effect of $CuSO_4$ filters can be explained by the increase in the R/Fr ratio and blue/red (B/R) ratio of the transmitted light (Rajapakse and Kelly, 1992) but also by the strong reduction in the light intensity (McMahon *et al.*, 1991; Hendriks and Ueber, 1995). $CuSO_4$ filters are not being used in practice (Hendriks and Ueber, 1995; Murakami *et al.*, 1997) because a 'fluid roof system' is difficult to apply on a greenhouse scale (Hendriks and Ueber, 1995; Oyaert *et al.*, 1999) and also due to the negative consequences for the total plant dry mass (TDM) (Mortensen and Strømme, 1987).

To overcome the problems of $CuSO_4$ filters the use of several selective plastic filters has been studied. Oyaert *et al.* (1999) evaluated the effect of different blue polyethylene (PE) films (low ϕ and high blue light transmission) on growth reduction of chrysanthemum plants, using different pigment concentrations (1, 2 and 3 % of blue pigment). These films were effective in reducing the growth of different chrysanthemum cultivars, at different seasons, under both natural and artificial assimilation light. The inhibition of stem elongation increased with increasing pigment concentration, with a maximum of 22 % growth reduction. However, blue filters also decreased total plant TDM (Oyaert *et al.*, 1999) due to lower light intensity of the transmitted light. The vaporised mica film is an alternative filter characterised by a higher light transmission capacity and higher R/Fr ratio but a lower B/R ratio, compared to blue PE films (Oyaert *et al.*, 1999). Therefore, despite the rather small reduction in chrysanthemum stem elongation (9 %), Oyaert *et al.* (1999) suggested that the vaporising technique should be improved as this film has the advantage of

reducing stem elongation without decreasing other plant quality aspects (no significant effect on branching rate, leaf area and plant TDM).

Photoperiod: Shorter LD period is also a frequently used method to achieve compact chrysanthemum plants (Hendriks and Ueber, 1995; Heuvelink *et al.*, 1998; McMahon and Kelly, 1999). Chrysanthemum is a short-day (long night) plant (Andersson, 1990; McMahon and Kelly, 1999) hence LD conditions stimulate vegetative growth. Thus, it was observed that reducing LD period decreases stem length and stem diameter (Heuvelink *et al.*, 1998). The stem length reduction is mainly a result of fewer internodes, due to earlier flower initiation (Heuvelink *et al.*, 1998), but also shorter internodes (Cathey, 1974).

When photoperiod extension is done during the natural SD period it was observed that the effect on stem elongation depends on the timing that chrysanthemum is exposed to light (night break lighting vs. morning or evening day extension). Given a night interruption of 4h, chrysanthemum showed a typical LD response (Cathey, 1974) resulting in longer internodes (Cathey, 1974) and taller plants (Cathey, 1974; McMahon and Kelly, 1999), than those lighted for the same duration but extending the photoperiod (Cathey, 1974). Moreover, internode length was considerably longer when chrysanthemum was exposed to light during the last 4 h of the dark period, rather than during the first 4 h (Cathey, 1974).

Relative humidity and CO_2 concentration: Chrysanthemum shoot length was positively correlated with relative humidity (RH), at constant temperature (Mortensen, 1986b; Gislerød and Nelson, 1997), which means a positive effect of absolute humidity. However, this effect was only significant after four weeks with high RH (95 % compared to 55 %) and no influence on stem TDM was observed (Gislerød and Nelson, 1997). Therefore, high RH levels have a negative influence on stem 'strength' (g cm^{-1}) of chrysanthemum.

CO_2 enrichment promotes both stem and shoot elongation (e.g. Lindstrøm, 1968; Mortensen and Moe, 1983; Eng *et al.*, 1985; Mortensen, 1986a) and stem diameter (Eng *et al.*, 1985; Mortensen, 1986a). Increasing CO_2 concentration (300-900 μmol mol^{-1}) favoured stem mass over elongation, resulting in higher stem 'strength' (Heij and De Lint, 1987). However, above a certain CO_2 concentration (saturation level around 1000 μmol mol^{-1}) there are no further significant effects on chrysanthemum growth (Mortensen and Moe, 1983).

Plant density: Besides climatic factors, plant density is also important in chrysanthemum quality (Huld and Andersson, 1997) as it is strongly related with the microclimate within the crop (Heins and Wilkins, 1979), namely with the light intensity and light quality. Chrysanthemum growing in a high plant density forms a leaf canopy which reduces light intensity and filters much of the R-light, while allowing larger amounts of Fr-light to pass through (Heins and Wilkins, 1979). These reductions in ϕ stimulate primary stem elongation and inhibit lateral branching (Heins and Wilkins, 1979) resulting in a lower stem 'strength' (Heij and De Lint, 1987).

In general, high plant density results in longer plants due to the 'etiolation effect' (competition for light), caused by low light intensity and low ϕ. However, increasing plant density to very high values (e.g. 83-125 plants m^{-2}) strongly reduced plant height (Huld and Andersson, 1997), especially during autumn and winter seasons (Langton *et al.*, 1999). These results can be explained by the low light penetration levels that combined with high amount of shaded leaves (acting as sinks) reduces chrysanthemum growth rate. Thus, the low assimilate availability can be seen as a factor limiting stem elongation at very high plant densities, which is consistent with the above discussed positive effect of supplementary light on stem length under low natural irradiance levels.

Leaf morphology

Temperature: It is generally recognised that increasing ADT in chrysanthemum results in higher rate of leaf appearance (or leaf unfolding rate: LUR) (Cockshull, 1979; De Lint and Heij, 1987; Karlsson *et al.*, 1989c; Heuvelink *et al.*, 1998). However, chrysanthemum has a determinate growth pattern (Pearson *et al.*, 1995), so the leaf initiation is stopped when flower induction occurs (De Lint and Heij, 1987). Thus, leaf number is not only dependent on LUR but also on the time to flower initiation. Therefore, below the optimum temperature for chrysanthemum flowering the final leaf number increases slowly with temperature (Cockshull, 1979; Pearson *et al.*, 1995), as the LUR increase is partly neutralised by the reduction in the time to flower initiation. Above that optimum value an increase in temperature results in a more rapid increase in the final leaf number (Cockshull, 1979; Pearson *et al.*, 1995), because of increased LUR and a strong delay in the flower initiation which allows more leaves to be formed, especially in temperature sensitive cultivars.

Neither DIF nor DROP temperature treatments, for controlling stem elongation (see stem morphology section), affected chrysanthemum leaf number (Cockshull *et al.*, 1995) or LA (Cuijpers and Vogelezang, 1992).

Light: Despite the positive effect of high photosynthetic photon flux (PPF) on LUR, chrysanthemum grown under these light conditions presents a lower final leaf number (Vince, 1960; Cockshull, 1979; Karlsson *et al.*, 1989c; Andersson, 1990), as flower initiation starts earlier (Cockshull, 1979). In contrast, Mortensen and Moe (1983) found that an increase in light level (44-395 $\mu mol\ m^{-2}\ s^{-1}$) increases leaf number. These results can be explained by the fact that the latter observations were done during the LD period and, therefore, leaf number is dependent only on LUR. At high irradiance intensity the leaf TDM also increases significantly (Gislerød and Nelson, 1997), as a consequence of higher assimilate availability, resulting in thicker leaves (Hughes and Cockshull, 1972).

In terms of light quality, blue light showed a significant effect only in reducing total LA at low photosynthetic active radiation levels (PAR = 8.0 or 12.6 mol m^{-2} day^{-1}) (Mortensen and Strømme, 1987). Similarly, chrysanthemum grown under blue PE filters (Oyaert *et al.*, 1999) or $CuSO_4$ filters (Rajapakse and Kelly, 1992), i.e. filters that reduce PAR intensity of the transmitted light, had a smaller LA (Oyaert *et al.*, 1999). This LA reduction is mainly attributed to smaller leaves and to a lesser extent to lower leaf number. Furthermore, both blue PE filters (Oyaert *et al.*, 1999) and $CuSO_4$ filters (Rajapakse and Kelly, 1992, 1995) induce a TDM translocation from stem to leaves and a higher specific leaf mass (SLM) (Rajapakse and Kelly, 1992). Khattak and Pearson (1997) suggested that low ϕ could also result in a reduced LA.

Relative humidity and CO_2 concentration: Chrysanthemum grown at high air humidity (Gislerød and Nelson, 1989; Bakker *et al.*, 1990; Gislerød and Nelson, 1997) and high CO_2 concentration (Gislerød and Nelson, 1989) tend to have larger LA per plant, on main and lateral stems. This is mainly the result of an increased area of the individual leaves (Gislerød and Nelson, 1997) and marginally due to higher number of leaves (Mortensen and Moe, 1983; Mortensen, 1986a; Heij and De Lint, 1987).

Increasing RH (from 55 % to 90 %) reduced chrysanthemum leaf dry mass content but leaf TDM was not affected (Gislerød and Nelson, 1997). Chrysanthemum leaf thickness increases with CO_2 concentration (Hughes and Cockshull, 1972).

Plant density: Once more, probably due to lower assimilate availability, an increase in chrysanthemum plant density decreases total LA per plant by reducing both leaf size (Huld and Andersson, 1997) and leaf number (Heij and De Lint, 1987; Huld and Andersson, 1997). Furthermore, the leaf thickness is also reduced under higher plant density (Huld and Andersson, 1997).

Flower characteristics

Temperature: Many studies in chrysanthemum have focused on the relationship between temperature and time to flowering. Time to flowering is often divided into time to flower initiation and time for subsequent flower development (Adams *et al.*, 1998). In chrysanthemum this is largely controlled by ADT (e.g. Cockshull *et al.*, 1986; Khattak and Pearson, 1997; Adams *et al.*, 1998; Larsen and Persson, 1999) and presents an optimum response to temperature (Fig. 2A). This optimum range of temperature, corresponding to the maximal rate of progress to flower (Adams *et al.*, 1998), is cultivar dependent (De Lint and Heij, 1987; Langton, 1987; Whealy *et al.*, 1987; Larsen and Persson, 1999) and may differ from the optimum for vegetative growth (Hughes and Cockshull, 1972).

A reduction in the uniformity of time to flowering within the plants was observed at non-optimum temperatures (Karlsson and Heins, 1986). In practice, such plant variability makes the choice for the right harvest moment quite difficult. An increase in PPF can partly compensate the flowering delay promoted by a certain range of high or low temperatures (Karlsson and Heins, 1986; Karlsson *et al.*, 1989b; Hidén and Larsen, 1994) (Fig. 2A). The extension of flowering delay also depends on the cultivar (Vince, 1960; Parups and Butler, 1982; Willits and Bailey, 2000) and on the timing and duration of the exposure to unfavorable temperature conditions (Carow and Zimmer, 1977; Whealy *et al.*, 1987). Vince (1960) reported that flowering of several chrysanthemum cultivars was delayed only when low NT was applied in the early part of SD period (before the bud had become visible macroscopically). A stronger flowering delay was observed when low temperature occurred during the last hours of the night period (10 °C for 5 h), compared with other periods of the night (Carow and Zimmer, 1977). Thus, according to Parups (1978) a split NT regime (low temperature for only a part of the night, during the SD period) could be adopted for some cultivars, to reduce energy costs, without a noticeable flower delay.

The temperature effect on chrysanthemum flower characteristics (size, mass and number) is strongly cultivar dependent. For example, in some cultivars a relative low NT had a positive effect on flower quality resulting in a larger diameter (Vince, 1960; Bonaminio and Larson, 1980), higher flower area per plant (Karlsson and Heins, 1986) and heavier flowers (Bonaminio and Larson, 1980). This flower TDM gain can be explained by a flowering delay, promoted by low NT, which results in a later harvest of the plants leading to an increase of the assimilate availability. In other cultivars, probably less temperature sensitive, size and/or number of flowers was not significantly affected by low NT (Parups, 1978) or negative DIF (Roeber *et al.*, 1995).

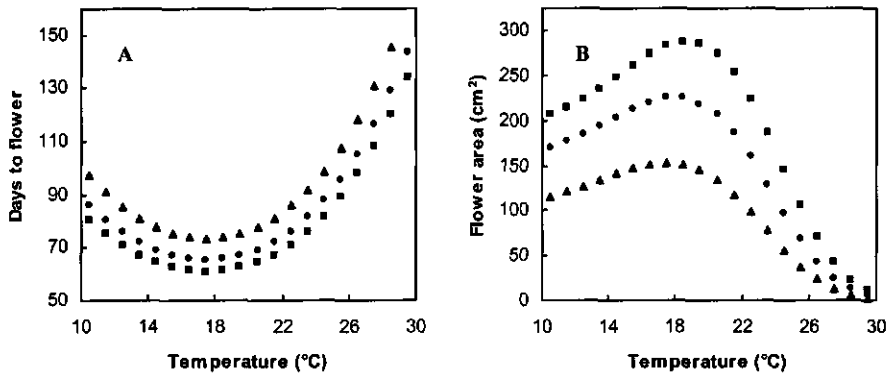

FIG. 2. Predicted number of days from start of SD to flowering (A) and total plant flower area (B) as influenced by a simultaneous increase in day and night temperature at PPF of 5 (▲), 10 (●) and 15 (■) mol day^{-1} m^{-2} in *Chrysanthemum* 'Bright Golden Anne'. The graph was created using a regression model. (Reprinted from *Scientia Horticulturae*, **39**, Karlsson *et al.*, 1989b, pages 262 and 264, copyright with permission from Elsevier Science).

Karlsson *et al.* (1989b) found that flower area in chrysanthemum presents an optimum response to temperature, as it increases to a maximum and falls down rapidly at higher temperatures (Fig. 2B). Furthermore, it was shown that NT influences final flower area to a larger extent than DT (Karlsson and Heins, 1986).

It has been reported that under high temperatures the percentage of lateral buds that fails to elongate increases (Faust and Heins, 1992; Schoellhorn *et al.*, 1996). Faust and Heins (1992), observed that at a DT of 35 °C, NT between 14 and 27 °C did not influence the percentage of axillary buds that developed lateral shoots in chrysanthemum stock plants. However, since they only tested at one extreme DT (35 °C), it would be unwise to conclude that NT does not play a role in lateral branching of chrysanthemum.

Light: In chrysanthemum, there is a clear relationship between the average daily light integral and both time to flower initiation and time for flower development (e.g. Hughes and Cockshull, 1971; Langton, 1987; Andersson, 1990). Thus, during the SD conditions an increase in the daily light integral reduces time to flowering (e.g. Cockshull and Hughes, 1972; Cockshull, 1979; Langton, 1987). This can be achieved, for instance, by increasing either intensity or duration of supplementary irradiance (Andersson, 1990) but still under SD conditions. Nevertheless, the absolute response

per integral increase declines as irradiance increases (Karlsson and Heins, 1986), following a light saturation process (Langton, 1987; Hidén and Larsen, 1994). Therefore, the effect of supplemental irradiation on time to flower is larger under low natural irradiance levels, compared with high levels (Cockshull and Hughes, 1972).

Light intensity also has a positive effect on chrysanthemum flower quality. In general, larger amount of assimilates accelerate axillary bud development and result in a higher number of shoots of greater size and mass (De Ruiter and Tromp, 1996; De Ruiter, 1997). Therefore, higher light intensity levels (natural or artificial light) enable more lateral buds to elongate (Acock *et al.*, 1979; Hicklenton, 1985; Holcomb *et al.*, 1988) and significantly promote flower number per plant (Parups, 1978; Eng *et al.*, 1985; Andersson, 1990). Furthermore, it was observed that the positive effect of PPF on the number of lateral branches is cultivar dependent (Schoellhorn *et al.*, 1996).

Considering flower size, a linear increase with PPF (from 1.8 to 21.6 mol day^{-1} m^{-2}) was observed, at a constant temperature of 20 °C (Karlsson *et al.*, 1989c). Larger flower buds TDM (Andersson, 1990), higher total flower area per plant (Fig. 2B) and greater uniformity of flowering were also present in chrysanthemum growing under higher light intensities (Karlsson and Heins, 1986; Karlsson *et al.*, 1989b).

Light quality also influences several flower characteristics of chrysanthemum. A decrease in ϕ (e.g. using colour filters with low R/Fr light transmission) or lower blue light transmission of the filter (e.g. yellow material) results in a flowering delay (Khattak and Pearson, 1997). However, the light transmitted through a $CuSO_4$ filter (high ϕ and high blue light transmission) also results in a flowering delay, by 1-2 weeks depending on the growing season (Rajapakse and Kelly, 1995). The explanation for this flowering delay could be the strong reduction of the light intensity promoted by the $CuSO_4$ filters.

The lateral branching in chrysanthemum is also influenced by light quality (Smith, 1982). Similarly, light with low R/Fr ratio (low ϕ) or low blue light transmission reduces both lateral branching (Heins and Wilkins, 1979; Mortensen and Strømme, 1987) and flower FM (Khattak and Pearson, 1997). It seems that ϕ has a stronger effect than blue light transmission level. Therefore, chrysanthemum grown under blue PE filters (low ϕ but high blue light transmission) had 33 % less FM, compared with control (Khattak and Pearson, 1997) and a lower number of axillary shoots was developed (Oyaert *et al.*, 1999).

As expected, the use of $CuSO_4$ filters results in a lower flower FM and smaller flowers (Rajapakse and Kelly, 1995). Nevertheless, $CuSO_4$ filters did not affect the number of flowers per plant (Rajapakse and Kelly, 1995; McMahon and Kelly, 1999).

Photoperiod: It is well known that daylength influences the flowering process of chrysanthemum (Langton and Cockshull, 1976; Hendriks and Ueber, 1995). As a short-day plant the natural generative phase takes place during the autumn (Kofranek, 1992). The critical day-length is cultivar dependent (Furuta, 1954) and it is shorter for flower development than for flower induction (McMahon and Kelly, 1999). Under photoperiods, longer than the critical one, chrysanthemum may also become generative (Furuta, 1954). However, flowering is delayed (McMahon and Kelly, 1999), not uniform and often results in abnormal or aborted buds (Furuta, 1954).

It has been observed that the use of different sources of light during the night period, for a sufficient duration of time, inhibits chrysanthemum flowering (Cathey, 1974; Heins and Wilkins, 1979; McMahon and Kelly, 1999). This effect of photoperiod extension depends on the timing that the plants are exposed to light and on the light quality. Therefore, a night break with incandescent light, for the same photoperiod, results in a significantly stronger flower delay than lighting in the last 4 hours of the night (Cathey, 1974). Considering the effect of the light quality, used to simulate LD conditions, Fr-light was the only light quality treatment that delayed chrysanthemum flowering, when applied in the last 4 h of the night (Cathey, 1974). This light treatment was also efficient in chrysanthemum when applied in the end of the day (McMahon and Kelly, 1999).

To improve winter-flowering chrysanthemum quality, a manipulation of photoperiod can be used by providing a LD period into the SD treatment after flower induction has taken place (Langton, 1987; Vonk Noordegraaf and Welles, 1995). The main aim is to elongate the peduncles, giving a more attractive presentation to the flowers (Langton, 1987). It has been suggested that this technique can also increase number and size of chrysanthemum flowers (Langton, 1987). Nevertheless, it delays the final harvest (Kofranek and Cockshull, 1985) and the starting time is critical for an effective treatment (Langton, 1987). An extended LD treatment (4 weeks, compared with 2 weeks) before starting SD treatment did not influence the number of flowers per plant (Heuvelink *et al.*, 1998).

Relative humidity and CO_2 concentration: There is little information about the influence of RH on the flowering process of chrysanthemum. It was found that higher RH increased both number of lateral shoots (Hicklenton, 1985; Gislerød and Nelson, 1989) and number of flower buds (Gislerød and Nelson, 1989). Hicklenton (1985) suggested that the increase of the lateral shoot number results from lower transpiration losses and higher water potential of the lateral buds.

Most likely as a result of improved assimilate status of chrysanthemum plants, flower quality responds positively to CO_2 enrichment. Increasing CO_2 level up to a certain concentration (saturation process as reported above) (Eng *et al.*, 1985) results in more lateral shoots (Hicklenton, 1985; Mortensen, 1986a; Gislerød and Nelson, 1989), more flowers per plant (Mortensen, 1986a; Gislerød and Nelson, 1989) and increases flower size (Potter, 1980). Nevertheless, the CO_2 level does not affect time to flowering in chrysanthemum (Potter, 1980; Mortensen, 1986a). This was previously reported by Vince (1960) but only for CO_2 enrichment during the LD period.

Plant density: Although the direct effect of plant density on the flower characteristics of chrysanthemum is not well described, it is well known that plant density is closely related to the intensity and quality of the light that passes through the canopy, as previously explained for stem morphology. Thus, an increase in plant density slightly delays time to flowering and reduces its uniformity within the plants. In autumn and winter, when light is limiting, this density response was stronger (Langton *et al.*, 1999). Furthermore, by increasing plant density (from 32 to 64 plants m^{-2}) a large reduction (48 %) of the flower number per plant was observed (Heuvelink *et al.*, 1998). This is mainly a result of a lower assimilate availability, less light interception per plant at high densities, and also partly due to lower ϕ of the light.

Existing quality models

Crop modelling has become an important research tool in horticulture (Gary *et al.*, 1998; Lentz, 1998; Marcelis *et al.*, 1998). In the past 10 years, models predicting chrysanthemum responses to different environmental conditions have been developed. However, very few models are actually being used in commercial production (Larsen and Persson, 1999). A model to be accepted by the growers has to be easy to use (Lentz, 1998) and flexible to adapt to new cultivars (Larsen and Persson, 1999).

The majority of the models for ornamental plants are focused on growth but some of these models also describe chrysanthemum quality aspects. For instance, stem elongation has been often predicted using mainly very simple descriptive models that estimate plant height or internode length (e.g. Karlsson *et al.*, 1989c; Khattak and Pearson, 1997; Langton and Cockshull, 1997b). Pearson *et al.* (1995) also developed a

model to predict the internode length but they went a step further by simulating the effect of a wide range of temperature conditions on the rate of internode extension. The side shoot elongation was also modelled in chrysanthemum, simulating the effect of daily light integral and temperature (Larsen and Gertsson, 1992; Karlsson and Heins, 1994).

Final leaf number can be used to obtain the number of internodes (same as leaf number) and results from the multiplication of average LUR and time to flowering initiation. The available models to simulate final leaf number are based on the influence of the ADT (Karlsson *et al.*, 1989c) or ADT and daily light integral (Larsen and Hidén, 1995) on LUR.

Much attention has been given to modelling time to flowering, based on temperature and light effects (e.g. Karlsson and Heins, 1985; Karlsson *et al.*, 1989b; Pearson *et al.*, 1993; Khattak and Pearson, 1997) and to a lesser extent on plant density effect (Langton *et al.*, 1999). Recently, Larsen and Persson (1999) improved a previous model that simulated the flower development in chrysanthemum in response to ADT and daily light integral (Hidén and Larsen, 1994). Thus, they included the cultivar response group as a component of the model, resulting in a more flexible model that fitted better the data and could be easily adapted to different cultivars.

Conclusions

Every external quality character in chrysanthemum is influenced by the growth conditions. In general, a good carbohydrate status is a basic condition to guarantee high external quality of chrysanthemum, as it reduces the competition between sinks. For instance, chrysanthemum growing under higher light intensity, higher CO_2 concentration or lower plant density results in taller plants, with greater stem diameter and more lateral branches. Leaf thickness is also increased as well as flower number and size. However, time to flowering in chrysanthemum does not respond to CO_2 enrichment (Potter, 1980; Mortensen, 1986a) but decreases with higher daily light integral (Cockshull, 1979; Langton, 1987; Andersson, 1990). Temperature also plays an important role in chrysanthemum external quality. The optimum temperature is cultivar dependent (Whealy *et al.*, 1987; Larsen and Persson, 1999) and differs according to the stage of development (Hughes and Cockshull, 1972). As a general rule extreme values should be avoided to enhance good quality plants and to prevent flowering delay.

Despite the extensive literature about different aspects of chrysanthemum growth

and development, several important quality aspects are still absent in the models. For example, stem 'strength', leaf size, flower number and flower size have never been estimated even by a descriptive model. Moreover, modelling morphogenesis should also focus on the spatial arrangement of the organ units (flower position on the stem) and not only on its number and dimension (Gary *et al.*, 1998). At present, the influence of the growth conditions on the flower position in chrysanthemum is not well described.

3

Effect of temperature

3.1

Internode and stem length: is everything explained by DIF?

Abstract

In many plant species, including chrysanthemum, a strong positive correlation between internode length and DIF (difference between day (DT) and night (NT) temperature) has been observed. However, Langton and Cockshull (1997, *Scientia Horticulturae* **69**: 229-237) reported no such relationship and showed that absolute DT and NT explained internode length rather than DIF. To investigate these conflicting results and to clarify the validity of the DIF concept, cut chrysanthemums (*Chrysanthemum* 'Reagan Improved') were grown in growth chambers at all 16 combinations of four DT and four NT (16, 20, 24 and 28 °C) with a 12 h daylength. Length of internode 10, number of internodes and stem length were measured on day 5, 10, 17, 22 and 27 after starting the temperature treatments. Internode length on day 10 showed a positive linear relationship with DIF (R^2 = 0.64). However, when internodes had reached their final length in all treatments (day 27), a much stronger positive linear relation was observed (R^2 = 0.81). A model to predict final internode length was developed based on the absolute DT and NT responses: both responses were optimum curves and no significant interaction between DT and NT occurred [Final internode length (mm) = -32.23 + 3.56DT + 1.08NT - 0.0687DT^2 - 0.0371NT^2; R^2 = 0.91]. It is shown that DIF can predict final internode length only within a temperature range where effects of DT and NT are equal in magnitude and opposite in sign (18-24 °C). Internode appearance rate, as well as stem length formed during the experiment, showed an optimum response to DT.

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Introduction

The control of stem length is particularly important in chrysanthemum cultivation since there are strict quality specifications for height (Karlsson and Heins, 1994). To achieve these quality requirements, chemical plant growth regulators are commonly used in both pot and cut chrysanthemums. However, their application is costly and environmentally unfriendly (Langton, 1998). The need to find effective environmental friendly alternatives for regulating plant height is a priority (Erwin and Heins, 1995; Pearson *et al.*, 1995; Khattak and Pearson, 1997).

Final stem length is determined both by number of internodes and internode lengths (Pearson *et al.*, 1995). In species with a determinate growth pattern, such as chrysanthemum, new internodes are formed up to flower initiation. After this stage, the increase in stem length depends on internode elongation only. Thus, the stem elongation process is strongly correlated with both internode appearance rate (IAR, equal to leaf unfolding rate) and internode elongation rate.

Several growth conditions are known to affect chrysanthemum stem elongation, such as temperature, light intensity, light quality, photoperiod, relative humidity, CO_2 concentration and plant density (Chapter 2). Efforts have been concentrated on temperature manipulation to regulate stem length (Myster and Moe, 1995), and this is already widely practised, based on the DIF concept: the difference between day (DT) and night (NT) temperature (Langton and Cockshull, 1997b). The observation that stem length shows a different response to temperature during the photoperiod compared with nyctoperiod was first investigated for tomato plants and termed 'thermoperiodicity' (Went, 1944). Since then, it has been reported for a wide range of plant species (e.g. Heuvelink, 1989; Erwin and Heins, 1995; Myster and Moe, 1995). Erwin *et al.* (1989) introduced the DIF concept when they found that plants of *Lilium longiflorum* Thunb. had the same final height when grown at the same DIF (using 25 combinations of DT and NT ranging from 14 to 30 °C), regardless of the mean temperature (MT). According to these authors, DT and NT influenced plant height in opposite ways. Increasing DT increased plant height, whereas increasing NT decreased plant height. Therefore, temperature combinations resulting in a negative DIF produced plants that were shorter than those grown under a positive DIF. Erwin *et al.* (1989) also reported a positive linear relationship between internode length and DIF. Thus, it was concluded that the absolute magnitude and sign of DIF were the critical factors determining internode and stem length. In fact, as later suggested by Langton and Cockshull (1997a), the temperature effect on stem length was exclusively

a result of the influence of DIF on internode elongation since terminal flowers had already been initiated and, therefore, the final number of internodes had been determined before the start of the treatment.

Many subsequent studies have shown similar results to those of Erwin *et al.* (1989), for several plant species, including pot chrysanthemum cultivars (e.g. Karlsson *et al.*, 1989c; Jacobsen and Amsen, 1992; Bertram and Karlsen, 1994; Cockshull *et al.*, 1995). However, the effects of day and night temperature have not always been equal in magnitude and opposite in sign (LePage *et al.*, 1984; Karlsson *et al.*, 1989c; Langton and Cockshull, 1997a), which is necessary for a clear DIF response. To clarify these responses, Langton and Cockshull (1997a) conducted a 10 day experiment, in which they grew pot chrysanthemum cultivar 'Bright Golden Anne' under 24 combinations of DT and NT, ranging from 12 to 32 °C. A photoperiod of 12 h was applied to give day and night equal weight. No relationship was found between internode length and DIF. According to these authors, stem elongation in chrysanthemum responded to the absolute DT and NT rather than to DIF, and DT appeared to be the dominant factor controlling internode length. It was thus concluded that DIF is an artefact, lacking real biological significance, that can obscure the real importance of the absolute temperatures at which the plants are grown (Langton, 1998). However, given the short duration of the experiment performed by Langton and Cockshull (only 10 days), the possibility exists that the measured internodes were not fully elongated, thereby invalidating their conclusions. Langton and Cockshull (1997a) were aware of this problem, but considered it unlikely that final internode lengths would have given a substantially better fit with DIF.

Despite numerous studies of the effects of temperature on extension growth of chrysanthemum (mainly pot chrysanthemum), this phenomenon is still not fully understood, leading to uncertainties over how to optimise the use of temperature (Langton, 1998). Furthermore, it is still not clear from the literature whether stem elongation in chrysanthemum is controlled by DIF. The aims of this paper are: (1) to test whether the conflicting results on DIF validity can be explained by differences in the stage at which internode length is measured; and (2) to identify the conditions where the use of DIF explains chrysanthemum internode length. To obtain more insight into the stem extension process, the time courses of internode length, number of internodes and stem length were measured by a non-destructive method, and analysed separately for 16 day and night temperature combinations, ranging from 16 to 28 °C.

Material and methods

Plant material and growth conditions

Block-rooted cuttings of *Chrysanthemum* 'Reagan Improved', obtained from a commercial propagator (Fides Goldstock Breeding, Maasland, The Netherlands), were planted on 16 May 2001 (replication 1) and 13 June 2001 (replication 2) in 14 cm pots containing a peat-based commercial potting compost (Lentse potgrond nr. 4; 85 % peat, 15 % clay). After two days in a common glasshouse environment (18 °C DT/ 16 °C NT and 18 h light), the temperature treatments were imposed. Plants were selected for uniformity (8.0 ± 1.0 leaves per plant; 12.1 ± 2.0 cm stem length) and distributed over four artificially lit growth chambers (l × w × h = 2.90 m × 2.20 m × 3.15 m).

Each growth chamber had a constant day and night temperature (16, 20, 24 or 28 °C). Since only four growth chambers were available, for each replication, plants were shifted at the start and end of each day according to their DT and NT treatment. Fluorescent tubes (Philips TL 58W, colour 84) were used continuously during the 12 h of daylight (between 0800 and 2000 h) providing 99 μmol m^{-2} s^{-1} photosynthetically active radiation at plant level (LI-COR, model LI-191SA; Lincoln, USA). This light level resulted in a daily light integral (4.3 mol m^{-2} d^{-1}) that was similar to that received by plants growing in commercial glasshouses during the winter in The Netherlands (lat. 52 °N). Plants were grown as individual plants under ambient CO_2 (growth chamber continuously ventilated) and at constant vapour pressure deficit (VPD = 0.57 kPa). Plants were watered by hand as required. The experiment ended when internode 10 had reached its final length in all temperature combinations studied. This occurred on day 26 (replication 1) and day 28 (replication 2) after the start of the treatments, but was considered to have occurred on day 27 for both replicates in the analyses.

Temperature and relative humidity were automatically recorded at 5 min intervals using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). The 24 h mean temperatures of each growth chamber did not differ from the set points (16, 20, 24 or 28 °C). The corresponding mean VPD was slightly different from its set point and there were small differences between the replicates (0.51 ± 0.02, 0.50 ± 0.03, 0.57 ± 0.00 and 0.57 ± 0.00 kPa).

Treatments

Sixteen temperature treatments were applied resulting from all combinations of four DT and four NT (16, 20, 24 and 28 °C) (Table 1). All plants were moved each day according to their DT and NT combination; plants in constant DT and NT treatments were also moved out of, and back into, their growth chambers. To test the effect of this movement on stem length, four extra treatments (16, 20, 24 and 28 °C with constant DT and NT) were initiated in which plants were kept permanently inside the growth chamber.

Periodic non-destructive measurements were conducted on ten plants per experimental plot on days 0, 5, 10, 17, 21 and 26 (replication 1) and days 0, 5, 10, 17, 24 and 28 (replication 2) after the start of the treatments. Length of internode 10, number of leaves on the main stem (≥ 10 mm; equal to number of internodes) and stem length were recorded. Internode 10 was chosen to guarantee that it developed under the treatment conditions, as it was not visible at the start of the treatments. A digital calliper was used to measure internode lengths.

Statistical design and analysis

The experimental set-up was a complete randomised block design with the two replications in time as blocks. Each replication consisted of ten plants per plot (treatment) and plants from the same treatment were placed in two separate trays (five pots per tray). Linear regression analysis and ANOVA were conducted and treatment effects were tested at the 5 % probability level. The statistical software package Genstat 5 (IACR- Rothamsted, UK) was used.

TABLE 1. DIF values (°C) resulting from the combination of four day temperatures and four night temperatures.

Night temperature (°C)	Day temperature (°C)			
	16	20	24	28
16	0	+4	+8	+12
20	- 4	0	+4	+8
24	- 8	- 4	0	+4
28	- 12	- 8	- 4	0

Results

Effect of temperature on internode length

Time patterns and temperature responses. Internode length followed a sigmoid time course for all the 16 day and night temperature combinations (Fig. 1). Five days after treatments had started, internode 10 was still not measurable, under any conditions. In general, rapid internode elongation was observed between days 5 and 17, followed by a plateau. In some temperature treatments (e.g. all 28 °C NTs), internode 10 was already fully elongated by day 17 (Fig. 1D), whereas in other treatments (e.g. 16 °C DT/16 °C NT) this was delayed by about 1 week (Fig. 1A). The experiment finished on day 27 when the final length of internode 10 had been achieved in all temperature treatments (Fig. 1).

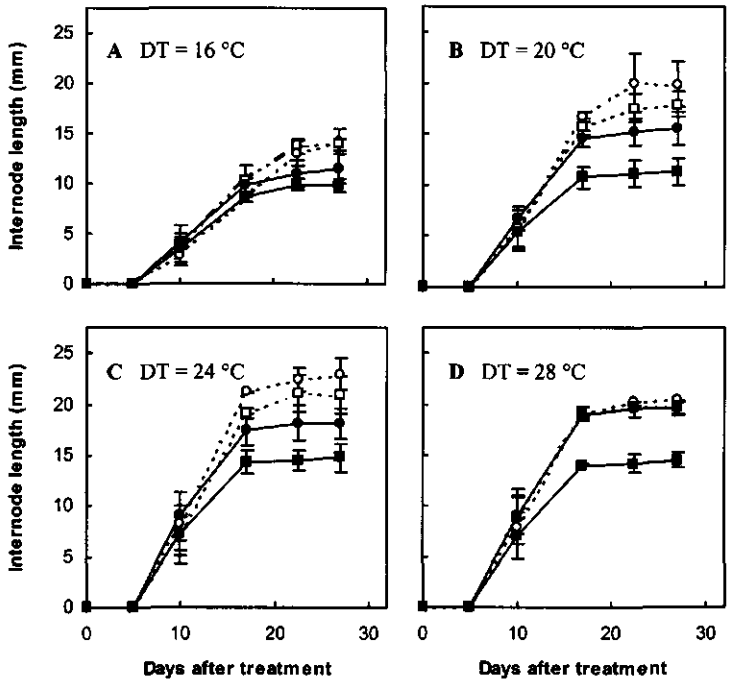

FIG. 1. Elongation patterns of internode 10 as a function of day temperature (DT) and night temperature (○ 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C) in *Chrysanthemum* 'Reagan Improved'. Vertical bars indicate s.e.m. (n = 2) when larger than symbols.

Ten days after the treatments were imposed, internode length showed a significant ($P < 0.001$) positive linear relationship with DT, which explained 84 % of its variance; no significant ($P = 0.97$) relationship was observed with NT. At the same time, although internodes were not yet full-grown, a significant ($P < 0.001$) positive linear relationship ($R^2 = 0.64$) between internode length and DIF was found (Fig. 2A). However, this relationship was much closer ($R^2 = 0.81$) when internodes had reached their final length in all the treatments (day 27) (Fig. 2B). Thus, plants grown under temperature combinations that resulted in a negative DIF had shorter internodes compared with plants grown under a positive DIF. For example, the mean final length of internode 10 at 16 °C DT/28 °C NT (-12 °C DIF) was 48 % less than that at the reciprocal combination 28 °C DT/16 °C NT (+12 °C DIF). Similarly, plants grown at the same DIF (e.g. -4 °C DIF: 16 °C DT/20 °C NT or 24 °C DT/28 °C NT) had similar final internode lengths. Linear regression analysis showed that among the temperature variables studied (DT, NT, MT and DIF), DIF gave the best fit to the final internode length, accounting for 81 % of its variance (Table 2). Furthermore, only a poor relationship ($R^2 = 0.51$) between internode length at day 10 and final internode length (day 27) was found (Fig. 3).

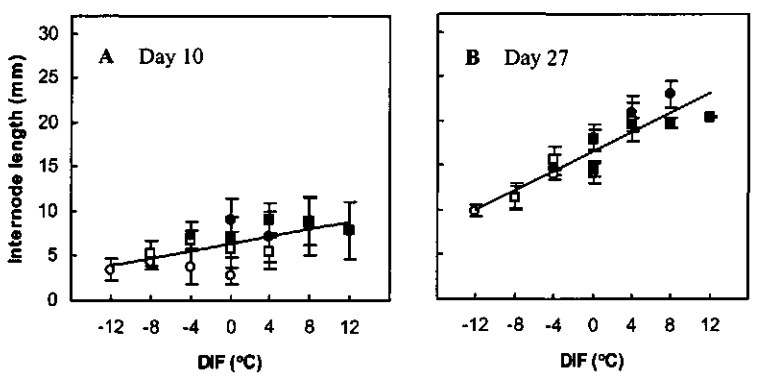

FIG. 2. Relationships between the length of internode 10 and DIF (°C) 10 days (A) and 27 days (B) after treatments started in *Chrysanthemum* 'Reagan Improved'. Symbols represent 16 day and night temperature combinations, with a day temperature of: o 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C. Regression lines: (A) y = 6.40 + 0.202x , $R^2 = 0.64$; (B) y = 16.57 + 0.547x , $R^2 = 0.81$. Vertical bars indicate s.e.m. (n = 2) when larger than symbols.

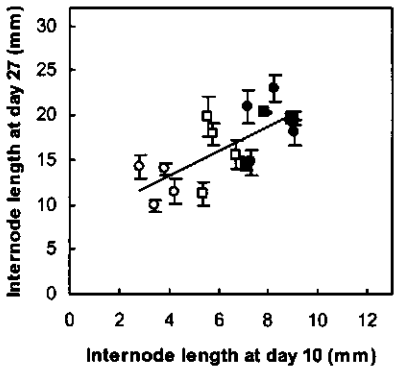

FIG. 3. Relationship between the length of internode 10 at 10 days and 27 days after treatments started in *Chrysanthemum* 'Reagan Improved'. Symbols represent 16 day and night temperature combinations, with a day temperature of: ○ 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C. Regression line: y = 7.89 + 1.36x , R^2 = 0.51. Vertical bars indicate s.e.m. (n = 2) when larger than symbols.

Final internode length: modelling DT and NT responses. The individual effect of absolute DT and NT on final length of internode 10 is given in Fig. 4. An ANOVA of final internode length showed no significant interaction between DT and NT (P = 0.091). The quadratic terms were tested and found to be significant for both DT (P < 0.001) and NT (P = 0.011). This resulted in the following regression equation:

$$\text{Final internode length (mm)} = -32.23 + 3.56DT + 1.08NT - 0.0687DT^2 - 0.0371NT^2 \quad (1)$$

Measured and predicted final internode length showed good agreement (R^2 = 0.91) (Fig. 4). Based on eqn (1), the optimum temperature for internode elongation was calculated, resulting in a much higher value for DT (25.9 °C) than for NT (14.6 °C). Thus, within the temperature range studied (16-28 °C), final internode length had an opposite response to DT and NT: a higher DT resulted in a quadratic increase in final internode length, whereas a higher NT resulted in a quadratic decrease in final internode length (Fig. 4).

TABLE 2. Regression models with one factor (DT, NT, MT and DIF) for final internode length, IAR and stem length formed during the experiment for *Chrysanthemum* 'Reagan Improved'.

Model	R^2_{adj}	s.e. [a]	F_{prob} [b]
Final internode length (mm)			
= 16.57 + 0.547 DIF	0.81	1.75	< 0.001
= 28.75 - 0.554 NT	0.42	3.08	< 0.001
= 4.69 + 0.540 DT	0.40	3.14	< 0.001
= 16.87 - 0.014 MT	0.01	4.03	0.330
IAR (number of internodes day $^{-1}$)			
= - 0.0022 DT^2 + 0.113 DT - 0.75 [c]	0.88	0.03	< 0.001
= - 0.0026 MT^2 + 0.131 MT - 0.99 [c]	0.61	0.07	< 0.001
= 0.281 + 0.0155 DT	0.72	0.05	< 0.001
= 0.228 + 0.0179 MT	0.51	0.06	< 0.001
= 0.622 + 0.0065 DIF	0.31	0.08	0.002
= 0.568 + 0.0024 NT	0.10	0.09	0.090
Stem length (cm)			
= - 0.146 DT^2 + 7.82 DT - 72.9 [c]	0.88	2.58	< 0.001
= - 5.18 + 1.40 DT	0.77	3.53	< 0.001
= 25.65 + 0.88 DIF	0.60	4.63	< 0.001
= 2.83 + 1.038 MT	0.18	6.63	0.021
= 33.66 - 0.364 NT	0.02	7.27	0.307

[a] Standard error of regression.
[b] F probability of regression.
[c] *Significant quadratic model tested based on the graphical presentation of the data.*

Using Eqn (1), final internode length was predicted for several ranges of DT and NT combinations, and was plotted against DIF (Fig. 5). Taking the first-order partial derivatives of Eqn (1) with respect to DT and NT showed that, at 22 °C, both derivatives had approximately the same absolute value and opposite sign. DIF values calculated within an interval of DT and NT combinations close to 22 °C, therefore, gave a good fit to the predicted internode length (Fig. 5A and B). DT and NT combinations in the range of 18-24 °C resulted in the best fit (R^2 = 0.95) between these two variables (Fig. 5A), followed by the studied temperature range (16-28 °C) where DIF could explain 89 % of the variance (Fig. 5B).

DIF showed a poor relationship with the predicted final internode length for combinations of DT and NT ranging between 12 and 22 °C (Fig. 5C), and between 22 and 32 °C (Fig. 5D). For these temperature ranges, plants growing under the same DIF would have a very different predicted internode length. For instance, at 0 °C DIF, the predicted internode length varied between 8 mm (12 °C DT/12 °C NT) and 19 mm (22 °C DT/22 °C NT) (Fig. 5C). Although a significant ($P < 0.001$) positive linear trend was observed for both temperature ranges (Fig. 5C and D), a lower DIF did not always result in shorter internodes. For example, the predicted internode length for plants grown at 18 °C DT/22 °C NT (-4 °C DIF) was 88 % larger than for plants grown at 12 °C DT/12 °C NT (0 °C DIF).

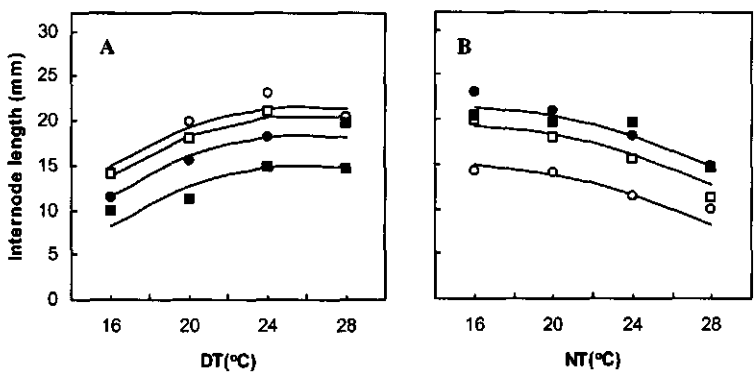

FIG. 4. Mean final length of internode 10 as a function of day temperature (DT) and night temperature (NT) in *Chrysanthemum* 'Reagan Improved'. Symbols represent measured values from 16 day and night temperature combinations, with: (A) NT of: ○ 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C; (B) DT of: ○ 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C. Solid lines represent regression model: Final internode length (mm) = -32.23 + 3.56 DT + 1.08 NT - 0.0687 DT^2 - 0.0371 NT^2, $R^2 = 0.909$. $LSD_{15;\,0.05} = 1.08$ mm.

Effect of temperature on internode appearance rate and stem length

Internode appearance rate (IAR) was calculated using the slope of the linear relationship between number of internodes and time (from 0 to 21 days after treatments started). A quadratic response to DT could explain 88 % of the variance observed in IAR (Table 2). IAR increased with DT up to an optimum at 25.7 °C.

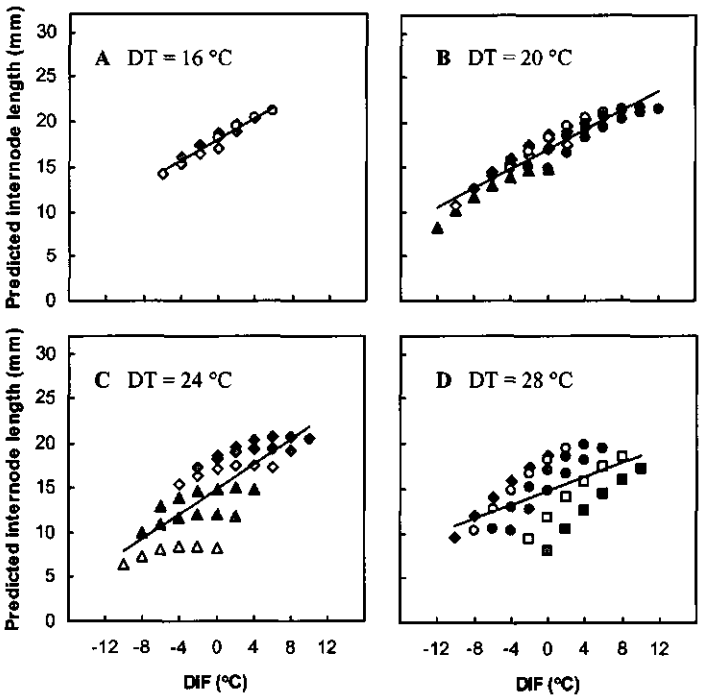

FIG. 5. Predicted final internode length in *Chrysanthemum* 'Reagan Improved', based on eqn (1), as a function of DIF (°C) in four temperature intervals: (A) 18-24 °C; (B) 16-28 °C; (C) 12-22 °C; (D) 22-32 °C. Symbols represent day and night temperature combinations, with a day temperature of △ 12 °C, ▲ 14 °C, ▲ 16 °C, ◊ 18 °C, ♦20 °C, ♦22 °C, ○ 24 °C, ● 26 °C, ● 28 °C, □ 30 °C, ■ 32 °C. Solid lines represent linear regressions on the data: (A) y = 18.07 + 0.578x, R^2 = 0.95; (B) y = 17.00 + 0.546x, R^2 = 0.89; (C) y = 14.88 + 0.704x, R^2 = 0.62; (D) y = 14.74 + 0.388x, R^2 = 0.32.

At this temperature, predicted IAR was 43 % higher than for plants grown at 16 °C DT. A further increase in DT, up to 28 °C, had a minor effect on this rate (Fig. 6A). MT and DIF also had a significant influence on IAR, but a lower percentage of variance was explained by these variables (Table 2). Stem length formed during the experiment (final stem length - initial stem length) was significantly influenced by DT, DIF and MT, but a quadratic model using DT only, explained the largest proportion of the variance (88 %) (Table 2). Increasing DT from 16 to 24 °C caused stem length to double, but a further increase had only a minor effect. However, this model overestimated the stem length of plants grown at 28 °C NT (Fig. 6B).

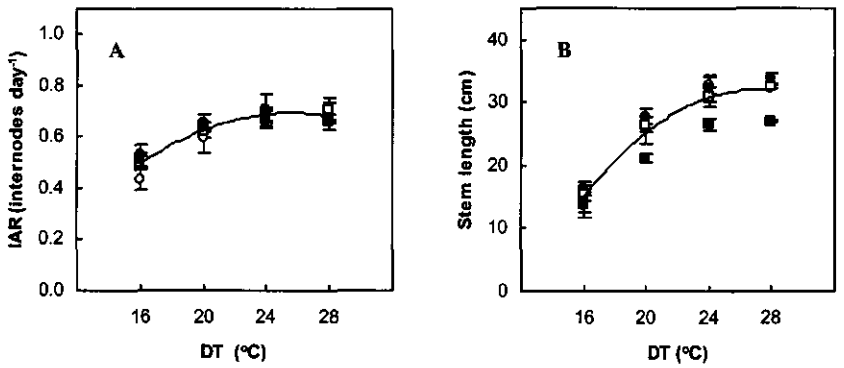

FIG. 6. IAR (A) and stem length formed during the experiment (B) as a function of day temperature (DT) in *Chrysanthemum* 'Reagan Improved'. Symbols represent 16 day and night temperature combinations, with a night temperature of: ○ 16 °C; □ 20 °C; ● 24 °C; ■ 28 °C. Regression curves: (A) $y = -0.0022 x^2 + 0.113x - 0.75$, $R^2 = 0.88$; (B) $y = -0.146 x^2 + 7.82x - 72.9$, $R^2 = 0.88$. Vertical bars indicate s.e.m. (n = 2) when larger than symbols.

Stem length was closely related to IAR ($R^2 = 0.82$) and to a lesser extent with final length of internode 10 ($R^2 = 0.71$). These two variables together explained 97 % of its variability.

Effect of movement on stem length

The daily movement between growth chambers that was imposed on the plants had no significant effect ($P = 0.097$) on stem length formed during the experiment under the temperature conditions tested (16, 20, 24 and 28 °C constant DT and NT).

Discussion

Within the experimental temperature range of 16-28 °C, a positive linear relationship between DIF and internode length was observed when internodes had reached their final size (Fig. 2B). In contrast to the assumption of Langton and Cockshull (1997a), it was shown that internode lengths recorded in early stages of development do not bear a close relationship to the final internode lengths (Fig. 3). A possible reason for these findings is the fact that internodes from plants grown under different temperature combinations were at different stages of elongation at day 10 (Figs 1 and 3). For instance, 10 days after the treatments started, internode 10 from plants grown at 20 °C

DT/16 °C NT had reached only 28 % of its final length, whereas at 20 °C DT/28 °C NT it had reached 47 % of its final length (Fig. 1B). This is a result of different durations of elongation period and different rates of internode elongation. Hence, the conclusion of Langton and Cockshull (1997a) that internode elongation is not related to DIF may be invalidated by the short duration of their experiment (10 days).

Besides the stage at which the internodes were measured, the range of temperatures also played a major role in the relationship with DIF. The predicted final internode length, based on a quadratic model for both DT and NT [Eqn (1)], showed a positive relationship with DIF (Fig. 5). However, a close relationship existed only within a certain temperature interval (Fig. 5A and B) when the positive effect of DT on final internode length was compensated by a similar negative effect of NT (Fig. 4), resulting in equal length at the same DIF. For temperature combinations outside that range (10-22 °C and 22-34 °C), predicted final internode length showed a poor relationship with DIF (Fig. 5C and D).

These results clearly demonstrate that the DIF concept is valid only if the effects of DT and NT on internode length have similar magnitudes and opposite signs. This leads to a conclusion similar to that drawn by Pearson *et al.* (1995) who reported that plants do not respond to DIF itself but to the combination of the independent effects of temperature during the day and night periods.

In previous studies on pot chrysanthemum, stem length was closely related to DIF (e.g. Karlsson *et al.*, 1989c; Cockshull *et al.*, 1995) because plants were grown under short day conditions, without a period of long days. Consequently, stem elongation was mainly the result of internode elongation alone since all the internodes had been previously formed. To analyse the effect of temperature on stem elongation of cut chrysanthemum, attention should also be paid to its influence on the number of internodes. For many plant species, including chrysanthemum, leaf unfolding rate (equal to IAR) has been reported to increase linearly with MT (Karlsson *et al.*, 1989c; Challa *et al.*, 1995). However, in the present study, IAR showed an optimum response to MT, and an even stronger one to DT alone (Table 2 and Fig. 6A). Larsen and Hidén (1995) were also unable to find a simple linear relationship between MT and LUR in chrysanthemum. These differences may be due to a cultivar effect.

Stem length formed during the experiment showed a closer relationship to DT than to DIF (Table 2). If stem length was dependent only on final internode length (mainly controlled by DIF), than plants grown at the same DIF, regardless of the actual DT and NT, would have a similar stem length. However, at the end of the experiment, plants had not reached their final stem length. Several internodes (above internode 10) were not fully elongated, but the number of internodes was already

defined in all the treatments, except in 28 °C NT combinations, where the apical flower bud was still not visible. This could explain why stem length showed a closer relationship with IAR (R^2 = 0.82) than with final length of internode 10 (R^2 = 0.71). Thus, it is expected that the response of final stem length to temperature would have been different. In general, as more internodes become fully elongated, the relationship between final stem length and DIF should improve.

Considering that chrysanthemum is commonly grown at temperatures between 17 and 23 °C and that the daily movement imposed to the plants did not affect stem length, these results can be extrapolated to commercial growth conditions. Although the DIF concept is simply a different parameterisation of the distinct responses to DT and NT (Langton and Cockshull, 1997a) it can still be a valid tool in the manipulation of final internode length. However, the use of different chrysanthemum cultivars (Hansen *et al.*, 1996) or different growth conditions (Myster and Moe, 1995) should be taken into account when evaluating the effectiveness of DIF. For instance, Myster and Moe (1995) suggested that there is a higher sensitivity of stem elongation to temperature fluctuations during the SD period rather than during the LD period for several pot plants.

Conclusions

The response of chrysanthemum final internode length to temperature is strongly related to DIF, but this response is simply the result of independent and opposite effects of day and night temperatures. It is concluded that, although the DIF concept does not have a biological meaning, it can be a good predictor of final internode length of chrysanthemum, within a temperature range of 16-28 °C.

3.2

Flower characteristics

Abstract

The sensitivity to temperature of number of flowers per plant (including flower buds; NoF), individual flower size, flower position and colour was investigated in cut chrysanthemum 'Reagan Improved'. Plants were grown both in a glasshouse, at two constant temperatures (17 and 21 °C), and in growth chambers, at 32 temperature combinations (from 15 to 24 °C). In the latter experiment, temperature treatments were applied dividing the cultivation period into three sequential phases: long-day period (phase I), start of short-day period to visible terminal flower bud (phase II) and end of phase II to harvest (phase III).

All flower characteristics were strongly affected by temperature, except for the flower position within the plant. A higher temperature increased NoF, mainly by increased number of flower buds, but decreased individual flower size, most likely due to competition for assimilates. The temperature effect was also dependent on the phase of the cultivation period. In general, flower characteristics were less sensitive to the temperature applied during the long-day period. NoF was affected positively by temperature mainly during phase III, whereas individual flower size increased with temperature during phase II but decreased with temperature during phase III. Lower temperatures during phase III significantly enhanced flower colour intensity. The importance of using a more dynamic heating strategy is discussed.

Submitted as:

Carvalho SMP, Abi-Tarabay H, Heuvelink E. 2003. Temperature in three phases of the cultivation period influences chrysanthemum flower characteristics.

Introduction

Temperature plays a very important role in flower initiation and development of numerous greenhouse crops (Hanan, 1998). According to Adams *et al.* (2001), research in flowering traditionally aimed at either understanding the underlying physiological processes or at quantifying the effects of the photo-thermal environment on the time to flowering. These latter studies were often concentrated on the effects of mean temperature applied during the complete cultivation period (Adams *et al.*, 2001).

Also in chrysanthemum many studies have focused on the relationship between temperature and time to flowering (e.g. Cockshull, 1979; Whealy *et al.*, 1987; Pearson *et al.*, 1993; Larsen and Persson, 1999). In spite of the major importance of several flower characteristics on chrysanthemum external quality and, therefore, on its price (Chapter 2), quantitative information on the effect of temperature on flower number, size, position and colour is still very limited and often not clear. For instance, when the effect of temperature on flower size is studied, total flower area per plant is commonly determined (e.g. Karlsson *et al.*, 1989b). As this represents the combined effects of individual flower size and number of flowers, no clear separation between these two characteristics is possible. Furthermore, despite the objectives and sensitivities of each phase of the cultivation may differ, and one phase may affect the succeeding one (Hanan, 1998), only few authors divided the cultivation period in a number of phases (Karlsson *et al.*, 1989a; Wilkins *et al.*, 1990). This is possibly due to the difficulty in assessing a clear differentiation among the distinct phases that chrysanthemum passes through before anthesis has been reached (Adams *et al.*, 1998). However, dividing the cultivation period in different phases allows a more detailed study of the thermo-morphogenic effects. For instance, Wilkins *et al.* (1990) reported that the first three weeks of the short-day period, when floral induction, initiation and beginning of flower development occur, are particularly critical, and appropriate temperatures must be present to avoid delays in time to flowering. Since little research exists to justify one strategy over another (Karlsson *et al.*, 1983), temperature control in most greenhouses is still based on fixed day and night set-points, which are applied for the complete cultivation period (Hendriks *et al.*, 1992). To minimise energy costs, by using more flexible and dynamic heating strategies, and yet produce a high quality chrysanthemum, a good understanding and quantification of the thermo-morphological effects on the important flower characteristics is, therefore, needed. Knowledge of the sensitivity of each important flower quality attribute to temperature at different phases of the cultivation will enable a more accurate temperature control, adjusted to the specific quality aims.

The aim of the present work is to study the effect of temperature on flower characteristics of cut chrysanthemum and to obtain a better insight into the underlying physiological processes. The overall effect of temperature was quantified under greenhouse conditions. Furthermore, possible differences in temperature sensitivity of flower characteristics in different phases of the cultivation were studied, in order to identify the most critical period, where temperature should be controlled more accurately. This study was done in growth chambers to allow for accurate temperature control and a constant light intensity in the different phases. The cultivation period was divided in three sequential and main macroscopically visible phases, which could be easily identified: (1) long-day (LD) period; (2) from the start of short-day (SD) period to the visible terminal flower bud (VB); and (3) from VB to harvest.

Material and methods

Experimental set-up

Two experiments were conducted using block-rooted cuttings of *Chrysanthemum* 'Reagan Improved', a dark pink coloured cultivar, obtained from a commercial propagator (Fides Goldstock Breeding, Maasland, The Netherlands).

Experiment 1 was carried out in four compartments (12.8 m × 12.0 m), that were part of a multispan Venlo-type glasshouse (Wageningen University, The Netherlands, lat. 52 °N). Cuttings were planted in 12 January 2000 in parallel soil beds, at a density of 48 plants m^{-2}. Day temperature heating set-point was 16 °C in two compartments (low temperature treatment, LT) and 20 °C in the other two (high temperature treatment, HT), for the whole cultivation period. Night temperature set points were one degree above day temperature set points. Ventilation temperature was set at one degree higher than the heating temperatures. During the first three weeks after planting, plants were grown under LD conditions, followed by a SD period up to the harvest. High-pressure sodium lamps (HPS, Philips SON-T Agro, 44 μmol $m^{-2}s^{-1}$ photosynthetically active radiation, PAR) were kept continuously on during the day hours of the LD (19 h, from 0500 to 2400 h) and SD period (10 h 40 min., from 0730 to 1810 h). This resulted in an average daily light integral of 7.1 mol m^{-2} d^{-1} PAR. Pure CO_2 was supplied when CO_2 concentration in the greenhouse was lower than 350 μmol mol^{-1} and dosing was stopped at 420 μmol mol^{-1}.

TABLE 1. Temperatures applied to *Chrysanthemum* 'Reagan Improved' during different phases of the cultivation period (phase I, II and III) in Exp. 2.

	Temperature (°C)	Cultivation period	Duration (d)
Phase I	18; 24	Planting to start SD [a]	14
Phase II	15; 18; 21; 24	Start SD to VB	19 - 22
Phase III	15; 18; 21; 24	VB to harvest	35 - 42 [b]

[a] i.e. LD period.
[b] Only when temperature in Phase III was 24 °C an important delay was observed.

Experiment 2 was conducted in four artificially lit growth chambers (l × w × h = 4.50 m × 3.25 m × 2.20 m). Cuttings were planted in 16 August 2001, in 14 cm pots containing a peat-based commercial potting compost (Lentse Potgrond nr. 4.85 % peat, 15 % clay; Lentse Potgrond, Lent, The Netherlands). Plants were placed at a density of 50 plants m^{-2}, on side by side trolleys, and were distributed over the growth chambers. During the first two weeks plants were grown under LD conditions at 18 °C or 24 °C constant day and night temperature (phase I). At the start of the SD period (phase II) and at the appearance of VB (phase III) plants were redistributed over the corresponding growth chambers (15, 18, 21 and 24 °C). This resulted in a total of 32 treatments (Table 1). Assimilation lamps (HPI-T plus and HPS SON-T Agro, Philips, 1:1, 370 µmol m^{-2} s^{-1} PAR) were continuously on during the 19 h of the LD period. In the SD period these lamps were on during 8 h followed by 3 h of incandescent light (13 µmol m^{-2} s^{-1} PAR). This light level resulted in an average daily light integral of 13.5 mol m^{-2} d^{-1} PAR. Plants were grown under ambient CO_2 (growth chamber continuously ventilated) and at constant vapour pressure deficit (VPD = 0.57 kPa). Plants were watered by hand as required. Fertilisation was done on a weekly basis, from 7 September to 12 October 2001 (Kristalon, N19-P6-K20-Mg3-Micro, 2 g l^{-1} of water; Hydro Agri, Vlaardingen, The Netherlands;).

In both experiments, PAR was measured at average plant height (0.5 m) using a 1.0 m line quantum sensor (LI-COR, model LI-191SA; Lincoln, USA). Plant protection was done according to an integrated pest management scheme (IPM), using both biological and chemical agents, and at the start of Exp. 2 a treatment against *Pythium* was applied (Previcur, 2.5 cc l^{-1} H_2O [a.i. propamocarb-hydrocloride]; Aventis Cropscience Benelux BV). No growth regulators were applied. Temperature, relative humidity and CO_2 concentration (only for Exp. 1) were automatically recorded at 5 min intervals using a commercial computer system (Hoogendoorn,

Vlaardingen, The Netherlands). In Exp. 1, the greenhouse 24 h mean temperature was 17.2 °C (LT) and 20.9 °C (HT). The daily mean CO_2 concentration, between 1000 and 1600 h, was 444 μmol mol^{-1}. Daily outside global radiation was obtained from a meteorological station located at about 100 m distance (23.4 mol m^{-2} d^{-1}). In Exp. 2 mean temperature did not differ from its set points.

Measurements

In both experiments, plants were harvested when at least three flowers per plant where fully open (ray florets in horizontal plane) and the first row of their disc florets had reached anthesis. This stage was reached at different times depending on the treatments. Six (Exp. 1) or five (Exp. 2) plants were harvested per experimental unit, leaving two rows (Exp. 1) or one row (Exp. 2) of border plants between different treatments. Number of flower buds (> 5 mm, but ray florets not yet separated from inflorescence disc), number of open flowers (florets separated from inflorescence disc), individual flower area of the fully open flowers (LI-COR, model 3100 Area Meter; Lincoln, USA), flower and total dry mass (ventilated oven at 105 °C during 15 h) and time to flowering were recorded in both experiments. No root measurements were done. In Exp. 2, also the flower position and colour were measured. Flower position was evaluated based on the distance between the highest and the lowest flower on the stem (flower distance) and based on the distribution of the open flowers within the stem (percentage of open flowers at the top 15 cm). As at harvest stage flower colour was homogeneous within each growth chamber, only four temperature treatments were analysed (18/15/15 °C, 18/18/18 °C, 18/21/21 °C and 18/24/24 °C, representing phase I/II/III). Flower colour intensity was measured using a 3CCD video camera (Hitachi Denshi, HV-C20E/K–S24, Japan) connected to a PC. Measurements were done at one fully open flower per plant, under constant light environment created with fluorescent tubes (Philips TL 16W, colour 84) and a diffuser plate. Flower image was separated into ray florets (pink) and disc florets (yellow) with a specialised colour learning software (KAS, ATO, Wageningen). Light intensities for red, green and blue (RGB) were separately averaged over all pixels from the ray florets. Only the red and blue values were used, as these are the basic colours responsible for the pink colour of the ray florets.

Statistical design and analysis

The experimental set-up was a complete randomised block design with two blocks. In Exp. 1, two greenhouse compartments were at 17 °C and the other two at 21 °C, grouped in two blocks. Similarly, during phase I of Exp. 2, two growth chambers were

at 18 °C and the other two at 24 °C, so each two chambers formed one block. However, at the beginning of phase II and III, plants were redistributed over the four chambers (15, 18, 21 and 24 °C), and treatments were replicated within each chamber.

Analysis of variance was conducted and treatment effects were tested at 5 % probability level. Mean separation was done by Student's t-test ($P = 0.05$). As for both experiments block effects were never significant, these were excluded from the statistical analysis and the sum of squares and degrees of freedom were added to the residual term. In Exp. 2, the effect of the quantitative factors was separated in a linear and a quadratic component, when more than two temperature levels were studied. A linear regression model was built for each studied variable, using as regressors the temperatures during phases of the cultivation period that were found to have a significant effect as well as the significant 2-way interactions. Possible 3-way interactions, resulting from the combination of the temperatures during the three different phases of the cultivation period, were not considered. The statistical software package Genstat 5 (VSN International Ltd; Herts, UK) was used. For calculated fractions the normality of the data was checked, using the 'Kolmogorov-Smirnov' test from SPSS package (SPSS Inc.; Chicago, USA). If the data were not normally distributed an arcsine square root transformation was applied (Montgomery and Peck, 1982).

Results

Number of flowers per plant

Total number of flowers: Higher temperature, during the whole cultivation period (Exp. 1), showed a significant positive effect on total number of flowers and flower buds per plant (NoF). Plants grown at 21 °C had 47 % higher NoF than plants grown at 17 °C (Table 2).

In the growth chamber experiment (Exp. 2), it was observed that only the temperature during phase II did not significantly affect NoF ($P = 0.381$). In contrast, temperature during phase I had a significant positive effect ($P < 0.001$) resulting, on average, in 3 or 4 more flowers per plant when temperature rose from 18 to 24 °C (Fig. 1). Furthermore, NoF increased linearly ($P < 0.001$) with the temperature during phase III (Fig. 1) and plants grown at 24 °C, during this phase, had 54 % more flowers than plants grown at 15 °C. A linear regression model, with temperature during phase I and III as regressors, could explain 73 % of the variance observed in NoF (Fig. 1).

This linear model also showed that the effect of temperature during phase III was approximately twice as large as temperature influence during phase I.

TABLE 2. Flower characteristics of *Chrysanthemum* 'Reagan Improved', as a function of temperature applied during the complete cultivation period (Exp. 1).

	17 °C	21 °C	F_{prob} [a]
Total number of flowers (plant $^{-1}$) [b]	19	28	*0.012*
Flower buds (%)	18.5	23.3	*0.319* [c]
Individual flower dry mass (g flower $^{-1}$) [d]	0.24	0.20	*0.030*
Individual flower area (cm^2 flower $^{-1}$) [d]	35.8	31.3	*0.019*
Total flower dry mass (g plant $^{-1}$)	2.69	2.96	*0.303*
Total plant dry mass (g plant $^{-1}$)	11.5	12.3	*0.073*
Flower mass ratio (fraction)	0.234	0.241	*0.661*
Total cultivation period (d)	83	76	

[a] F probability (significant when < 0.05).
[b] Including flower buds.
[c] ANOVA based on transformed data.
[d] Average of fully open flowers (at least 3 flowers per plant).

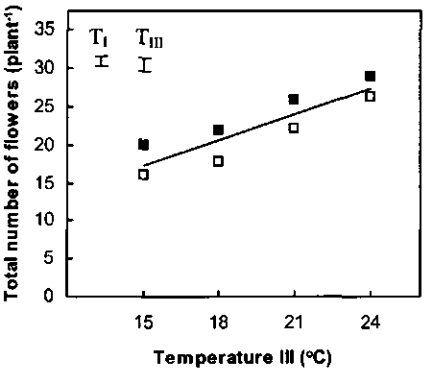

FIG. 1. Total number of flowers per plant including buds of *Chrysanthemum* 'Reagan Improved', as a function of temperature during phase I and III. Overall regression model: $y = -11.4 + 0.58T_I + 1.10T_{III}$, $R^2 = 0.73$. Symbols represent temperature during phase I: □ 18 °C; ■ 24 °C. Vertical bars indicate LSD = 1.29 (phase I) and LSD = 1.83 (phase III).

Percentage of flower buds: In both experiments, the proportion of flowers in a bud stage was not normally distributed. Statistical analysis performed on the transformed data showed that although the percentage of flower buds increased from 18.5 to 23.3 %, when plants were grown during the complete cultivation period at 21 °C compared to 17 °C, this effect was not significant (Table 2). In Exp. 2, where a wider temperature range was applied, an overall regression model showed that temperature could explain 76 % of the variance observed in the percentage of flower buds per plant (Fig. 2). Percentage flower buds was significantly influenced by the interaction between temperature during phase I and II ($P = 0.017$) and the interaction between temperature during phase II and III ($P = 0.022$). The general trend was similar to the one described for Exp. 1, as higher temperature usually resulted in an increased percentage of flower buds (Fig. 2). Temperature during phase I only had a significant effect when the temperature during the following phase was 15 °C. In that case, plants grown at higher temperature during phase I had 7 % more buds per plant (Fig. 2A). When temperature during phase II increased from 15 to 21 °C the percentage of buds was only slightly affected, but a further temperature increase up to 24 °C strongly promoted it (Fig. 2). The interaction between temperature in phase II and III resulted in a wide range of percentages of flower buds per plant (Fig. 2B).

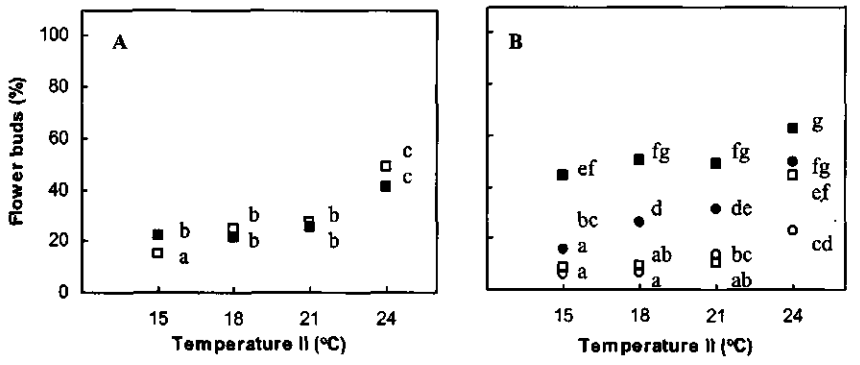

FIG. 2. Percentage of flower buds per plant of *Chrysanthemum* 'Reagan Improved, as a function of the interaction between temperature during: (A) phase I and II; (B) phase II and III. Overall regression model for the transformed data: $y = -2.76 + 0.0564T_I + 0.121T_{II} + 0.0761T_{III} - 0.00287T_IT_{II} - 0.00156T_{II}T_{III}$, $R^2 = 0.76$. Symbols represent temperature during: (A) phase I: □ 18 °C; ■ 24 °C; and (B) phase III: ○ 15 °C; □ 18 °C; ● 21 °C; ■ 24 °C. Different letters indicate significant differences between treatments based on transformed data.

The amount of flower buds per plant ranged from 6 % for the lowest temperatures (15 °C during the complete SD period), to 63 % for the highest temperatures (24 °C during the complete SD period). Furthermore, when plants were grown at 24 °C during phase III at least 44 % of their flowers were in a bud stage. In contrast, treatments with 15 °C during phase III had less than 23 % of buds, regardless the temperatures that they were previously subjected to (Fig. 2B).

Individual flower size

In contrast to NoF, individual flower size decreased significantly with increased temperature (Table 2). Plants grown at 21 °C during the complete cultivation period had on average 17 % lighter and 13 % smaller fully open flowers, compared to plants grown at 17 °C. Also in Exp. 2 individual flower dry mass (R^2 = 0.70) and area (R^2 = 0.77) were closely related to temperature (Fig. 3). Only the temperature during the LD period (phase I) showed neither a significant effect on individual flower dry mass (P = 0.244) nor on individual flower area (P = 0.710). Temperatures during phase II and III significantly influenced flower size, but they acted in opposite ways. Individual flower dry mass ($P < 0.001$) and area ($P < 0.001$) increased linearly with temperature during phase II. For plants grown at 24 °C during phase II the fully open flowers were 25 % heavier and 22 % larger than for plants grown at 15 °C (Fig. 3).

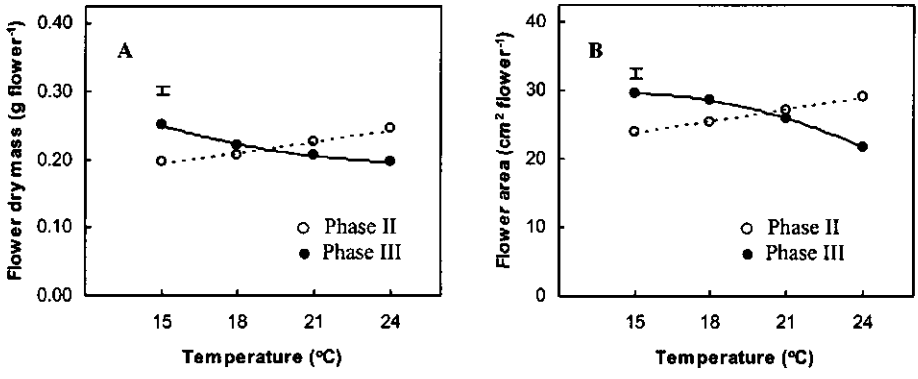

FIG. 3. Individual flower dry mass (A) and individual flower area (B) of *Chrysanthemum* 'Reagan Improved', as a function of temperature during phase II and phase III. Overall regression models: (A) $y = 0.42 + 0.0055T_{II} - 0.0262T_{III} + 0.00052T_{III}^2$, $R^2 = 0.70$; (B) $y = -0.10 + 0.57T_{II} + 2.54T_{III} - 0.087T_{III}^2$, $R^2 = 0.77$. Vertical bars indicate LSD = 0.012 (A) and LSD = 1.6 (B).

In contrast, increased temperature during phase III significantly decreased the flower dry mass ($P = 0.032$) and area ($P = 0.006$), according to a quadratic relationship. For plants grown at 24 °C during phase III flowers were 26 % lighter and 33 % smaller than for plants grown at 15 °C. Predicted minimum flower dry mass was 0.20 g per flower and occurred at a temperature of 25.0 °C during phase III (Fig. 3A). Flower area showed an optimum value of 29.6 cm^2 per flower at 14.6 °C (Fig. 3B). As a result of the opposite effect of temperature during phase II and III, plants that received either 15 °C or 24 °C during all the SD period (phase II and III), resulted in an individual flower dry mass of 0.22 g per flower.

Flower position

Flower distance: Only a poor relationship between temperature and the distance between the highest and the lowest flower on the stem (flower distance) was found ($R^2 = 0.40$) (Fig. 4). Temperature during LD period had no significant effect on the flower distance ($P = 0.199$), and although a significant temperature effect was observed in each of the following phases of the cultivation period, flower distance varied between 29 and 35 cm only (Fig. 4).

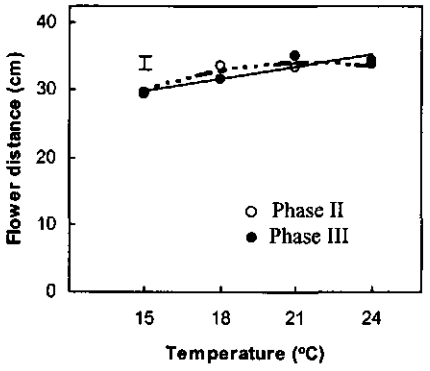

FIG. 4. Flower distance of *Chrysanthemum* 'Reagan Improved', as a function of temperature during phase II and phase III. Overall regression model: $y = -22.9 + 4.17T_{II} + 0.614T_{III} - 0.097T_{II}^2$, $R^2 = 0.40$. Vertical bar indicates LSD = 2.1.

Percentage of open flowers at the top 15 cm: The number of open flowers, located at the first 15 cm from the top of the plant, as a percentage of the total number of open flowers on the plant was analysed. This percentage was significantly influenced by the interaction between temperature during phase I and II ($P = 0.031$) and the interaction between temperature during phase II and III ($P = 0.003$). However, the overall regression model for these temperature effects only explained 36 % of the observed variance (Fig. 5). Plants grown at higher temperature during phase I showed a significantly lower percentage of open flowers, at the top part of the plant, except for 15 °C during phase II (Fig. 5A). In general, increasing temperature during phase II from 15 to 21 °C resulted in relatively less open flowers at the top of the plant (Fig. 5). A further increase to 24 °C, depending on the temperature during the following phase, did not affect the percentage of flowers at the top (e.g. 15 °C during phase III) or even increased it (e.g. 18 and 21 °C during phase III) (Fig. 5). No clear pattern for the effect of temperature in phase III could be distinguished.

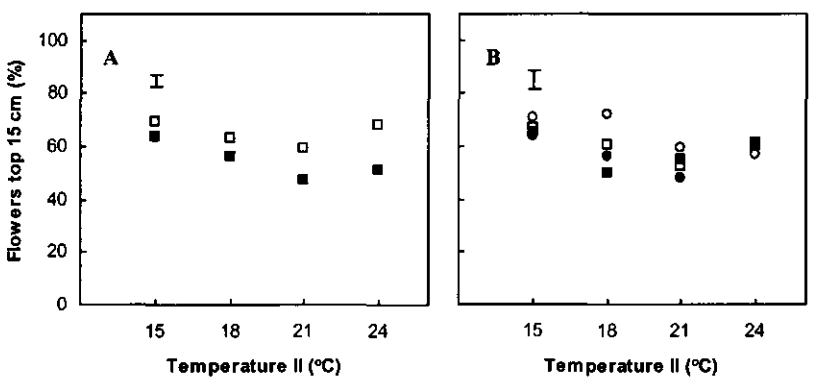

FIG. 5. Number of open flowers located at the first 15 cm from the top of the plant, as a percentage of total number of open flowers on the plant for *Chrysanthemum* 'Reagan Improved', as a function of the interaction between temperature during: (A) phase I and II; (B) phase II and III. Overall regression model: $y = 66.1 + 2.39T_I + 1.09T_{II} - 2.10T_{III} - 0.199T_IT_{II} + 0.148T_{II}T_{III}$, $R^2 = 0.36$. Symbols represent temperature during: (A) phase I: □ 18 °C; ■ 24 °C; and (B) phase III: ○ 15 °C; □ 18 °C; ● 21 °C; ■ 24 °C. Vertical bars indicate LSD = 5.2 (A) and LSD = 7.3 (B).

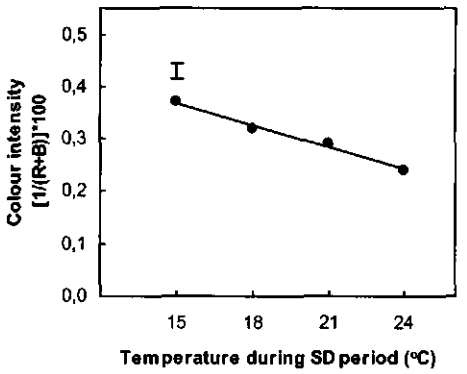

FIG. 6. Colour intensity of the ray florets of *Chrysanthemum* 'Reagan Improved', as a function of temperature during the complete SD period. Regression line: $y = 0.58 - 0.014T_{SD}$, $R^2 = 0.95$. Vertical bar indicates LSD = 0.031.

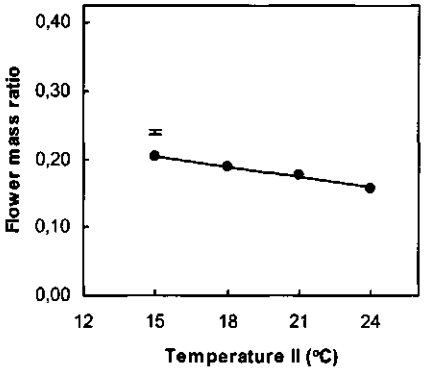

FIG. 7. Flower mass ratio of *Chrysanthemum* 'Reagan Improved', as a function of temperature during phase II. Regression line: $y = 0.28 - 0.0051T_{II}$, $R^2 = 0.71$. Vertical bar indicates LSD = 0.0069.

Flower colour

Colour intensity was expressed as [1/(red+blue)] × 100 to show the observed decay in the pink intensity (Fig. 6). Temperature during the complete SD period (phase II and III) showed a significant ($P < 0.001$) negative linear relationship with the colour intensity of the ray florets ($R^2 = 0.95$). The ray florets from plants grown at 15 °C during the SD period had a strong pink colour, whereas at 24 °C florets were white-pinkish.

Dry mass partitioning to the flowers

Relative dry mass partitioning towards the flowers (flower mass ratio, FMR), i.e. the ratio between total flower dry mass (TDM_f) and total aerial plant dry mass (TDM_p), did not significantly differ between plants grown at 17 or 21 °C during the complete cultivation period (Table 2). This was a consequence of no significant temperature effect on neither TDM_f nor TDM_p, over this range of temperatures.

When a larger temperature range was studied, temperature during phase II showed a significant negative linear effect on FMR ($P < 0.001$), which decreased from 0.20 to 0.15 as temperature increased from 15 to 24 °C (Fig. 7). This negative effect on FMR was only a result of a significant increase in TDM_p since a regression model with the overall temperature effect on TDM_f could only explain 27 % of the variance in TDM_f (data not shown). Actually, temperature could explain a larger part of the variation in TDM_p ($R^2 = 0.55$) and phase II was the period where TDM_p was most sensitive to temperature, showing a positive quadratic response with a minimum at 15.9 °C (data not shown). An increase in temperature from 15.9 °C to 24 °C, during this phase, resulted in 17 % TDM_p increase. FMR was neither significantly influenced by the temperature during phase I ($P = 0.521$), nor by temperature during phase III ($P = 0.056$), resulting in an overall average of 0.18.

TABLE 3. Summary[a] of the effects of temperature during different phases of the cultivation period on several flower characteristics in *Chrysanthemum* 'Reagan Improved' (Exp. 2).

	Temperature effect		
	Phase I (18, 24 °C)	Phase II (15, 18, 21, 24 °C)	Phase III (15, 18, 21, 24 °C)
Number of flowers & flower buds (NoF)	+	0	++
Percentage flower buds	+[b]	+[b]	+[b]
Individual flower dry mass	0	+	-
Individual flower area	0	+	-
Flower distance	0	+	+
Open flower distribution[c]	+[b]	+[b]	+[b]
Flower colour	0	0	--
Flower mass ratio	0	-	0

[a] *++ large positive effect, + positive effect, 0 no effect, - negative effect, -- large negative effect.*

[b] Interaction between temperatures in phase I and II, and between temperatures in phase II and III.

[c] Number of open flowers located at the first 15 cm from the top of the plant, as a percentage of total number of open flowers on the plant.

Discussion

The present study clearly demonstrates that temperature has a large impact on several flower characteristics in cut chrysanthemum and, therefore, it strongly affects external quality at harvest (Table 3). It was shown that the influence of temperature depends on the phase of the cultivation and on the flower characteristic itself. In general, flower characteristics were less responsive to temperature applied during the LD period (phase I), compared to the SD period (phase II and III). For instance, individual flower size (Fig. 3) and flower distance (Fig. 4) were only sensitive to temperature, during the SD period, whereas NoF slightly increased with temperature during phase I (Fig. 1). The reason for this smaller response of the flower characteristics to temperature during the LD period, is related to the fact that chrysanthemum is a SD plant and, therefore, flower initiation and development are induced by SD conditions (Cockshull and Hughes, 1971; Horridge and Cockshull, 1989). Karlsson *et al.* (1989a) divided the SD period in four phases and observed that flower development rate depends not only on the prevailing temperature, but also on the temperature during the preceding phase. This is in agreement with the present results as significant interactions, between temperatures applied during different phases of the cultivation, were observed for some flower characteristics, but they involved sequential phases only as no interaction between phases I and III was found (Fig. 2 and 5).

Number of flowers per plant and individual flower size

Higher temperatures increased NoF, mainly because more flower buds were produced (Table 3). A positive effect of temperature (14 to 18 °C) on NoF was also reported by LePage *et al.* (1984). It is often thought that only during the first weeks of SD (phase II), temperature is important for flower initiation. Therefore it seems unexpected, that NoF mainly responded to temperature during phase III, whereas no effect was observed during phase II (Fig. 1). This response is possibly related to the chrysanthemum basipetal progression of flower initiation and development under SD conditions (Langton, 1992). Hence, flower initiation starts to take place in the terminal and surrounding meristems around the first 6 to 8 days of the SD period (Cockshull, 1979), which is common to all the treatments. These flower buds already start to develop during phase II (Adams *et al.*, 1998), but buds originating from more basipetal branches and from the second order shoots will initiate only in the second part of the SD period (phase III). The higher the NoF, the more buds were initiated in this phase. This was the case of the higher temperatures during phase III.

The influence of the temperature during LD on NoF (Table 3) is probably the result of more leaves and internodes initiated at higher temperature (not shown), so more possibilities (axillary buds) for the formation of lateral branches. Besides, it also agrees with an observed positive effect of assimilate availability on chrysanthemum flower number (Carvalho *et al.*, 2002), in this case as a result of increased leaf unfolding rate (Karlsson *et al.*, 1989c), which increased light interception.

In Exp. 1, increasing temperature from 17 to 21 °C had a negative influence on flower size (Table 3). This is in agreement with previous findings where higher night temperature resulted in smaller flowers in several cultivars (Willits and Bailey, 2000). However, no overall effect was observed on Exp. 2 due to the opposite effect of temperature during phase II and III that counteracted each other. A possible reason for the difference observed between the two experiments is the higher irradiance at which the plants were grown in Exp. 2 (13.5 mol $m^{-2}d^{-1}$, as compared to 7.1 mol $m^{-2}d^{-1}$ in Exp. 1). This might have reduced the sensitivity of the plants to higher temperatures, as described by Karlsson and Heins (1996) and Karlsson *et al.* (1989b). This interaction between temperature and irradiance was also reported in Chapter 4.1. Flower size depends on the number of florets and the size of individual florets. Higher temperature hastened floret initiation and development resulting in an increased number of florets: 7-8 rows of florets after 21 SD at 15 °C, instead of only 4-5 rows after 28 SD at 10 °C (Karlsson and McIntyre, 1990). This is a possible explanation for the positive effect of temperature during phase II on flower size, although from the paper of Karlsson and McIntyre (1990) it is not clear whether also final number of florets was increased with temperature. Furthermore, supra-optimal temperatures (26/30 °C instead of 18/22 °C) strongly reduced the number of rows of florets initiated, observed after 28 SD (Whealy *et al.*, 1987).

Only the temperature during phase III influenced both NoF and individual flower size, and a higher temperature enhanced NoF at the expense of individual flower size. This is most likely due to competition for assimilates, because of the increased flower number (number of sinks). Furthermore, reduced assimilate availability because of higher maintenance respiration at higher temperatures may also contribute to this. This opposite effect of temperature during phase III, on NoF and individual flower size, suggests that this phase is a critical period where temperature should be adjusted according to the quality aim (more smaller flowers, or less bigger ones).

Percentage of flower buds and flower position

Higher temperatures increased NoF, but plants had also relatively more flowers in the bud stage (Table 3). For instance, plants grown at 24 °C during phase III had about 50 % of their flowers in the bud stage, independently of the temperature during the previous phases. This increase in the number of buds at higher temperatures is because of continued flower initiation and development on more basipetal lateral branches and on second order shoots. These buds are later initiated compared to the apical and surrounding meristems and, therefore, experienced strong competition for assimilates from them, resulting in more flowers remaining at the bud stage.

The effect of temperature on flower position within the plant was studied based on flower distance and percentage of open flowers located at the top 15 cm. In both cases temperature explained only a small percentage of their variation (Fig. 4 and 5). Plant density is probably a more important factor than temperature in explaining flower position, as high plant density results in a lower red:far-red ratio that stimulates primary stem elongation and inhibits lateral branching (Heins and Wilkins, 1979). Hence, flower distance is expected to decrease and percentage of open flowers located at the top 15 cm is expected to increase with plant density.

Flower colour

At harvest stage flower colour was homogeneous within each growth chamber, which leads to the conclusion that only phase III was involved in the colour formation process. This is in agreement with Weiss (2000) who reported that the accumulation of anthocyanins, pigments responsible for the pink colour, occurs during later stages of petal (ray florets) development. Furthermore, Whealy *et al.* (1987) observed that floret colour of *Chrysanthemum* 'Orange Bowl' was negatively affected (yellow rather than orange-yellow, which is typical of the cultivar) by exposure to high temperatures, only after the seventh week of short days.

The colour intensity of the ray flowers decreased linearly with increasing temperature from 15 to 24 °C during the (last part of the) SD period (Fig. 6). This is consistent with previous studies where 15 °C was the optimum temperature for the conversion of sugars into anthocyanins (Stickland, 1974; De Jong, 1978). Furthermore, temperature also plays a role in the availability of sugars in the plant, as at higher temperatures more sugars are consumed for maintenance respiration (De Jong, 1978).

Flower mass ratio

The dry mass partitioning to the flowers was only affected by temperature in phase II, and higher temperatures resulted in a smaller proportion of assimilates diverted to the flowers (Fig. 7). At beginning of phase II, vegetative growth in the apical meristem is still going on until flower induction has started (Cockshull, 1979). This negative effect on FMR may be explained by an increased production of gibberellins in the young leaves at higher temperatures, as observed in tomato (Abdul and Harris, 1978), which makes these vegetative organs stronger competitors for assimilates in a very important period for flower initiation. Total biomass production increased (faster build up of leaf area and hence light interception) but because of the stronger competition from the vegetative parts, flowers could not take their proportional share, resulting in a decreased FMR.

Conclusions

Temperature influence on chrysanthemum flower characteristics varied greatly with the phase of the cultivation period and with the flower characteristic itself. Thus, it is not possible to determine one optimum temperature as no consistently better temperature was found within each of the studied phases of the cultivation period. For example, temperature during phase III affected number of flowers and individual flower size in opposite ways. Therefore, priorities must be defined, as the desired temperature cannot disregard the quality aim.

A practical limitation of using a more dynamic heating strategy, adjusted to the phase of the cultivation period, can exist in a continuous chrysanthemum production program. In such conditions, it may occur that plants grown in the same compartment are at different stages of development (Hanan, 1998). Attention should also be paid when generalising this information to other chrysanthemum cultivars, since temperature effects on some flower characteristics such as number and size are highly cultivar dependent (Chapter 2).

4

Effect of assimilate availability

4.1

Flower characteristic and plant height: an integrated study

Abstract

The influence of assimilate availability on the number of flowers per plant, individual flower size and plant height of chrysanthemum was investigated in different seasons, integrating the results from eight greenhouse experiments. Increased assimilate availability was obtained by higher light intensity, higher CO_2 concentration, lower plant density or longer duration of the long-day (LD) period. Within each experiment, conditions that were expected to increase assimilate availability indeed resulted in higher total aerial dry mass of plant (TDM_p). In contrast, flower mass ratio was hardly affected, except for the increased duration of the LD period that significantly reduced the partitioning towards the flowers. Consequently, an increase in total flower dry mass with assimilate availability was observed and this was mainly a result of higher number of flowers per plant, including flower buds (NoF). Individual flower size was only influenced by assimilate availability when average daily incident PAR during short-day period was lower than 7.5 mol $m^{-2}d^{-1}$, resulting in lighter and smaller flowers. Excluding the positive linear effect of the duration of the LD period, assimilate availability hardly influenced plant height (< 10 % increase). It is concluded that within a wide range of growth conditions chrysanthemum invests additional assimilates, diverted to the generative organs, in increasing NoF rather than in increasing flower size. Irrespective of the growth conditions and season a positive linear relationship (R^2 = 0.90) between NoF and TDM_p was observed. This relationship was cultivar-specific. The generic nature of the results is discussed.

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Introduction

Chrysanthemum is produced year-round in greenhouses and it represents one of the most intensive and controlled crop production systems in horticulture (Machin, 1996). Since prices are to a great extent based on visual quality attributes (Vonk Noordegraaf and Welles, 1995; Trip *et al.*, 2000), growers aim at high and constant quality throughout the year (Langton *et al.*, 1999). Number of flowers per plant, flower size and plant height are key attributes of chrysanthemum quality and they depend on the cultivation measures and climate conditions (Chapter 2). Therefore, to reduce the seasonal fluctuations in quality, growers adapt plant density and duration of the long-day (LD) period to the cultivation season (Chapter 5.2). The use of supplementary assimilation light, during periods of poor natural light conditions, and CO_2 enrichment is becoming a common practice in chrysanthemum production in The Netherlands (Spaargaren, 2002), which leads to an improvement of the winter quality.

Although many studies on the effects of the growth conditions on plant growth and development have been conducted in chrysanthemum, only few have focused on the external quality attributes, especially on the flower characteristics. Furthermore, only a limited number of experiments have addressed several growth conditions simultaneously, which is important to establish functional relationships between the environment and plant responses (Karlsson and Heins, 1986). However, to achieve high quality chrysanthemum year-round an integrated and quantitative evaluation of the effect of the growth conditions is of utmost importance (Chapter 2). It has been long since it was reported that increased light intensity and CO_2 concentration produced higher total flower dry mass (Cockshull and Hughes, 1967; Hughes and Cockshull, 1972). Since then, several studies showed a positive effect of light intensity on number of flowers per plant (Parups, 1978; Eng *et al.*, 1985; Andersson, 1990) and on total flower area (Karlsson *et al.*, 1987, 1989b). Similarly, higher CO_2 level was shown to enhance number of flowers per plant (Mortensen, 1986a; Gislerød and Nelson, 1989) and to increase flower diameter of 'standard' chrysanthemum cultivars (Lindstrøm, 1968). Nevertheless, as no systematic study was carried out on this topic, it remains unclear whether an increase in total flower dry mass results from an increased number of flowers per plant, a larger individual flower size or from a combination of both aspects. A recent study by Carvalho *et al.* (2002) showed that higher light intensity and lower plant density resulted in heavier plants with increased number of flowers per plant including flower buds (NoF), whereas individual flower size was not significantly affected.

Temperature is the major climatic factor used to control plant height in chrysanthemum, based on the DIF concept (Chapter 3.1). Besides, supplementary light, under low natural irradiance levels (Hughes and Cockshull, 1972; Parups, 1978; Eng *et al.*, 1985), or increased CO_2 concentration (Mortensen and Moe, 1983; Mortensen, 1986a) resulted in taller plants. Moreover, increasing plant density to more than 83 plants m^{-2} strongly reduced plant height (Huld and Andersson, 1997), especially in autumn and winter crops (Langton *et al.*, 1999). These findings suggest that higher levels of assimilates sustain stem elongation (Bertram and Karlsen, 1994) and that low assimilate availability can be a limiting factor for elongation (Chapter 2).

In the present work, the total dry mass of the plant is considered as a measure of the assimilate availability, as heavier plants reflect higher source activity. This implicitly assumes one common 'pool of assimilates' and, hence, no compartmentation in source-sink units within the plant. Assimilates are distributed from this pool based on the organ 'sink strength', as described by Heuvelink (1995a). The hypothesis that, irrespective of the growth conditions, higher assimilate availability increases flower quality (NoF and individual flower size) and plant height is tested for cut chrysanthemum. This study aims at quantifying the effect of assimilate availability on these external quality attributes using an integrated approach. Assimilate availability is taken as the integrating factor among different experiments, rather than focusing on specific growth conditions. Thus, several light intensities, plant densities, durations of the LD period and CO_2 concentrations were applied to achieve a wide range of assimilate availability levels. To determine the generic nature of the observed effects, the experiments were conducted in different seasons of the year and in one experiment three cultivars were used.

Material and methods

Experimental set-up

Eight experiments were conducted in two to four compartments (12.8 m × 12.0 m) from a multispan Venlo-type glasshouse (Wageningen University, The Netherlands, lat. 52 °N), between Sep. 1999 and Jul. 2001 (Table 1 and 2). Before each experiment the nutrient condition was adjusted based on a soil analysis (BLGG, Naaldwijk, The Netherlands) and soil was steam-sterilised in Dec. 1999 and Dec. 2000.

Block-rooted cuttings of *Chrysanthemum* 'Reagan Improved', 'Goldy' and 'Lupo' (Fides Goldstock Breeding, Maasland, The Netherlands) were planted in soil beds on the dates indicated in Table 1. The 'santini type' cultivars, 'Goldy' and

'Lupo', were used in Exp. 8 only. Each compartment contained eight parallel soil beds (1.125 m × 10.25 m per bed), from which the two outer beds acted as borders. Light intensity was varied using supplementary lighting in the autumn and winter crops (Exp. 1, 6 and 7) and creating two shade levels in summer (Exp. 5). In experiments with supplementary light as a treatment, the compartments were split in two halves and only the two middle soil beds of each half compartment were used to allocate the treatments. Each shade level was obtained by installing a white plastic net located on top (1.5 m height from the ground) and sides of two contiguous soil beds. Two different meshes were used, resulting in 66 % and 43 % light intensity compared to the unshaded control.

Single-stem plants were grown supported by a wire mesh (0.125 m × 0.125 m), connected to a movable frame that included the heating pipes. Plants were initially submitted to LD conditions followed by a short-day (SD) period up to the final harvest (Table 1). A plant density of 64 plants m^{-2} and a LD period of 21 days were used in all experiments, but when these factors were studied more levels were included (Table 2). In autumn and winter, light from either incandescent lamps (control - Exp. 1; 7 μmol $m^{-2}s^{-1}$ photosynthetically active radiation, PAR) or high-pressure sodium lamps (control - Exp. 6 and 7; HPS Philips SON-T Agro, 9 μmol $m^{-2}s^{-1}$ PAR) was applied to the crop to extend the natural photoperiod. Furthermore, in some treatments a higher intensity assimilation light (Assim., HPS Philips SON-T Agro, from 44 to 58 μmol $m^{-2}s^{-1}$ PAR) was used to supplement the natural sunlight (Table 1). PAR was measured at a constant height of 0.5 m from the ground, using a 1.0 m line quantum sensor (LI-COR, model LI-191SA; Lincoln, USA). In most experiments, except in Exp. 3, lamps were kept continuously on during the 19 day hours of the LD (from 0500 to 2400 h) and 11 h of the SD period (from 0800 to 1900 h). In Exp. 3, the lamps were switched off when outside global radiation was higher than 150 W m^{-2}. In spring and summer plants were grown under natural light conditions during the LD period, which resulted in approximately 16 h (Exp. 4 and 8) or 17.5 h (Exp. 5) of light per day. The SD conditions were achieved closing the blackout screen for 13 h a day (from 1900 to 0800 h).

Irrigation was provided as required by two pipe systems with micro sprinklers. Overhead irrigation was applied from planting to visible flower buds and from then on, up to harvest, plants were irrigated using the system located on the ground. In all experiments, plant protection was applied according to an integrated pest management scheme (IPM), using both biological and chemical agents. No growth regulators were applied to the crop.

TABLE 1. General information on eight greenhouse experiments using *Chrysanthemum* 'Reagan Improved' (all Exp.) or 'Reagan Improved', 'Goldy' and 'Lupo' (Exp. 8). Dates are expressed as day of the year (day 1 = 1 Jan.).

Experiment	Year	Season	Planting Dates	Duration of Long-day (d)	Duration of Short-day (d)	Harvest Dates
1	1999	Autumn	273	21	60-61	354-355
2	1999	Autumn	273	21	57-60	351-354
3	2000	Autumn	250, 257, 264	21, 14, 7	61-63	332-334
4	2001	Spring/ Summer	129, 139, 150	21, 11, 0	55-61	205-211
5	2000	Summer	160	21	55-60	236-241
6	2001	Winter	24, 33, 45	21, 12, 0	58-65	103-110
7	2001	Winter	24	21	57-59	102-104
8	2000	Spring/ Summer	124	21	48-54	193-199

Experiment	Outside Global Radiation [x] ($mol\ m^{-2}d^{-1}$)	Incident PAR [x] ($mol\ m^{-2}d^{-1}$)	Temperature [y] (°C)	CO_2 [z] ($\mu mol\ mol^{-1}$)
1	18.4	4.2 (C) 5.9 (Assim.)	19.3	583
2	18.5	5.9 (Assim.)	19.1	410
3	25.4	6.3- 7.3 (Assim.)	20.0	392
4	90.9	18.1-18.3	22.6	345
5	77.6	17.0 (100 %) 11.2 (66 %) 7.3 (43 %)	22.2	345
6	28.6	6.3- 7.7 (C) 8.6- 9.8 (Assim.)	19.6	508
7	27.3	6.3- 6.4 (C) 8.5- 8.6 (Assim.)	19.6	398 (Control) 623 (High CO_2)
8	81.9	17.7	21.5	379

[x] Averaged over the whole cultivation period. For incident PAR the range indicates different treatments. C = treatments with no supplementary assimilation light; Assim. = treatments with supplementary assimilation light.

[y] 24 h average greenhouse temperature, averaged over the whole cultivation period.

[z] From 1000 to 1600 h average greenhouse CO_2, averaged over the whole cultivation period.

The terminal flower bud was pinched as soon as it was separated from the other crown buds (< 5 mm). In each experiment, plants were harvested when the first row of disc florets had reached anthesis in at least three inflorescences (flowers) per plant. Since within each experiment flower development rate slightly differed among the treatments, harvest was spread over 2 to 8 days (Table 1).

Greenhouse climate

For all the experiments, day temperature was set at 18.5 °C and night temperature was 1 °C higher, which is common practice in commercial chrysanthemum production in The Netherlands. Ventilation temperature set point was either 1 °C higher than the heating temperatures (Exp. 1, 2, 3, 5 and 8) or was 20.5 °C continuously (Exp. 4, 6 and 7). In general, pure CO_2 was supplied when CO_2 concentration in the greenhouse was lower than 350 μmol mol^{-1} and dosing was stopped at 400 μmol mol^{-1}. In Exp. 1, 6 and 7 the CO_2 set points were higher, with a maximum of 700 μmol mol^{-1} in Exp. 6 (high CO_2 treatment) but decreasing gradually when the ventilators were open. Greenhouse temperature was measured using PT500 elements. CO_2 concentration was measured with a CO_2 analyser (URAS G, Hartmann & Braun; Frankfurt, Germany).

Greenhouse temperature, relative humidity and CO_2 concentration were automatically recorded each five minutes by a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Daily outside global radiation was obtained from a meteorological station located at about 100 m distance from the greenhouse compartments. Actual values are shown in Table 1.

Daily incident PAR takes into account the loss of radiation during the SD period and the additional PAR from the supplementary light. Calculations were done based on measured daily integral of outside global radiation and measured glasshouse transmissivity of 0.49 (average of 42 positions measured on a cloudy day). From daily global radiation integral, 30 minutes values for diffuse and direct PAR were calculated according to Gijzen (1992) and instantaneous greenhouse transmissivity was determined using Bot's (1983) model, parameterised as in Heuvelink *et al.* (1995b). This model predicts transmissivity for direct radiation based on solar position, orientation of the greenhouse, greenhouse roof angle, dimensions of the roof construction parts and transmissivity of the glass panes. To correct for the lower transmissivity of the present greenhouses, compared to the 0.62 transmissivity described by Heuvelink *et al.* (1995b), calculated instantaneous greenhouse transmissivity was divided by 0.62 and multiplied by 0.49.

Treatments

Several levels of light intensity, CO_2 concentration, plant density and duration of the LD period were applied in different seasons of the year, resulting in a total of 47 treatments (Table 2). In Exp. 1 to 4 only one factor was analysed, whereas in the remaining experiments more factors were combined to test possible interactions among them. In the latter experiments, the number of treatments resulted from all combinations of the studied factor levels. In Exp. 8, the 'santini type' cultivars were chosen due to their typical flower characteristics, i.e. high NoF of small size. 'Goldy' and 'Lupo' were selected because they belong to the same response group as 'Reagan Improved' (i.e., 7.5 weeks from the start of the SD period up to harvest).

TABLE 2. Experimental set-up applied to eight greenhouse experiments using cut chrysanthemum.

Exp.	Factors	Levels [x]	Number of		Statistical design [y]
			Compart.	Blocks	
1	Light intensity	C, Assim.	2	2	CRBD
2	Density (plants m^{-2})	32, 48, 64	2	2	CRBD
3	Duration of LD (d)	7, 14, 21	2	6	CRBD
4	Duration of LD (d)	0, 11, 21	3	3	CRBD
5	Light intensity (%) Density (plants m^{-2})	100, 65, 45 32, 64, 80	3	3	Split-plot Main factor: Light Split factor: Density
6	Light intensity Duration of LD (d)	C, Assim. 0, 12, 21	4	2	Split-plot Main factor: Light Split factor: LD
7	CO_2 (µmol mol^{-1}) Light intensity Density (plants m^{-2})	350, 700 C, Assim. 32, 48, 64	4	2	Split-split-plot Main factor: CO_2 Split factor: Light Split-split factor: Density
8	Density (plants m^{-2}) Cultivar	32, 64, 80 Reagan, Goldy, Lupo	1	3	Split-plot Main factor: Density Split factor: Cultivar

[x] C = treatments with no supplementary assimilation light; Assim. = treatments with supplementary assimilation light.

[y] CRBD = Complete randomised block design.

Measurements

For each experiment one destructive measurement was carried out at harvest stage, using five (Exp. 3 to 8) or six (Exp. 1 and 2) plants per experimental unit, resulting in 10 to 30 plants per treatment (Table 2). Plants were randomly selected, excluding the two border rows on each side of the bed and at least two rows between different treatments. Total dry mass of the plant excluding the roots (TDM_p) and total dry mass of the flowers (TDM_f) were measured (ventilated oven, 105 °C for at least 15 h). Number of flowers and flower buds (> 5 mm), as well as plant height were recorded. Both individual flower dry mass and individual flower area (LI-COR, model 3100 Area Meter; Lincoln, USA) were determined for the fully open flowers only (i.e., inflorescences with ray florets in horizontal plane). These last two measurements were performed in all experiments, except for Exp. 5. In Exp. 8 the three cultivars were measured but only at 64 plants m^{-2}. No root measurements were conducted.

Statistical design and analysis

The statistical design was laid out in two up to six blocks (Table 2). Depending on the experiments a block consisted of two consecutive soil beds (Exp. 3 and 8), one compartment (Exp. 1, 2, 4 and 5) or two adjacent compartments (Exp. 6 and 7). An analysis of variance was conducted for each experiment and treatment effects were tested at 5 % probability level. Mean separation was done using Student's *t*-test (P = 0.05). Except for Exp. 3, block effects were never significant and were, therefore, removed from the statistical analysis. Sum of squares and degrees of freedom were added to the residual term. The effect of the quantitative factors was separated in a linear and a quadratic component, when more than two levels were studied. The statistical software package Genstat 5 (VSN International Ltd; Herts, UK) was used. When fractions were calculated, the normality of the data was checked with the 'Kolmogorov-Smirnov' test, from SPSS package (SPSS Inc.; Chicago, USA). The data were considered normally distributed and, therefore, no transformations were necessary.

Results

Dry mass production and partitioning to the flowers

Plants grown under higher light intensity (Table 3), lower plant density (Table 4) and longer duration of the LD period (Table 5) were significantly heavier than the control

plants. Plant density was the factor that influenced TDM_p the most within the applied levels (Table 4). The strongest effect was observed in summer (Exp. 5), as a wider range of plant densities was used. Hence, TDM_p of 'Reagan Improved' grown at 32 plants m^{-2} was approximately two times as high as at 80 plants m^{-2} (Table 4, Exp. 5). Although CO_2 concentration did not have a significant effect on TDM_p, plants grown at 625 μmol mol^{-1} were 15 % heavier than the ones grown at 400 μmol mol^{-1} (Table 6). A comparison between plants from different experiments, which were grown under the same plant density and duration of the LD period, revealed a seasonal effect. For instance, plants that received 21 LDs and were planted in summer (Exp. 4) were 45 % and 20 % heavier than when planted in autumn (Exp. 3) and winter (Exp. 6), respectively (Table 5).

In spite of the large variation in TDM_p, the flower mass ratio (FMR = TDM_f / TDM_p; i.e. proportion of dry mass allocated to the flowers) observed within each experiment, was not significantly influenced by the light intensity, plant density nor CO_2 concentration, or only a very small effect was found (Tables 3, 4 and 6). For instance, varying plant density had a very strong impact on TDM_p of the three studied cultivars (Fig. 1), but FMR of 'Reagan Improved', 'Goldy' and 'Lupo' was hardly affected: 0.19, 0.21 and 0.22, respectively (data not shown). However, plants that received a different duration of the LD period differed significantly in their FMR (Table 5). An increase in the number of LDs strongly reduced the partitioning towards

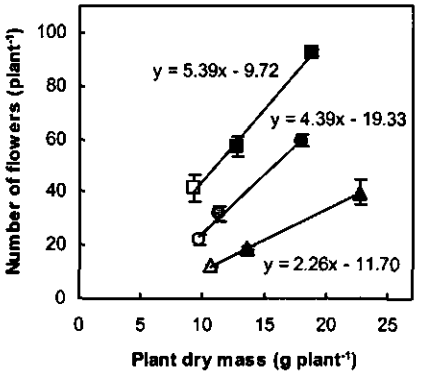

FIG. 1. Relationship between total number of flowers per plant, including buds, and total dry mass per plant at harvest of *Chrysanthemum* cultivar 'Reagan Improved' (triangles), 'Lupo' (circles) and 'Goldy' (squares) grown at 32 plants m^{-2} (black symbols), 64 plants m^{-2} (grey symbols) and 80 plants m^{-2} (white symbols) (Exp. 8). Lines represent linear regression. Vertical bars indicate s.e.m.

the flowers, especially when comparing plants that received no LD treatment with plants that received 11 to 21 LDs (Table 5 and Fig. 2). A significant interaction between light intensity and duration of the LD period was observed in Exp. 6 (Fig. 2). FMR showed a consistent negative linear response to the duration of the LD period but a small, though significant, negative effect of light intensity on FMR was only found at 21 LDs (Fig. 2). Similarly to the total plant dry mass, a seasonal effect was observed for FMR. In general, considering a 'standard' situation of no supplementary assimilation light, 64 plants m^{-2} and 21 LDs, plants grown in autumn had the lowest FMR, ranging from 0.111 (Table 5, Exp. 3) to 0.158 (Table 4, Exp. 2). In summer and winter crops FMR was higher, with a minimum of 0.183 (Exp. 5, data not shown) and a maximum of 0.230 (Fig. 2).

TABLE 3. Effect of light intensity on total dry mass of the plant excluding the roots (TDM_p), flower mass ratio (FMR), number of flowers and flower buds (NoF), individual flower dry mass and plant height at harvest of *Chrysanthemum* 'Reagan Improved'.

Exp.	Incident PAR (mol $m^{-2}d^{-1}$) [x]	TDM_p (g $plant^{-1}$)	FMR	NoF ($plant^{-1}$)	Individual flower DM (g $flower^{-1}$) [z]	Plant height (cm)
1	4.2 (C)	5.5	0.125	7.6	0.110	98.4
	5.9 (Assim.)	7.7	0.150	12.2	0.133	102.4
	F_{prob} [y]	***0.021***	*0.123*	***0.036***	*0.251*	*0.143*
5	7.3 (43 %)	9.1	0.167	16.1	---	Sig. Int. *[1]
	11.2 (66 %)	11.1	0.177	18.3	---	
	17.0 (100 %)	13.3	0.189	20.2	---	
	F_{prob} [y]	*< 0.001*	*0.027*	*0.006*		
	Linear	***< 0.001***	***0.010***	***0.002***		
	Quadratic	*0.098*	*0.691*	*0.307*		
6	6.9 (C)	6.7	Sig. Int. *[2]	11.4	0.218	83.8
	9.2 (Assim.)	8.3		14.6	0.217	88.6
	F_{prob} [y]	***0.006***		***0.005***	*0.855*	***0.020***
7	6.4 (C)	10.1	0.233	18.4	0.201	98.1
	8.6 (Assim.)	13.3	0.219	24.7	0.206	106.5
	F_{prob} [y]	***0.034***	*0.168*	*0.053*	*0.609*	*0.070*

[x] C = treatments with no supplementary assimilation light; Assim. = treatments with supplementary assimilation light.

[y] F probability (significant levels < 0.05 presented in bold).

[z] Average of the fully open flowers (at least 3 flowers per plant).

--- : Not measured.

Sig. Int. = Significant interaction with: *[1] plant density (not shown); *[2] duration of the LD period (Fig. 1).

TABLE 4. Effect of density on total dry mass of the plant excluding the roots (TDM_p), flower mass ratio (FMR), number of flowers and flower buds (NoF), individual flower dry mass and plant height at harvest of *Chrysanthemum* 'Reagan Improved'.

Exp.	Density (plants m^{-2})	TDM_p (g $plant^{-1}$)	FMR	NoF ($plant^{-1}$)	Individual flower DM (g $flower^{-1}$) [y]	Plant height (cm)
2	32	11.4	0.169	22.4	0.145	98.1
	48	9.9	0.160	17.1	0.142	100.5
	64	7.5	0.158	11.3	0.137	101.5
	F_{prob} [x]	*0.078*	*0.439*	*0.072*	*0.036*	*0.548*
	Linear	***0.036***	*0.263*	***0.032***	***0.016***	*0.323*
	Quadratic	*0.685*	*0.621*	*0.928*	*0.651*	*0.783*
5	32	15.6 c	0.179 ab	29.0 c	---	Sig. Int.
	64	9.7 b	0.172 a	14.4 b	---	
	80	8.1 a	0.182 b	11.2 a	---	
	F_{prob} [x]	*< 0.001*	*0.093*	*< 0.001*		
	Linear	*< 0.001*	*0.860*	*< 0.001*		
	Quadratic	***0.017***	***0.033***	***0.012***		
7	32	14.1	0.232	28.8	0.204 ab	100.9
	48	11.5	0.228	20.5	0.210 b	101.8
	64	9.4	0.216	15.3	0.196 a	104.4
	F_{prob} [x]	*< 0.001*	*0.007*	*< 0.001*	*0.069*	*0.011*
	Linear	***< 0.001***	***0.003***	***< 0.001***	*0.172*	***0.004***
	Quadratic	*0.607*	*0.265*	*0.290*	***0.050***	*0.286*

[x] F probability (significant levels < 0.05 presented in bold). Different letters indicate significant differences between treatments based on LSD at 5 % level.

[y] Average of the fully open flowers (at least 3 flowers per plant).

--- : Not measured.

Sig. Int. = Significant interaction with light intensity (not shown).

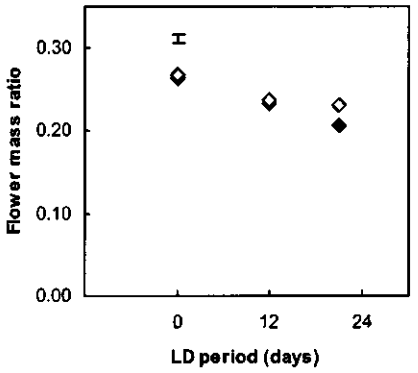

FIG. 2. Flower mass ratio as a function of light intensity (◊ treatments with no supplementary assimilation light; ♦ treatments with supplementary assimilation light) and duration of the LD period at harvest of *Chrysanthemum* 'Reagan Improved' (Exp. 6). Vertical bar indicates LSD = 0.011.

TABLE 5. Effect of duration of long-day (LD) period on total dry mass of the plant excluding the roots (TDM_p), flower mass ratio (FMR), number of flowers and flower buds (NoF), individual flower dry mass and plant height at harvest of *Chrysanthemum* 'Reagan Improved'.

Exp.	LD period (days)	TDM_p (g plant^{-1})	FMR	NoF (plant^{-1})	Individual flower DM (g flower^{-1}) [y]	Plant height (cm)
3	7	5.5	0.096	11.2	0.147	79.5
	14	6.5	0.107	12.1	0.140	92.6
	21	8.0	0.111	12.6	0.133	110.6
	F_{prob} [x]	*< 0.001*	*0.044*	*0.006*	*0.250*	*< 0.001*
	Linear	***< 0.001***	***0.016***	***0.002***	*0.116*	***< 0.001***
	Quadratic	*0.495*	*0.537*	*0.626*	*1.000*	*0.086*
4	0	6.6	0.272	11.2	0.217	70.3
	11	9.8	0.213	17.1	0.187	92.1
	21	11.6	0.199	16.8	0.207	110.7
	F_{prob} [x]	*0.002*	*0.002*	*0.067*	*0.317*	*< 0.001*
	Linear	***< 0.001***	***< 0.001***	***0.044***	*0.576*	***< 0.001***
	Quadratic	*0.406*	*0.083*	*0.181*	*0.169*	*0.596*
6	0	5.4	Sig. Int.	9.9	0.220	69.1
	12	7.5		13.3	0.216	88.5
	21	9.6		15.8	0.215	101.1
	F_{prob} [x]	*< 0.001*		*< 0.001*	*0.460*	*< 0.001*
	Linear	***< 0.001***		***< 0.001***	*0.231*	***< 0.001***
	Quadratic	*0.341*		*0.932*	*0.804*	*0.553*

[x] F probability (significant levels < 0.05 presented in bold).
[y] Average of the fully open flowers (at least 3 flowers per plant).
Sig. Int. = Significant interaction with light intensity (see Fig. 1).

TABLE 6. Effect of CO_2 concentration on total dry mass of the plant excluding the roots (TDM_p), flower mass ratio (FMR), number of flowers and flower buds (NoF), individual flower dry mass and plant height at harvest of *Chrysanthemum* 'Reagan Improved'.

Exp.	CO_2 (μmol mol^{-1})	TDM_p (g plant^{-1})	FMR	NoF (plant^{-1})	Individual flower DM (g flower^{-1}) [y]	Plant height (cm)
7	400	10.9	0.228	19.2	0.205	99.8
	625	12.5	0.223	23.9	0.202	104.9
	F_{prob} [x]	*0.065*	*0.504*	***0.028***	*0.698*	*0.088*

[x] F probability (significant levels < 0.05 presented in bold).
[y] Average of the fully open flowers (at least 3 flowers per plant).

Flower characteristics

Total number of flowers: Over a wide range of growth conditions, assimilate availability had a clear positive effect on the NoF. This positive effect was always significant with the exception of Exp. 7 where, nevertheless, plants grown under

supplementary assimilation light had 34 % more flowers per plant (Table 3). In general, increasing light intensity, duration of the LD period or CO_2 concentration resulted in 12.5 % (Table 5, Exp. 3) up to 61 % (Table 3, Exp. 1) higher NoF (Tables 3, 5 and 6). As described above for TDM_p, plant density treatments had the greatest impact on NoF (Table 4). Similarly to TDM_p, a significant negative linear response was found within the range of 32 to 64 plants m^{-2} and a further increase to 80 plants m^{-2} showed a quadratic effect on NoF. In the summer experiment, where the widest density range was studied, for plants grown at 32 plants m^{-2} average NoF was 29, which represented 2.0 and 2.6 times more flowers per plant than at 64 and 80 plants m^{-2}, respectively (Exp. 5, Table 4). Likewise, plant density had also a strong negative effect on NoF for the 'santini' cultivars. Plants grown at 32 plants m^{-2} had 2.2 ('Goldy') and 2.7 ('Lupo') higher NoF compared to plants grown at 80 plants m^{-2} (Fig. 1).

Besides the effect of the cultivation measures a strong seasonal effect was also observed on NoF. For example, chrysanthemum grown at 64 plants m^{-2} and receiving 21 LDs had 7.6 NoF when planted in Sep. (Table 3, Exp. 1) and 16.8 NoF when planted in May (Table 5, Exp. 4).

Individual flower size: In contrast with NoF, assimilate availability had in most experiments no significant effect on the average individual flower dry mass of the fully open flowers (Tables 3 to 6). Therefore, individual flower dry mass was found to be rather constant within each experiment. However, a clear seasonal effect was observed and basically two groups of flower dry mass could be distinguished. Plants with lighter fully open flowers were found only in autumn crops, showing an average mass between 0.121 g (Table 3, Exp. 1) and 0.145 g (Table 4, Exp. 2) per flower. Interestingly, plants grown during the winter, spring or summer resulted in heavier flowers with an average flower dry mass that varied between 0.204 g (Table 3 and 4, Exp. 4 and 7) and 0.218 g (Table 3, Exp. 6). In addition, flower size differed significantly between cultivars (Exp. 8). At 64 plants m^{-2} 'Reagan Improved' had the heaviest flowers (0.223 g $flower^{-1}$) followed by 'Lupo' and 'Goldy' which were, respectively, 33 and 49 % lighter.

Individual flower area showed the same response as individual flower dry mass (data not shown). On average the area of a fully open flower from a plant grown during autumn varied between 23.4 and 26.7 cm^2 per flower, whereas in the other seasons larger flowers were obtained (from 31.8 up to 32.7 cm^2).

Plant height

Increased assimilate availability normally resulted in taller plants, but a strong effect was only observed when plants received a different duration of the LD period. Thus, increasing the number of LDs resulted in a linear increase of plant height (Table 5). For instance, plants that received 21 LDs before starting the SD period were 46 % (winter, Exp. 4) or 57 % (summer, Exp. 6) taller compared to plants which received no LD period. Interestingly, plants that received no LD period still reached a marketable height of around 70 cm, in both seasons. For the remaining growth conditions, differences in plant height were always lower than 10 % within each experiment (Tables 3, 4 and 6). For example, the largest variation in plant height was observed in Exp. 5, where a significant interaction between light intensity and plant density was found, but still plant height ranged only between 104 and 114 cm. In this experiment, higher plant density enhanced the height of plants grown at 100 % and 66 % light, whereas it reduced plant height at 43 % light. Despite the significant difference in plant height of the three studied cultivars, with 'Reagan Improved' being on average 10 cm taller than 'Goldy' and 22 cm taller than 'Lupo', a positive linear increase of plant height with plant density was observed (no cultivar x plant density interaction; data not shown). Unlike the flower characteristics, plant height remained similar throughout the year.

A regression model for flower characteristics

Total number of flowers as a function of plant dry mass: Based on the observed similarities between TDM_p and NoF, in response to the various growth conditions (Tables 3 to 6), the correlation between these two variables was analysed for chrysanthemum 'Reagan Improved'. Using all the treatments from all the experiments, which resulted in 41 data points, a positive linear relationship was found and TDM_p could explain 90 % of the observed variance in NoF (Fig. 3A). This relationship covered a wide range of TDM_p and NoF. Plants that received no LD period and were grown at 64 plants m^{-2} without supplementary assimilation light during winter (Exp. 6), were the lightest (4.9 g $plant^{-1}$) and had only around 8 flowers including buds per plant. In contrast, plants that received 21 LDs and were grown at 32 plants m^{-2} during summer (Exp. 8), TDM_p was almost five times as high as the former and the highest NoF was obtained (40 flowers $plant^{-1}$).

To test whether this relationship was influenced by the growing season the experiments were divided into two groups: autumn and winter compared to spring and summer crops. Although a slightly better fit between NoF and TDM_p was observed for

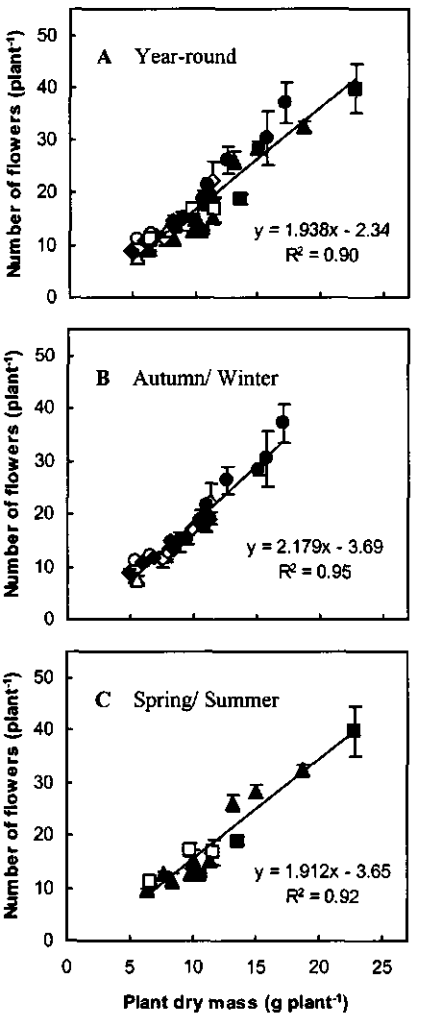

FIG. 3. Relationship between total number of flowers per plant, including buds, and total dry mass per plant at harvest of *Chrysanthemum* 'Reagan Improved' in all the experiments (A), in the autumn and winter experiments (B) and in the spring and summer experiments (C). Symbols represent: △ Exp. 1, ◊ Exp. 2, ○ Exp. 3, □ Exp. 4, ▲ Exp. 5, ♦ Exp. 6, ● Exp. 7, ■ Exp. 8. Line represents linear regression. Vertical bars indicate s.e.m. when larger than symbols.

the autumn and winter experiments (R^2 = 0.95), both seasons showed a very similar relationship (Fig. 3). Furthermore, a positive linear relationship between NoF and TDM_p was also found for the 'santini' type cultivars but these cultivars showed a higher slope compared to 'Reagan Improved' (Fig. 1). For instance, increasing TDM

from 10 g to 15 g would increase NoF by 27 for 'Goldy' and 22 for 'Lupo', whereas in 'Reagan Improved' NoF would increase by 11 only.

Individual flower size as a function of average daily incident PAR: Taking into account that season was the only factor that substantially influenced individual flower size (Tables 3 to 6) and that radiation represented the largest difference among seasons (Table 1), the relationship between radiation and individual flower size was studied. For each treatment, where individual flower size has been determined (30 treatments), average daily incident PAR for the complete cultivation period and specifically for the SD period was calculated. Average daily incident PAR for the complete cultivation period did not clearly explain the observed variation in the individual flower dry mass (Fig. 4A). For instance, plants that received 6.3 mol $m^{-2}d^{-1}$ PAR averaged over the whole cultivation period produced, depending on the season, flowers with a dry mass of 0.147 g $flower^{-1}$ (Sep.) or 0.218 g $flower^{-1}$ (Jan.).

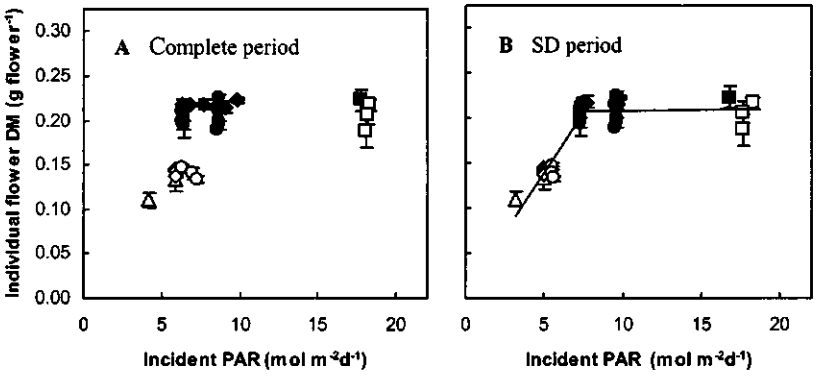

FIG. 4. Relationship between average individual flower dry mass of the fully open flowers and daily incident PAR, averaged over the complete cultivation period (A) and over the SD period (B), at harvest of *Chrysanthemum* 'Reagan Improved'. Symbols represent: △ Exp. 1, ◊ Exp. 2, ○ Exp. 3, □ Exp. 4, ▲ Exp. 5, ♦ Exp. 6, ● Exp. 7, ■ Exp. 8. Regression lines: $y = 0.0271x + 0.0042$; $R^2 = 0.92$, if PAR < 7.5 mol $m^{-2}d^{-1}$; $y = 0.208$, if PAR ≥ 7.5 mol $m^{-2}d^{-1}$. Vertical bars indicate s.e.m. when larger than symbols.

In contrast, individual flower dry mass was closely related to the daily incident PAR averaged over the SD period only. Thus, when the average daily incident PAR during the SD period varied between 7.5 and 18.3 mol $m^{-2}d^{-1}$ individual flower dry mass was constant (0.208 g $flower^{-1}$), but it decreased linearly with PAR for values below this critical light level (Fig. 4B).

Once more, individual flower area had a very similar response to the one described for individual flower dry mass (data not shown). Individual flower area increased linearly with daily incident PAR averaged over the SD period up to 7.1 mol $m^{-2}d^{-1}$ ($y = 2.81x + 11.7$; $R^2 = 0.95$) and a further increase in PAR had no effect, resulting in a constant individual flower area (31.6 cm^2 $flower^{-1}$).

Discussion

The present work provides an integrated view on the effects of a large range of growth conditions, which have in common their influence on assimilate availability in cut chrysanthemum. These growth conditions successfully influenced TDM_p that varied between 4.9 and 22.7 g, when applied in different seasons (Fig. 3A). Seasonal variations were mainly caused by differences in daily outside global radiation, and consequently daily incident PAR, as this represents the major difference among experiments conducted in distinct seasons (Table 1). A strong seasonal effect on TDM_p due to different daily light integral was also previously described (Langton, *et al.*, 1999; Lee *et al.*, 2002b). Furthermore, though to a lesser extent, differences in temperature could also partly result in a seasonal effect when comparing autumn and winter (19.5 °C) with spring and summer experiments (22.1 °C). Higher temperatures in spring and summer resulted from higher outside temperatures and radiation levels, combined with the closure of the blackout screens during the last hours of the day.

Flower mass ratio

According to Heins *et al.* (1984), increasing TDM_p does not necessarily result in higher quality, as the dry mass partitioning is a critical factor that should not be disregarded. In the present work it was observed that higher TDM_p resulted always in higher TDM_f, as the partitioning towards the flowers, i.e. FMR, remained unaffected within a wide range of growth conditions (Tables 3 to 6). The present observations are in agreement with Cockshull and Hughes (1967) who suggested that increasing TDM_p is the only way of obtaining higher TDM_f, as FMR at harvest stage was hardly affected by light intensity and CO_2 concentration. Likewise, Karlsson and Heins (1992) also reported that the proportion of dry mass allocated to the chrysanthemum flowers at harvest was not strongly correlated with light intensity. However, Langton *et al.* (1999) observed a large increase in FMR with increasing plant density. A possible reason for this difference may be the wider range of plant densities used in that study (41-121 plants m^{-2}). Furthermore, it has been shown for fruit vegetables that

assimilate partitioning is primarily regulated by the sinks, whereas source strength or assimilate availability has no direct influence (Marcelis, 1993b; Heuvelink, 1995c). On the long-term, source strength may influence fruit set or abortion and therefore dry matter partitioning (Marcelis and De Koning, 1995). This also happens in cut chrysanthemum (more flowers when more assimilates are available; Fig. 3). However, apparently, the extra number of flowers increases vegetative and generative sink strength in the same way, resulting in a constant FMR, which may be explained by the extra side shoots formed to support the extra flowers.

Within each season, FMR was only strongly affected by the duration of the LD period. Plants that received no LD period showed a higher partitioning towards the flowers compared to plants that received 11 to 21 LDs (Table 5, Fig. 2). This seems obvious, as chrysanthemum is a SD plant (Andersson, 1990). Under LD conditions all assimilates are partitioned to the vegetative parts, as no generative growth takes place. The longer the duration of the LD period the later the flower initiation (Cockshull, 1976) and consequently more assimilates are allocated to the vegetative organs during this phase, especially at higher light intensity (Fig. 2). The seasonal influence on FMR was also observed by Langton *et al.* (1999), who reported the lowest FMR in autumn, which is also shown in the present data (Tables 3 to 6). This may be the result of decreased individual flower size under a critical PAR level in the SD period (Fig. 4).

Individual flower size and number of flowers

Individual flower dry mass and area were to a large extent independent of light intensity, within the same experiment (Table 3). Nevertheless, a seasonal effect was observed, resulting in smaller flower size when chrysanthemum was planted in Sep. only (Exp. 1, 2 and 3). This seasonal effect was clearly related to the average daily incident PAR during the SD period, rather than during the whole cultivation period (Fig. 4). Individual flower size of the fully open flowers was only influenced by assimilate availability when average daily incident PAR, during the SD period, was lower than 7.5 mol $m^{-2}d^{-1}$ (Fig. 4B). These results might be explained by the observation that higher sensitivity to low light intensity occurs after two weeks of SD period, i.e. on the transition from leaf formation to the initiation of the flower receptacle (Cockshull and Hughes, 1971). Furthermore, these authors also observed a delay in floret initiation at low light intensity, which would possibly result in a lower number of florets and, hence, in a smaller flower.

The present experiments clearly show that an increase in TDM_f was mainly a result of higher NoF as individual flower size hardly changed. Therefore, within a wide range of growth conditions chrysanthemum invests additional assimilates, which

were diverted to the generative organs, in increasing the number of flowers rather than in increasing their size. This information is new, as in literature results are scattered over many reports and individual flower size is hardly ever measured. However, Langton *et al.* (1999) stated, without showing data, that increases in total flower fresh mass, resulting from lower plant density, were reflected both in increased NoF and higher mass per individual flower. Unfortunately, it is not clear how these characteristics were measured.

Prediction of number of flowers

It has been reported that increased light intensity (Eng *et al.*, 1985), lower plant density (Lee *et al.*, 2002b and Chapter 5.2), longer duration of the LD period (Chapter 5.2) or higher CO_2 concentration (Eng *et al.*, 1985; Mortensen, 1986a; Gislerød and Nelson, 1989) resulted in heavier plants with higher NoF, which was also found in this study. However, the present work goes further, showing that one simple linear function could describe the relation between TDM_p and NoF, based on all treatments from eight experiments with 'Reagan Improved', resulting in an excellent fit to the data (R^2 = 0.90; Fig. 3A). This relationship is very important for modelers as using a photosynthesis-driven model to predict TDM_p (e.g. CHRYSIM; Lee, 2002), NoF can be further estimated.

In Chapter 3.2 it was observed that NoF increased from 19 to 28 when temperature rose from 17 to 21 °C. Surprisingly, the regression model that relates NoF to TDM_p in 'Reagan Improved' showed no season effect and, therefore, no clear temperature sensitivity (Fig. 3B and C). A possible explanation is the fact that plants grown under higher light intensity (spring and summer experiments) became less temperature sensitive. For instance, it was observed that higher irradiance levels can partly compensate the negative effect of high temperature on time to flowering and on total flower area (Karlsson and Heins, 1986; Karlsson *et al.*, 1989b).

Plant height

Increased assimilate availability resulted in taller plants, but except for the positive linear effect of the duration of the LD period, the growth conditions had only a minor effect on plant height (< 10 %). In Chapter 5.2 it was reported that the positive effect of the duration of the LD period on plant height is mainly due to increased number of internodes rather than increased average internode length. The positive influence of plant density on the height of the plants (Table 4, Exp. 7) can be described as the 'shade avoidance' strategy. This strategy results in the enhancement of elongation growth, mediated by the reduction in the ratio of red to far-red radiation that occurs at

increased plant density (Smith and Whitelam, 1997). Nevertheless, when plants were grown at higher densities (32, 64 and 80 plants m^{-2}) combined with three light intensities, this strategy was not observed at the lowest light intensity (Exp. 5). These results reinforce the hypothesis that low assimilate availability can limit chrysanthemum stem elongation (Chapter 2).

Cultivar effects

To extend this study to other chrysanthemum cultivars one extra experiment was performed (Exp. 8). As plant density was very effective to obtain different TDM_p (Table 4), two 'santini' cultivars were compared with 'Reagan Improved' at three plant densities. Although 'Goldy' and 'Lupo' were morphologically different from 'Reagan Improved' (shorter plants, with more flowers of smaller size), the responses of TDM_p and NoF to plant density were very much alike (Fig. 1). Also for the 'santini' type cultivars a positive linear relationship between NoF and TDM_p was found, but the relationship was cultivar specific. Both 'santini' cultivars showed a higher slope, which reflects that for the same increase in TDM_p the plant produces a higher NoF (Fig. 1). This may be explained by their smaller flower size that allowed, for the same TDM_f, more flowers to be formed.

In addition, FMR and plant height were also not affected by plant density in these 'santini' cultivars, which shows that the validity and applicability of the results in this paper are not limited to one cultivar.

Conclusions

In chrysanthemum, TDM_p and NoF are strongly and positively related to assimilate availability, whereas individual flower size, FMR and plant height are hardly influenced. Assimilate availability only limits individual flower size when light intensity during the SD period is below a critical level. In general, only increasing the duration of the LD period results in significantly taller plants and with lower FMR. The linear relationship between NoF and TDM_p (irrespective of the growth conditions and season) as well as the rather constant individual flower size and FMR (observed within each season) are particularly valuable conclusions when aiming at developing a model for chrysanthemum quality. Although the three studied cultivars showed a similar behaviour, future investigations should be carried out to validate these findings for a wider range of cultivars.

4.2

Sink-source ratio affects flower size

Abstract

Sink-source ratio was manipulated in cut chrysanthemum by flower bud removal (leaving one, two or four flowers and a control), by axillary shoot removal and by varying light intensity. The influence of flower position within the stem on flower size was investigated for the terminal flower and for different lateral flowers.

Reducing the competition for assimilates (i.e. decreasing sink-source ratio by leaving fewer flowers per plant, removing axillary shoots or using supplementary assimilation light) resulted in a significantly higher individual flower dry mass and area in treatments where the number of flowers was imposed. Nevertheless, the control plants responded to the supplementary assimilation light with an increased number of flowers rather than by producing larger flowers. Flower position was found to have a negligible effect on flower size in both the disbudded and control plants, except that the second order lateral flowers were significantly smaller than the first order ones. Monoflower plants without side shoots represented the potential (maximum) flower size; they had flowers up to 2.4 times as heavier and 76 % as larger in area as the control plants. Interestingly, the ratio of the flower disc diameter to the total diameter was maintained at 0.2. Higher leaf starch content and lower specific leaf area (thicker leaves) were observed in the monoflower treatments. Plant dry mass was only reduced at the lowest sink strength treatment, whereas flower mass ratio (FMR) showed a saturation response to the number of flowers per plant.

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Introduction

Flower size is an important visual quality attribute of cut chrysanthemum (Chapter 2), which is characteristic of a given cultivar, and it is frequently described by the commercial propagators (Spaargaren, 2002). Indeed, in a previous study it was shown that individual flower size was rather constant under a large range of growth conditions, but it differed depending on the cultivar (Chapter 4.1). Nevertheless, when the daily incident photosynthetically active radiation (PAR) during the short-day period was under a certain threshold (< 7.5 mol $m^{-2}d^{-1}$), individual flower dry mass was reduced by 13 % per mol $m^{-2}d^{-1}$ (Chapter 4.1). On the other hand, it is well known that the size of the sink organs (e.g. flower and fruit size) increases with lower plant sink-source ratio (Heuvelink, 1997). Also when competing flower buds were removed in an early stage in chrysanthemum, flower size was enhanced (Cockshull, 1982; Lee *et al.*, 2001; Carvalho *et al.*, 2002). Carvalho *et al.* (2002) showed that the size of a cut chrysanthemum flower from a plant with only one lateral flower and without side shoots, had 50 % larger area compared to the flower size from the control spray-type plants.

Sink-source ratio can be manipulated by varying the sink strength (demand for assimilates), the source strength (supply of assimilates, i.e. plant photosynthetic rate) or by manipulating both. Although it is clear that both sink strength and source strength can influence flower size, this topic has been poorly addressed in cut chrysanthemum, and several important questions still remain. It is not known whether one flower per plant reflects the potential (maximum) flower size, i.e. the size reached under conditions of non-limiting assimilate availability (Marcelis, 1996), and whether this potential size is cultivar-specific and whether it can be reduced under low light conditions. Furthermore, the effect on flower size of removing flower buds or removing complete axillary shoots at an early stage of plant development may differ: developing axillary shoots are competitors for assimilates, but because of leaf development they may become sources later on. Therefore, it is not clear when axillary shoots will act as a sink or source organs, i.e. organs that are, respectively, a net importer or exporter of carbon assimilates (Marcelis, 1996). Finally, it is not known whether, and to what extent, flower size depends on its position on the plant, e.g. can a flower bud initiated later reach the same size as an early initiated bud?

Sink-source ratio has also been described to have an effect on the hormonal balance and on the carbohydrate status of the plant, which can interfere with stomatal aperture, mesophyll conductance etc, and consequently affect photosynthesis (Guinn and Mauney, 1980). Accumulation of starch in the leaves can indicate a reduced sink-

source balance and this has been used as an explanation for a negative feedback control on photosynthesis at low sink demand, the so-called end-product inhibition (Guinn and Mauney, 1980; Foyer, 1988). This situation occurs when the assimilate production exceeds the capacity of utilisation by the sink organs (Foyer, 1988). Therefore, under conditions of non-limiting assimilate availability accumulation of starch is expected, and the potential flower size can be determined. Sink strength, i.e. the competitive ability of a flower to accumulate assimilates (Marcelis, 1996), is quantified by the potential flower growth rate, and it may be used for simulating the partitioning of assimilates towards the flowers (Heuvelink, 1996).

The present work aims at quantifying and understanding the effects of sink and source strength on flower size of cut chrysanthemum. It is attempted to separate the effects of the number of flowers (competition of generative sinks), axillary shoots (competition of vegetative sinks), and flower position, on flower size. Also, the possible negative effect of strongly reduced sink strength on source strength is studied. Most work was conducted on cultivar 'Reagan Improved', but to test possible cultivar effects extreme sink-source ratio treatments were also applied to two 'santini' cultivars.

Material and methods

Experimental set-up

Four experiments with different planting dates (Table 1) were carried out in 12.8 m × 12.0 m compartments, which were part of a multispan Venlo-type glasshouse (Wageningen University, The Netherlands, lat. 52 °N). The experiments were conducted in one (Exp. 2 and 4) or two (Exp. 1 and 3) compartments. Each compartment contained eight parallel soil beds (1.125 m × 10.25 m per bed), of which the two outer beds acted as guard rows. In Exp. 3, the compartments were split into halves, and to avoid light interference from the adjacent light environment only the two middle soil beds, of each half compartment were used to allocate the treatments.

Block-rooted cuttings of *Chrysanthemum* 'Reagan Improved', 'Goldy' and 'Lupo' were obtained from a commercial propagator (Fides Goldstock Breeding, Maasland, The Netherlands) and were planted in soil beds at 64 plants m^{-2}, except for Exp. 1 (48 plants m^{-2}). 'Goldy' and 'Lupo' were used in Exp. 4 only. Plants were grown under long-day (LD) conditions for two (Exp. 2) or three (Exp. 1, 3 and 4) weeks, followed by a short-day (SD) period up to the final harvest. In autumn and winter, supplementary light was applied to extend the natural photoperiod (Exp. 1

and 3). In Exp. 3, light from either incandescent lamps (– HPS, control light treatment; 7 μmol $m^{-2}s^{-1}$ PAR) or assimilation lamps (+ HPS treatment; HPS Philips SON-T Agro, 44 μmol $m^{-2}s^{-1}$ PAR) was used (Table 1). In Exp. 1, only the assimilation lamps were used. Lamps were kept continuously on during the 19 day hours of the LD (from 0500 to 2400 h) and 11 h of the SD period (from 0800 to 1900 h). In spring and summer, plants were grown under natural light conditions during the LD period, which resulted in approximately 16 h of light per day (Exp. 2 and 4). The SD conditions were achieved by closing the blackout screens for 13 h a day. For all the experiments, day temperature was set at 18.5 °C and night temperature at 19.5 °C. In autumn and winter experiments, pure CO_2 was supplied to the crop. Greenhouse temperature and CO_2 concentration were automatically recorded each five minutes by a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Daily outside global radiation was obtained from a meteorological station located at about 100 m distance from the greenhouse compartments. PAR, from the supplementary light, was measured at a constant height of 0.5 m from the ground, using a 1.0 m line quantum sensor (LI-COR, model LI-191SA; Lincoln, USA). Daily incident PAR takes into account the additional PAR from the supplementary light and the loss of radiation during the SD period (blackout screens). Calculations were done based on measured daily integral of outside global radiation and measured glasshouse transmissivity for diffuse radiation (0.49). Details on the calculations are given in Chapter 4.1. Average values for each experiment are shown in Table 1.

TABLE 1. General information on four greenhouse experiments using *Chrysanthemum* 'Reagan Improved' (all Exp.) or 'Reagan Improved', 'Goldy' and 'Lupo' (Exp. 4). Dates are expressed as day of the year (day 1 = 1 Jan.).

Exp.	Year	Planting Date	Harvest Dates	Outside Global Radiation [x] (mol $m^{-2}d^{-1}$)	Incident PAR [x] (mol $m^{-2}d^{-1}$)	Temperature [y] (°C)	CO_2 [z] (μmol mol^{-1})
1	2000	12	84-88	22.2	6.8	20.9	456
2	2001	129	196-205	89.4	18.2	22.2	346
3	1999	273	348-355	18.4	4.2 (– HPS) 5.9 (+ HPS)	19.3	583
4	2000	124	192-199	81.9	17.7	21.5	379

[x] Averaged over the whole cultivation period. – HPS = absence of supplementary assimilation light; + HPS = presence of supplementary assimilation light.

[y] 24 h average greenhouse temperature, averaged over the whole cultivation period.

[z] From 1000 to 1600 h average greenhouse CO_2, averaged over the whole cultivation period.

Plant protection was applied according to an integrated pest management scheme (IPM), using both biological and chemical agents. No growth regulators were given to the crop. Irrigation was provided when needed with micro sprinklers. As common practice in commercial cut chrysanthemum production, the terminal flower bud was pinched as soon as it was separated from the other crown buds (< 5 mm). This procedure was not applied to the terminal flower treatment (1F T*, Fig. 1). In each experiment, plants were harvested at a constant development stage, i.e. when the first row of disc florets had reached anthesis in at least three inflorescences (flowers) per plant. Dates of final harvest for each experiment were extended over a period of four to nine days, depending on the treatment (Table 1).

Treatments

Sink-source ratio was varied using different levels of flower bud removal (leaving one, two, or four flowers or a control), keeping or removing axillary shoots, and applying supplementary assimilation light. The effect of flower position on flower size was analysed for the top apical flower, the first order lateral flowers (on node 1, 5, 1 to 4 and 5 to 8 from the top of the plant) and the second order lateral flower (Table 2). In Exp. 4, 'Goldy' and 'Lupo' were also studied because of their characteristic small flower size ('santini type' cultivars).

TABLE 2. Overview of the treatments [x] applied and of the studied effects, in four greenhouse experiments using *Chrysanthemum* 'Reagan Improved' (all Exp.) or 'Reagan Improved' (R), 'Goldy' (G) and 'Lupo' (L) (Exp. 4).

Exp.	All Treatments	Studied effects		
		Flower number	Axillary shoots	Flower position
1	1F L1*, 2F L1-2*, C	1F L1*/ 2F L1-2* [y]		
2	1F L1*, 1F L1, 1F L5, 1F 2nd, 2F L1-2, C	1F L1/ 2F L1-2 [y] 1F L1/ C1 1F L5/ C5 1F 2nd/ C2nd	1F L1*/ 1F L1	1F L1, 1F L5, 1F 2nd C1/ C2/ C5/ C2nd
3	1F T*, 1F L1*, 4F L1-4, 4F L5-8, C (in – HPS and + HPS) [w]	4F L1-4, 4F L5-8/ C		1F T*/ 1F L1* 4F L1-4/ 4F L5-8
4	1F L1*, C (in R, G and L) [z]			

[x] Flower bud and axillary shoot removal according to the formula: *n*F *p* where *n* is the number of flowers retained per plant (*n* = 1, 2 or 4); *p* is the position of those flowers from the top of the plant (L *i* = lateral flower on node *i*, T = top flower, 2nd = second order lateral flower). C *i* represents flower from the control treatment on node *i* and * (asterisk) treatments refer to plants without axillary shoots.

To determine: [y] potential flower size; [w] light effect; [z] cultivar effect.

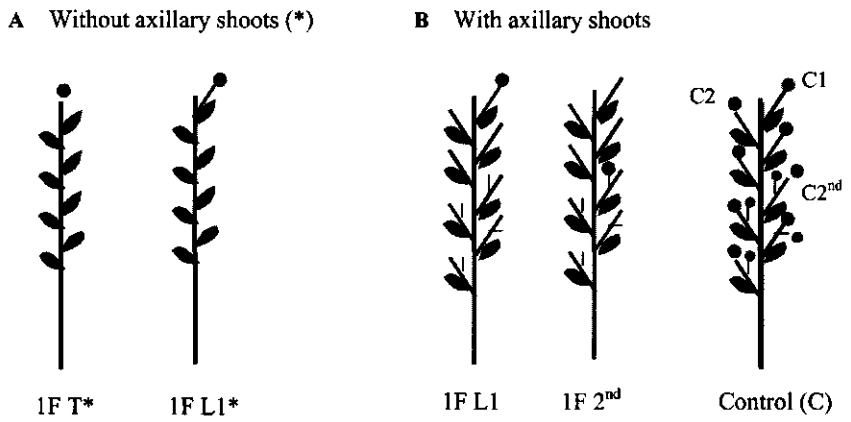

FIG. 1. Examples of the major practices of flower bud and axillary shoot removal applied in different experiments with cut chrysanthemum. Legend of the treatments in Table 2.

A total of two to six pruning treatments was applied to the plants, with only the treatments 1F L1* and C being common to all experiments (Table 2). These treatments represented a situation of low sink-source ratio (1F L1*: only the first order lateral flower, at node 1, was left on the plant) and of high sink-source ratio (C: control spray-type, grown without interference except for the removal of the terminal apical bud). The main pinching practices are illustrated in Fig. 1. In the treatments without axillary shoots (*, Table 2), the buds located in the axil of the leaves on the main stem were regularly removed as soon as bud outgrowth had started (Fig. 1A). In the other treatments, the axillary shoots were allowed to grow and only their flower buds were removed (Fig. 1B).

Measurements

Periodic destructive measurements were conducted on the monoflower (1F T*, 1F L1*) and control treatments from plants grown under supplementary assimilation light in Exp. 3 (Table 2). Individual flower dry mass (ventilated oven, 105 °C for at least 15 h) was recorded two times a week, from the visible flower bud stage to the final harvest, using six plants per experimental unit. In the control plants, only the first order lateral flower at node 1 was measured as this was to be compared with the monoflower treatment (C1, Fig. 1).

In each experiment destructive measurements were carried out at the final harvest, using five (Exp. 2 and 4) or six (Exp. 1 and 3) plants per experimental unit. Plants were randomly selected from an experimental plot of at least 54 plants, from which the two border rows on each side of the bed and at least two rows between different treatments were excluded. Individual flower dry mass, individual flower area (LI-COR, model 3100 Area Meter; Lincoln, USA) and plant dry mass (excluding roots) was determined for all treatments. In Exp. 4 disc and total flower diameter were also recorded, on 1F L1* and on C1, using a digital calliper. In the control plants the number of flowers including flower buds (> 5mm) were counted and only the fully open flowers (i.e., inflorescences with ray florets in horizontal plane), were used to determine the average flower dry mass and area. The dry mass from the removed shoots and buds was added to the final plant dry mass.

A starch analysis was performed in all the treatments from Exp. 1 and 2, except for 2F L1-2* (Table 2), using three plants per experimental unit. For these treatments, the dry mass of leaves in the main stem, as well as the leaf area (LA), was measured. Carbohydrate accumulation in the leaves is known to vary with the developmental stage of the plant and with the leaf position along the stem (Bertin *et al.*, 1999). Therefore, as accumulation of starch is especially expected at the end of the growth period, after a longer exposure to low sink demand (Marcelis, 1991), and in leaves that are fully exposed to sunlight (Chang, 1979), measurements were conducted at a fixed developmental stage (final harvest) and on the leaf from the 3rd node on the main stem for both experiments. In Exp. 1, starch analysis was also performed on leaves from node 8th and 13th, to test the leaf position effect. The leaves were collected as soon as the blackout screens opened, as the carbohydrate content is less variable with time early in the morning (Challa, 1976). Samples were immediately frozen in liquid nitrogen and then stored at -21 ± 1 °C. Prior to the starch analysis (after Van Meeteren *et al*, 1995), leaves were freeze dried and powdered in a ball mill.

Statistical design and analysis

In Exp. 1 and 2 the statistical design was a complete randomised block design. A split-plot design was applied in Exp. 3 and 4, where light and cultivar were, respectively, the main factors and sink-source ratio was the split factor. Depending on the experiment a block consisted of two consecutive soil beds (Exp. 2 and 4) or one compartment (Exp. 1 and 3). The number of blocks was two (Exp. 1 and 3) or three (Exp. 2 and 4).

An analysis of variance was conducted for each experiment and treatment effects were tested at 5 % probability level. Mean separation was done using Student's *t*-test

(P = 0.05). In Exp. 1, 3 and 4, block effects were not significant and were removed from the statistical analysis to increase the degrees of freedom for the residual term. Block sum of squares and degrees of freedom were added to the residual term. The statistical software package Genstat 5 (VSN International Ltd; Herts, UK) was used. When fractions were calculated, the normality of the data was checked with the 'Kolmogorov-Smirnov' test from SPSS package (SPSS Inc.; Chicago, USA). The data were considered normally distributed and, therefore, no transformations were necessary.

Results

Sink-source ratio effects on flower size

Competition from generative sinks: Doubling the number of flowers per plant, from one to two flowers, had no significant influence on either flower dry mass (Fig. 2A and B) or flower area (Fig. 3A and B). This was observed both in plants with (Exp. 2) or without (Exp. 1) axillary side shoots in summer and winter, respectively. Nevertheless, when competition for assimilates was further increased, flower size was strongly reduced. For instance, in Exp. 2 an average flower from the control treatment (with 19 competing flowers, Fig. 7) was at least 46 % lighter and 25 % smaller than an average flower from its correspondent monoflower treatment (Fig. 2B and 3B). Likewise, in Exp. 3 increasing the number of retained flowers from four (4F treatments) to eight (– HPS, control), produced no effect on flower size, whereas an increase to 12 flowers (+ HPS, control) resulted in significantly lighter and smaller flowers (Fig. 2C and 3C).

Flower dry mass increased exponentially from visible bud stage to harvest stage, in both monoflower (1F T* and 1F L1*) and control plants (Fig. 4). However, the monoflower plants showed a higher flower relative growth rate compared to the control treatment.

Competition from vegetative sinks: The influence of retaining the axillary shoots was tested in monoflower plants (Exp. 2, Table 2). The presence of axillary shoots had a significant negative effect on flower size, resulting in a 16 % lighter (Fig. 2B) and 13 % smaller flower (Fig. 3B).

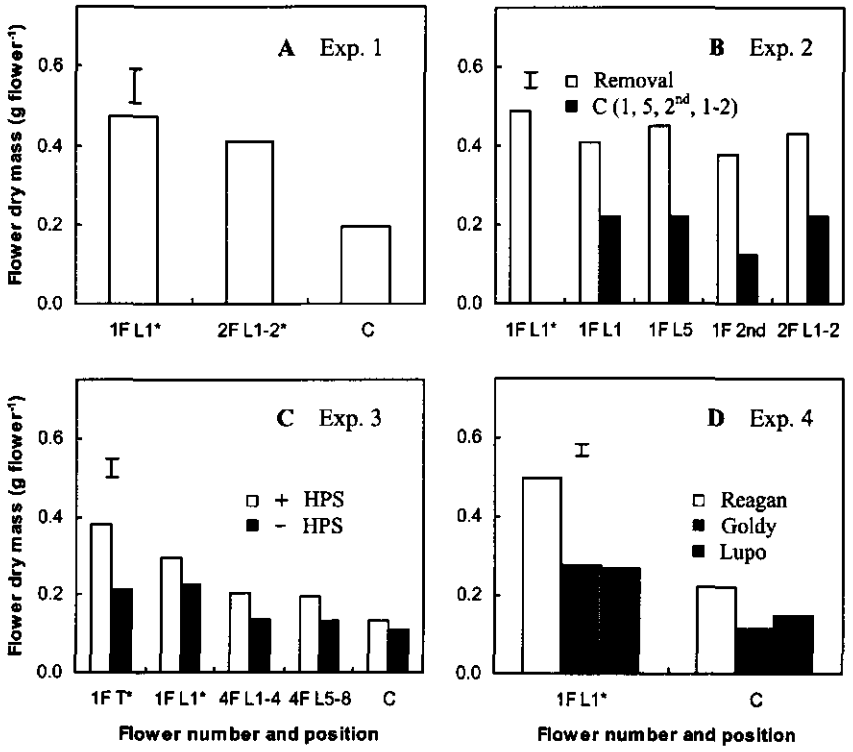

FIG. 2. Individual flower dry mass at harvest as a function of plant sink-source ratio of *Chrysanthemum* 'Reagan Improved' (A, B, C and D) and 'Goldy' and 'Lupo' (D), under presence (+ HPS) or absence (– HPS) of supplementary assimilation light. In Fig. B, white bars refer to the flower from treatments with removal of sinks and black bars refer to the equivalent flower from the control treatment (node 1, 5, 2nd order and average 1-2). Vertical bars indicate LSD: 0.084 (A), 0.038 (B), 0.051 (C) and 0.028 (D). Legend of the treatments in Table 2.

Cultivar effects: The two extreme sink-source ratios were applied to the three studied cultivars (Table 2). As described for 'Reagan Improved', also the flower size of the 'santini' cultivars was significantly affected by the sink-source ratio. Thus, the monoflower plants without side shoots (lowest sink-source ratio) had a significantly larger flower in terms of dry mass (Fig. 2D) and area (Fig. 3D). In relative terms, this gain was higher for 'Goldy' and 'Reagan Improved', compared to 'Lupo' (Table 3). Interestingly, in all three cultivars the proportionality between flower disc diameter and total diameter was maintained when flower size increased (Fig. 5). For instance, in 'Reagan Improved' the diameter of the disc (disc florets) represented 22 % of the

total diameter, both for the monoflower and the lateral flower on the first node of the control. However, the proportion of the disc diameter was cultivar specific, being much larger in the 'santini' cultivars (around 37 %).

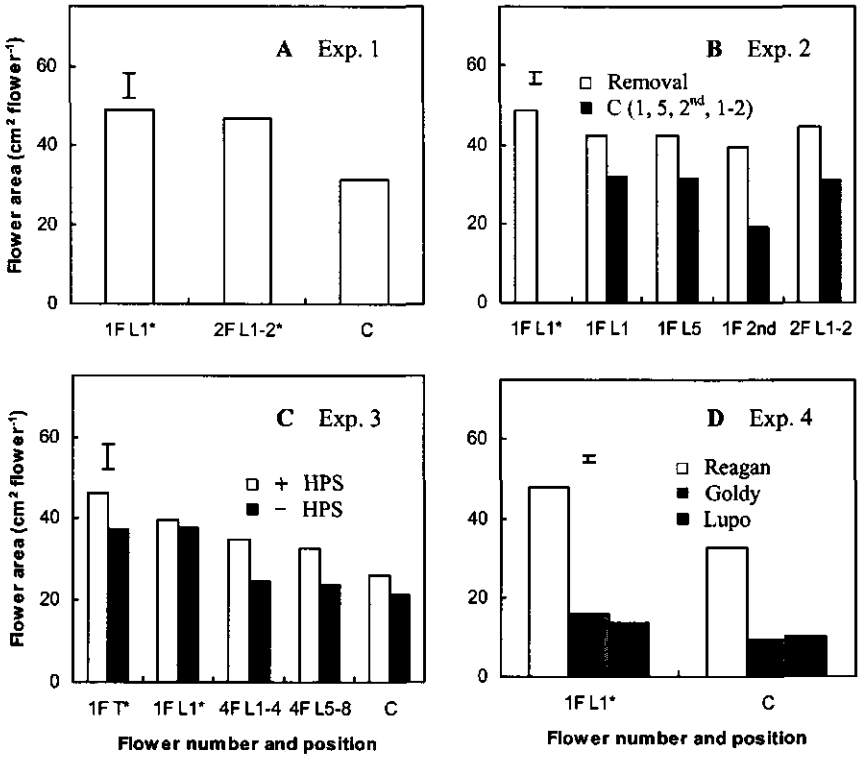

FIG. 3. Individual flower area at harvest as a function of plant sink-source ratio of *Chrysanthemum* 'Reagan Improved' (A, B, C and D) and 'Goldy' and 'Lupo' (D), under presence (+ HPS) or absence (– HPS) of supplementary assimilation light. In Fig. B, white bars refer to the flower from treatments with removal of sinks and black bars refer to the equivalent flower from the control treatment (node 1, 5, 2nd order and average 1-2). Vertical bars indicate LSD: 6.42 (A), 3.12 (B), 6.10 (C) and 1.70 (D). Legend of the treatments in Table 2.

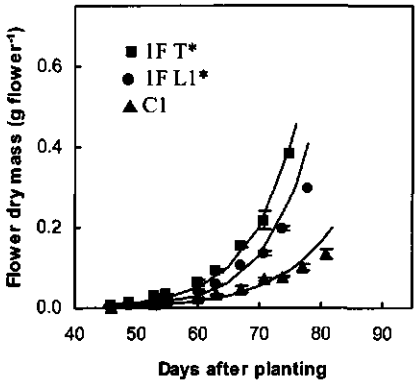

FIG. 4. Dynamics of individual *Chrysanthemum* 'Reagan Improved' flower dry mass from the monoflower plants and the control (Exp. 3, + HPS = presence of supplementary assimilation light). Plants were grown under supplementary assimilation light. Solid lines represent regression curves: $y = 1E\text{-}05e^{0.1388x}$ (1F T*); $y = 6E\text{-}06e^{0.1436x}$ (1F L*); $y = 3E\text{-}05e^{0.1096x}$ (C1). Vertical bars indicate s.e.m. when larger than symbols. Legend of the treatments in Table 2.

TABLE 3. Potential and actual flower dry mass and flower area of *Chrysanthemum* 'Reagan Improved' (all Exp.) or 'Reagan Improved', 'Goldy' and 'Lupo' (Exp. 4). Legend of the treatments in Table 2.

Experiment		Flower dry mass (g flower^{-1})			Flower area (cm^2 flower^{-1})		
		potential (1F L1*)	actual (C1)	ratio (1F L1*/C1)	potential (1F L1*)	actual (C1)	ratio (1F L1*/C1)
1		0.48	0.20	2.4	48.8	31.3	1.6
2		0.49	0.22	2.2	48.8	31.9	1.5
3	– HPS	0.23	0.11	2.1	37.7	21.4	1.8
	+ HPS	0.30	0.13	2.2	39.8	26.2	1.5
4	Reagan	0.50	0.22	2.2	47.7	32.7	1.5
	Goldy	0.28	0.11	2.4	15.5	9.2	1.7
	Lupo	0.27	0.15	1.8	13.5	10.1	1.3

– HPS = absence of supplementary assimilation light;
+ HPS = presence of supplementary assimilation light.

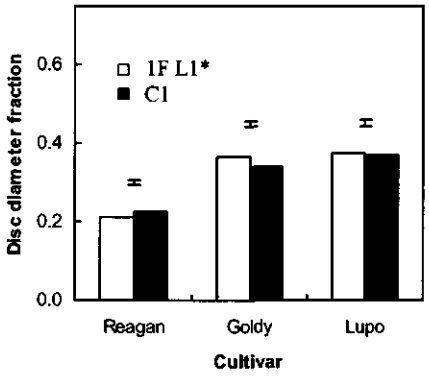

FIG. 5. Disc diameter fraction at harvest of chrysanthemum flowers as a function of plant sink-source ratio and cultivar. Vertical bars indicate LSD: 0.010 ('Reagan Improved'), 0.015 ('Goldy') and 0.018 ('Lupo'). Legend of the treatments in Table 2.

Flower position effects on flower size

When comparing first order flowers located in different nodes of the control treatment (Fig. 2B and 3B), no significant influence of the flower position within the stem was found on flower size. Also, in plants with a fixed number of flowers and no axillary shoot removal, the average flower size of the four upper flowers (node 1 to 4) or of the lower four flowers (node 5 to 8) did not differ significantly at both light levels (Fig. 2C and 3C). However, the second order flower from the control plants ($C2^{nd}$) was reduced by around 40 % in dry mass and area, compared to a first order flower (C1, C2 or C5). Similarly, in the monoflower treatments the smallest flower was developed on the second order axillary shoot (1F 2^{nd}) (Fig. 2B and 3B).

The top apical flower (1F T*) was significantly larger than the lateral flower on the first node (1F L1*), in plants grown in autumn under supplementary assimilation light (Fig. 2C and 3C). No differences among these flowers were, however, observed when plants were grown without assimilation light.

Sink-source ratio effects on dry mass production, starch content and partitioning to the flowers

When a larger number of combinations of sink-source ratio was studied (Exp. 2 and 3), only the treatment with the lowest sink-source ratio (1F L1*) differed significantly from the control, in terms of total dry mass production (Fig. 6). In general, 1F L1* plants showed 12 to 17 % lower total assimilate production than the

control plants from 'Reagan Improved'. This reduction was even stronger for the 'santini' cultivars, where the 'Goldy' monoflower plants without side shoots produced 37 % less dry mass per plant compared to the control. Interestingly, when the axillary shoots were retained (e.g. 1F L1), or when the top flower was left on the plant (1F T*) rather than the first other lateral one (1F L1*), plants showed a similar total dry mass production to the control (Fig. 6B and C).

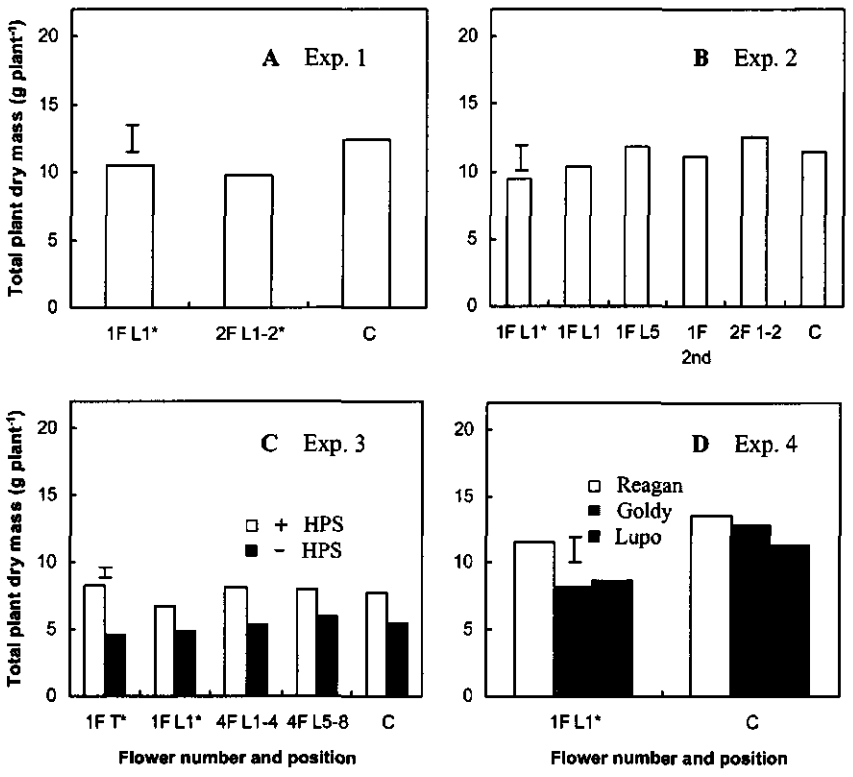

FIG. 6. Total plant dry mass (excluding roots) at harvest as a function of plant sink-source ratio of *Chrysanthemum* 'Reagan Improved' (A, B, C and D) and 'Goldy' and 'Lupo' (D), under presence (+ HPS) or absence (– HPS) of supplementary assimilation light. Vertical bars indicate LSD: 2.03 (A), 1.76 (B), 0.80 (C) and 1.83 (D). Legend of the treatments in Table 2.

Both during winter and summer, a significantly higher leaf starch content and lower specific leaf area (thicker leaves) were observed in the monoflower treatments, compared to the control plants (Table 4). In Exp. 1 similar results were observed for the 3rd, 8th and 13th leaf on the main stem, but although not significant ($P = 0.056$), starch content decreased when moving towards the lower leaves of the main stem (data not shown). In the summer experiment (Exp. 2), starch accumulation was very sensitive to the level of sink manipulation. When the number of flowers left on the plant was doubled from one (1F L1) to two (2F L1-2), the starch content of the 3rd main leaf was no longer significantly different from the spray type plants (Table 4). Flower position had no significant effect on starch accumulation nor on SLA, whereas removal of axillary shoots significantly increased leaf thickness.

In contrast with total plant dry mass, flower mass ratio (FMR, i.e. the ratio between total flower dry mass and total aerial plant dry mass), was strongly influenced by the number of flowers on the plant. For instance, plants with one flower and without side shoots had a FMR of 0.047, whereas in a plant with two flowers this increased to 0.086 (Exp. 1). FMR showed a saturation-type response to the number of flowers per plant, with a maximum of 0.22 (Fig. 7).

TABLE 4. Effect of axillary shoot removal, flower bud removal and flower position on the characteristics of the leaves on the main stem (main leaves) and on the axillary shoots (side leaves) of *Chrysanthemum* 'Reagan Improved'. Legend of the treatments in Table 2.

Experiment		Main leaves		Side leaves
		Starch content [x] (mg g^{-1}DM)	SLA [y] (cm^2 g^{-1})	LA [w] (cm^2)
1	1F L1*	57.8	271	---
	Control	19.5	368	---
	F_{prob} [z]	*<0.001*	*0.003*	
2	1F L1*	23.6 b	200 a	
	1F L1	24.3 b	244 b	159 b
	1F L5	27.8 b	239 b	195 b
	1F 2nd	26.9 b	239 b	161 b
	2F L1-2	22.4 ab	235 b	200 b
	Control	15.0 a	294 c	125 a
	F_{prob} [z]	*0.049*	*<0.001*	

[x] Leaf starch content of the 3rd top leaf on the main stem.
[y] Average specific leaf area (SLA) from all the leaves on the main stem.
[w] Average leaf area (LA) from all the leaves on the axillary shoots.
[z] F probability (significant when < 0.05). Different letters indicate significant differences between treatments based on LSD at 5 % level.

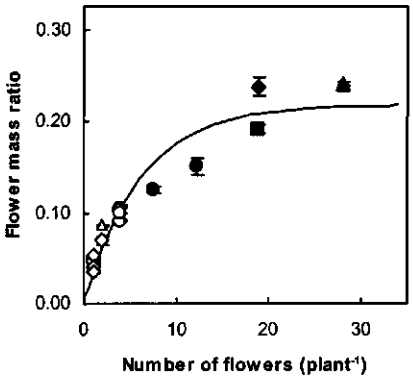

FIG. 7. Relationship between flower mass ratio and total number of flowers and flower buds per plant at harvest of *Chrysanthemum* 'Reagan Improved' in △ Exp. 1, ◊ Exp. 2, ○ Exp. 3, and □ Exp. 4 (black symbols refer to the control plants). Solid line represents regression curve: $y = 0.219(1-e^{-0.0354x/0.219})$; $R^2 = 0.93$. Vertical bars indicate s.e.m.

Discussion

Influence of plant sink-source ratio on flower size

Treatments with a fixed number of flowers per plant proved that flower size has potential to increase at lower sink-source ratio, i.e. when the number of competing sinks for assimilates was reduced (e.g. partial flower bud removal or axillary shoot removal) or the source activity was increased (e.g. supplementary assimilation light) (Fig. 2 and 3). This agrees with earlier findings that a higher individual flower dry mass was observed in pot chrysanthemum as a result of axillary flower bud removal (Cockshull, 1982; Lee *et al.*, 2001). However, in the control plants an increase in light intensity resulted in higher number of flowers rather than increased flower size. As shown in Chapter 4.1, chrysanthemum invests the additional assimilates in producing more flowers rather than producing larger flowers. This natural response could not occur in the treatments with a fixed number of flowers per plant, because flower buds were constantly removed.

Potential flower size: In the present study it was shown that doubling the competition for assimilates by increasing the number of flowers from one to two flowers is not enough to affect flower dry mass and area (Fig. 2A, 2B, 3A and 3B).

This leads to the conclusion that, in general, the monoflower plants experienced an abundance of assimilates, which is supported by their higher starch leaf content and thicker leaves (lower SLA) (Table 4). However, it was found that the removal of axillary shoots, in the monoflower treatment, had an additional positive effect on flower size (Fig. 2B and 3B), which means that the axillary shoots acted as net sink organs (rather than net source organs), competing with flowers for assimilates (Marcelis, 1996). Therefore, the monoflower plants without axillary side shoots (1F L1*) reflect the potential flower size, i.e. flower size under conditions of non-limiting assimilate supply (Marcelis, 1996). As both monoflower treatments, with or without side shoots, did not differ significantly in terms of starch accumulation (Table 4) the effect of the axillary side shoots removal on flower size is possibly related to the timing of the sink removal. In fact, the axillary shoots started to be removed 2 to 3.5 weeks after planting (respectively in summer and autumn experiments), whereas for flower buds this was only done several weeks later (as the top flower bud became visible around 3 weeks after start of SD period) (data not shown). Therefore, 1F L1* plants experienced non-limiting assimilate supply in an earlier stage than 1F L1, which allowed a higher assimilate availability during flower initiation and further development, resulting in bigger flowers. This agrees with earlier studies that showed a delay in floret initiation at low light intensity, specially when applied two weeks after the start of SD period, which would possibly result in a lower number of florets and, hence, in a smaller flower (Cockshull and Hughes, 1971).

The maximum size that a flower can achieve (potential flower size) was found to be cultivar specific and partly related to the growing season (Table 3). 'Reagan Improved' showed a higher potential flower size compared to the 'santini' type cultivars. The potential flower size of the former cultivar was rather constant for all the experiments (average of 0.49 g and 48.4 cm^2), except for the autumn experiment (Exp. 3). In autumn this size was reduced by 53 % in mass and 22 % in area. The same trend was observed for the actual flower size. This was previously reported to be related to the low light intensity during the SD period (Chapter 4.1) which delays floret initiation when under a certain threshold and thus has a negative impact on the final number of florets per inflorescence (Cockshull and Hughes, 1971). Despite the seasonal differences observed in the flower size, the ratio between potential and actual flower dry mass varied from 2.1 to 2.4 within all the experiments, as both responded in a similar way (Table 3). This means that in chrysanthemum average sink strength, in the phase where flower buds are formed is about twice as high as source strength. The ratio between potential and actual flower area varied between 1.5 and 1.8, which is less than the mass ratio. Hence, flowers grown under non-limiting assimilate supply

showed a higher specific dry mass. The same average sink-source ratio was reported by De Koning (1994) for tomato, whereas Marcelis (1994) reported for cucumber a maximum potential plant growth rate which was on average three times as bigger as the actual growth rate.

Influence of flower position on flower size

The first order lateral flowers showed a similar flower size independent of their position within the stem, both in control plants (node 1, 2 or 5) and in plants with a fixed number of flowers (node 1, 5, 1 to 4 and 5 to 8) (Fig. 2B, 2C, 3B and 3D). Nevertheless, the second order flower in the control plant ($C2^{nd}$) was significantly smaller than a first order one, and its potential flower size was found to be lower ($1F\ 2^{nd}$) (Fig. 2B and 3B). Furthermore, the top monoflower (1F T*) under supplementary assimilation light was heavier and larger than the first lateral monoflower (1F L1*) (Fig. 2C and 3C). Considering that chrysanthemum has a basipetal progression of flower initiation and development (Langton, 1992), the observed effect of flower position on flower size can be partly explained by the hypothesis that flowers which initiate first maintain their lead throughout development. This could be clearly seen on the flower dry mass pattern in time (Fig. 4). This effect was previously described for the fruit development in banana (Jullien *et al.*, 2001). Once more, the timing of when the competing sinks were removed seemed to play a role in flower size, and this could explain the differences observed between monoflower on top, first lateral and 2^{nd} order. As these flowers were sequentially initiated, the younger flowers were subjected to greater competition for assimilates, in their early stage of development, than were older flowers.

Sink-source ratio effect on dry mass production and partitioning to the flowers

In previous studies in chrysanthemum it was concluded that the removal of the most dominant sinks for assimilates enabled less dominant ones, particularly leaves, to become important sites for the net accumulation of dry mass (Cockshull and Hughes, 1968; Cockshull, 1982). Also in the present experiments plants reacted to low sink-source ratio with increased vegetative growth, producing more lateral branches (higher side LA), thicker leaves (lower SLA) and with higher starch accumulation in the leaves on the main stem (Table 4). When sink-source ratio was reduced a decrease in SLA has been also observed in tomato (Heuvelink and Buiskool, 1995) and cucumber (Marcelis, 1991). Sinks other than flowers could therefore be used as sites for the accumulation of assimilates, thus preventing a possible negative feed back control on photosynthesis. This was the case in plants with intermediate level of sink-

source ratio, such as monoflower plants where the axillary side shoots were retained (Fig. 6B). In contrast, a very drastic reduction of the number of sinks for assimilates (no axillary shoots and leaving one lateral flower only) resulted in a significantly lower total dry mass production (Fig. 6). As the roots were not measured we cannot be sure that a negative feedback control on photosynthesis had happened, since the partitioning towards the flowers could have increased in 1F L1* plants.

Except when comparing 1F T* with 1F L1*, flower position did not influence total plant dry mass (Fig. 6). This is probably because the top flower (1F T*) is a stronger sink (higher sink strength) than the lateral flower (1F L1*), which is consistent with its higher flower dry mass.

In contrast to total plant dry mass, flower mass ratio (FMR) was strongly influenced by the number of flowers per plant. FMR showed a saturation-type response to the number of flowers per plant, with a maximum value of 0.22 (Fig. 7). In fruit vegetables the dry mass partitioning towards the fruits was also observed to be strongly dependent on the number of the fruits per plant, and a saturation response was observed (Heuvelink and Buiskool, 1995; Marcelis, 1991, 1993a). A large deviation from this pattern was, however, found in the control plants from Exp. 2 and 4. Both plants produced 19 flowers but in Exp. 2 experiment FMR was 0.24 whereas in Exp. 4 only 0.19 was reached. As the growth conditions were identical the higher FMR in Exp. 2 is likely to have resulted from the shorter duration of the LD period (1 week less than for Exp. 4). In Chapter 4.1 it was shown that longer LD period in cut chrysanthemum, promotes partitioning towards the vegetative organs, since the generative development is delayed. This could also be the reason for a higher starch accumulation in Exp. 1 (2 weeks LD period), compared to Exp. 2 (3 weeks LD period).

Conclusions

It is concluded that low sink-source ratio strongly increases flower size in cut chrysanthemum and that one flower per plant, in plants without axillary side shoots, represents the potential flower size. However, this maximum flower size seems to be cultivar specific and is reduced at low light intensity during the short-day period.

The effect of flower position within the stem, on flower size is only relevant in cut chrysanthemum when comparing flowers located on the first order with flowers on second order axillary shoots, the last ones being 40 % smaller. Models simulating photosynthesis in spray cut chrysanthemum can neglect a negative feedback caused by low sink demand.

5

Modelling external quality aspects

5.1

Temperature effect on internode elongation

Abstract

The DIF concept states that equal internode length can be achieved with the same difference between day and night temperature irrespective of the mean 24 h temperature. However, the physiological background of the DIF concept is unclear. An attempt to model internode elongation is presented based on three plausible processes, namely (1) the accumulation of elongation requirements during the day, (2) elongation during the night using elongation requirements and (3) the limitation of internode length due to low turgor pressure unable to counter cell wall elasticity. Each reaction rate constant, one per process, depends on temperature according to Arrhenius' Law. The resulting process-based model describes internode elongation in time and was calibrated on a chrysanthemum data set. Chrysanthemum plants were grown in growth chambers with rigorously defined day and night temperatures. In total, 16 temperature treatments were applied, resulting from the combination of four day and four night temperatures (16, 20, 24 and 28 °C). Internode elongation was measured for the tenth internode in ten plants per treatment. The percentage variance accounted for, R^2_{adj}, was almost 91 %. Transferability of model parameters was shown to exist by cross validation. Simulation of the internode length in time as function of mean 24 h temperature and DIF showed that the DIF concept is not apparent after a growth period of 10 d, but is visible after 20 d. This model structure for describing internode elongation might also be applicable for other plants that show the DIF concept.

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Introduction

The DIF concept states that plants grown with the same difference between day (DT) and night (NT) temperature will have equal final internode length, regardless of the mean temperature (Jacobson and Willits, 1998 and Chapter 3.1). In several species including tomato, lilium and chrysanthemum, temperature combinations resulting in a negative DIF produced shorter plants with smaller internodes, compared with plants growing under a positive DIF (Erwin *et al.*, 1989; Karlsson *et al.*, 1989c; Jacobsen and Amsen, 1992; Bertram and Karlsen, 1994; Cockshull *et al.*, 1995). Total stem length results from the average internode length multiplied by the number of internodes (Pearson *et al.*, 1995), and the DIF effect on stem length is mainly a result of its influence on internode elongation (Langton and Cockshull, 1997a and Chapter 3.1).

Despite being the object of a large number of studies, the physiological background of the DIF concept remains an open question (Bertram and Karlsen, 1994; Langton, 1998). Many descriptive models have been developed for stem length in chrysanthemum (e.g. Karlsson *et al.*, 1989c; Pearson *et al.*, 1995; Khattak and Pearson, 1997). However, these models lack insight into the internode elongation process and only a few have included the DIF effect on stem elongation (e.g. Jacobson and Willits, 1998 and Chapter 3.1). The aim of the present work was to develop a process-based model to describe internode elongation as function of temperature. This model was calibrated by re-analysing the data from Chapter 3.1.

Material and methods

Conceptual model

The model was developed using a system of problem decomposition (Sloof, 2001). This system is oriented towards underlying processes that cause the observed phenomena, rather than towards the phenomena themselves.

Chrysanthemum, capsicum, salvia and petunia plants have higher stem elongation rates (SERs) during the night than during the day (Bertram and Karlsen, 1994). The main difference in SER between chrysanthemum plants grown under constant daily temperature and under a negative DIF occurred at night (Bertram and Karlsen, 1994). In chrysanthemum, capsicum, salvia and petunia, SER is affected by the irradiance level, such that high irradiance during the day induces high SERs during the following night (Bertram and Karlsen, 1994). Apparently, the presence of sufficient levels of

elongation requirements (ERs), obtained during the preceding day, affects stem elongation during the night.

The nature of the ERs is unclear. As irradiance during the preceding day is important for elongation during the night, it might be that ERs are photosynthates. On the other hand, gibberellin (GA) metabolism is involved in the stem elongation process. The average stem length of tomato plants grown for 14 days in contrasting day-night temperature regimes increased considerably following addition of exogenous GA_{4+7}, irrespective of the DIF regime applied and whether GA-deficient mutants or wild-type plants were used (Langton, 1998). It has been suggested that temperature affects elongation by influencing the GA concentration and not by changing the sensitivity of the plant (Langton, 1998). Temperature regimes that stimulate extension growth for bellflower showed an increase of physiologically active GA_1 and its precursors GA_{19} and GA_{44}. Reciprocal temperature regimes were accompanied by an increase in the inactive hydroxylated form of GA_{53}, the precursor of GA_{44} (Jensen *et al.*, 1996). Exactly how GA promotes stem elongation is not completely clear. It was reported for pea that GA changes the orientation of microtubules and cellulose microfibrils, making the cells swell more in length (Duckett and Lloyd, 1994). Another effect of GA is that the activity of xyloglucan endotransglycosylase, the enzyme that hydrolyses and then re-links hemicellulose, is increased, enhancing wall stretching in pea (Potter and Fry, 1993).

Phytochrome-mediated changes in GA production triggered stem elongation in cowpea (Fang *et al.*, 1991). Langton (1998) hypothesised that a light-on or a light-off signal triggers active GA production or interconversion. This might suggest that parallel to the accumulation of photosynthates during the day, accumulation of inactive GAs takes place, which are converted to the active form by a light-off signal. The first proposition for the internode length model is that accumulation of ERs is governed only by DT. The second proposition is that only NT governs the conversion of ERs into elongation.

GA enhances the synthesis of enzymes such as invertase, which converts sucrose to glucose and fructose, thereby lowering the water potential which helps to maintain turgor (Miyamoto *et al.*, 1993, 2000). As stem elongation progresses, it will become increasingly hard to sustain turgor in the stem cells as their volume increases. It can be suggested that elongation may be limited by low turgor pressure insufficient to counter cell wall elasticity. The third proposition for the internode length model is that the elongation process is limited by cell wall elasticity, which, as it is mainly a physical process, is governed both by DT and NT. The third proposition implies that, during the day, internode length decay may occur. Indeed, SERs appeared to become

negative for small periods during the day when chrysanthemum, salvia and capsicum were grown in a glasshouse, and these periods of apparent negative SER increased considerably when plants were grown under long-day compared to short-day treatments (Bertram and Karlsen, 1994). This indicates that stem elongation in glasshouses, started during the night, is sometimes turned into a stem length decay during the day; the shorter the night, the stronger the effect.

Mathematical model

The model is based on kinetic mechanisms, assumed and plausible for that particular process, and which have been developed using well-known rules of chemical kinetics (Segel, 1993; Whitaker, 1994). The three propositions for the internode length model are indicative for the processes of accumulation of elongation requirements (*ER*) [eqn (1)], internode length (*L*) growth [eqn (2)], and the limitation of *L* [eqn (3)], respectively.

$$\xrightarrow{k_{er}} ER \tag{1}$$

$$ER \xrightarrow{k_f} L \tag{2}$$

$$L \xrightarrow{k_d} \tag{3}$$

where k_{er}, k_f and k_d are the reaction rate constants for the formation of ERs, the formation of *L* and the decay of *L*, respectively. These processes describe a system that is expressed by the following set of differential equations [eqns (4) and (5)]:

$$\frac{\partial ER}{\partial t} = k_{er} - k_f \cdot ER \tag{4}$$

$$\frac{\partial L}{\partial t} = k_f \cdot ER - k_d \cdot L \tag{5}$$

This set of equations can be solved analytically for constant external conditions [eqn (6)].

$$L(t) = ER_0 \cdot \frac{k_f \cdot (e^{-k_d \cdot t} + e^{-k_f \cdot t})}{k_d - k_f} + L_0 \cdot e^{-k_d \cdot t} + k_{er} \cdot \frac{-k_f \cdot (1 - e^{-k_d \cdot t}) + k_d \cdot (1 - e^{-k_f \cdot t})}{k_d \cdot (k_d - k_f)} \tag{6}$$

where ER_0 and L_0 are the amounts of ERs and L when internode elongation starts.

As internode length was not visible when the treatments started, $L_0 = 0$ and therefore also $ER_0 = 0$. The internode length development in time can then be expressed according to eqn (7).

$$L(t) = k_{er} \cdot \frac{k_d \cdot (1 - e^{-k_f \cdot t}) - k_f \cdot (1 - e^{-k_d \cdot t})}{k_d \cdot (k_d - k_f)} \qquad (7)$$

Equation (8) describes the development of the internode length in time, starting at a length of zero ($t = 0$) and finishing at k_{er}/k_d (mm, $t = +\infty$). However, the experiment did not start at $t = 0$, but earlier when internode elongation was still absent. To take into account that every internode starts to grow at a different point in time, a time shift factor, t_{shift}, is introduced, defined as $t = t_{start} - t_{shift}$, where t_{start} is the experimental point in time when internode elongation starts. Incorporating the time shift factor results in the internode elongation equation used in the data analysis [eqn (8)].

$$L(t) = k_{er} \cdot \frac{k_d \cdot (1 - e^{-k_f \cdot (t_{start} - t_{shift})}) - k_f \cdot (1 - e^{-k_d \cdot (t_{start} - t_{shift})})}{k_d \cdot (k_d - k_f)} \qquad (8)$$

Temperature dependence

Each of the reaction rate constants mentioned (k_{er}, k_f and k_d) depends on temperature, presumably according to Arrhenius' Law [eqn (9)].

$$k_i = k_{i,ref} \cdot e^{\frac{E_i}{R_{gas}} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)} \qquad (9)$$

with R_{gas} being the gas constant (8.314 J $mol^{-1}K^{-1}$). The parameter $k_{i,ref}$ stands for the reaction rate constant at the arbitrarily chosen reference temperature T_{ref} (K). The energy of activation E_i expresses the dependence of the reaction rate constant k_i on temperature, with i = er, f, or d. T (K) is the DT or NT of the growth chambers applicable for the accompanying process. According to the propositions, k_{er} is only governed by DT only, k_f by NT only, and k_d by both DT and NT. The application of k_d being dependent on night and day temperature is solved by using two sub-parameters, $k_{d\,DT}$ and $k_{d\,NT}$, which are only dependent on day and night temperature, respectively, but have a common value of $k_{d,ref}$.

Experimental set-up and plant measurements

Cuttings of *Chrysanthemum* 'Reagan Improved' were obtained from a commercial propagator and planted in 14 cm pots containing a peat-based commercial potting compost on 16 May 2001 (replication 1) and 13 June 2001 (replication 2). Plants were selected for uniformity (8 ± 1 leaves per plant; 12 ± 2 cm stem length). After 2 days in a common glasshouse environment (18 °C DT, 16 °C NT and 18 h light), temperature treatments were imposed. A total of sixteen temperature treatments was applied, resulting from the combination of four DTs and four NTs (16 ± 0.1, 20 ± 0.1, 24 ± 0.1 and 28 ± 0.1 °C). Plants were grown in growth chambers (2.90 m × 2.20 m × 3.15 m), as individual plants, under ambient CO_2 and at a constant vapour pressure deficit of 0.57 kPa. Fluorescent tubes [Philips TL-58W, colour 84, 99 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at average plant level] were used for 12 h per day. This light level was similar to that received by plants growing in commercial glasshouses during winter in The Netherlands. Every 5 min, temperature was recorded using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Since only four growth chambers were available plants were shifted at the start and end of each day according to their DT and NT treatment. No effect on stem length of moving the plants was observed (Chapter 3.1). A more detailed description is given in Chapter 3.1.

Length of internode 10 of ten plants per temperature treatment was measured non-destructively using a digital calliper. This internode was chosen because it was not visible when the treatments started. Measurements were repeated for replication 1 on days 0, 5, 10, 17, 21 and 26 and for replication 2 on days 0, 5, 10,17, 24 and 28 after the start of the treatments. The experiments ended before day 30 as internode 10 had apparently reached its final length for all temperature treatments as deduced by the lack of any clear increase in internode length over time. Each replicate consisted of ten plants per treatment divided between two trays. Plants were randomly distributed over the treatments and trays.

Statistical analysis

Experimental data on internode elongation were analysed statistically using the non-linear regression routine of Genstat 5 (release 3.2; Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The equations and mathematical description of the model were developed using Maple V (release 4; Waterloo Maple Software, Waterloo, Canada). The data set of the first replication was analysed using the model formulation of eqn (8) together with the temperature dependence according to the

Arrhenius equation [eqn (9)]. These data were analysed using temperature and time simultaneously as explaining variables estimating the time shift factor (t_{shift}) per plant and the kinetic parameters (k_{er}, k_f, k_d, E_{er}, E_f, and E_d) in common for all plants in one optimisation. The internode length data set of the second replication was analysed using the model formulation of eqn (8) and the kinetic parameters obtained from the analysis of the first replication data set, estimating t_{shift} per plant. The reference temperature for the Arrhenius equation (T_{ref}) was in both analyses 289 K (16 °C).

Results

Kinetic parameters of the internode length model

The data set of the first replication was used to calibrate the model parameters per tray. Internode length measurements were used, without transformation, for $L(t)$ in eqn (8). Separate analysis per tray showed that a high percentage of variance (R^2_{adj}) was accounted for, on average more than 92 %. Using the average parameter values, R^2_{adj} was almost 91 % for both analyses (Table 1). Experimental and simulated data, i.e. applying the estimated parameters from Table 1 and estimated t_{shift} values for all plants in the first tray of replication 1, are shown in Fig. 1. At the moment the internode started growing, a sigmoidal behaviour in time was encountered depending on DT and NT in combination with t_{shift}. For some treatments, for instance 16 °C DT and 16 °C NT, it is obvious that the final internode length is not reached at day 30.

TABLE 1. Parameter estimates and their standard error (s.e.) for the analysis of the data set of replication 1.

Parameter	Value	s.e.
$k_{er,ref}$ (mm d^{-1})	2.256	0.151
$k_{f,ref}$ (d^{-1})	0.1053	0.0204
$k_{d,ref}$ (day^{-1})	0.0422	0.0086
E_{er} (J mol^{-1})	63810	7487
E_f (J mol^{-1})	57011	8323
E_d (J mol^{-1})	94696	2935
T_{ref} (K)	289 (16 °C)	
R^2_{adj} (%)	90.8	
n[a]	780	

[a] Number of observations.

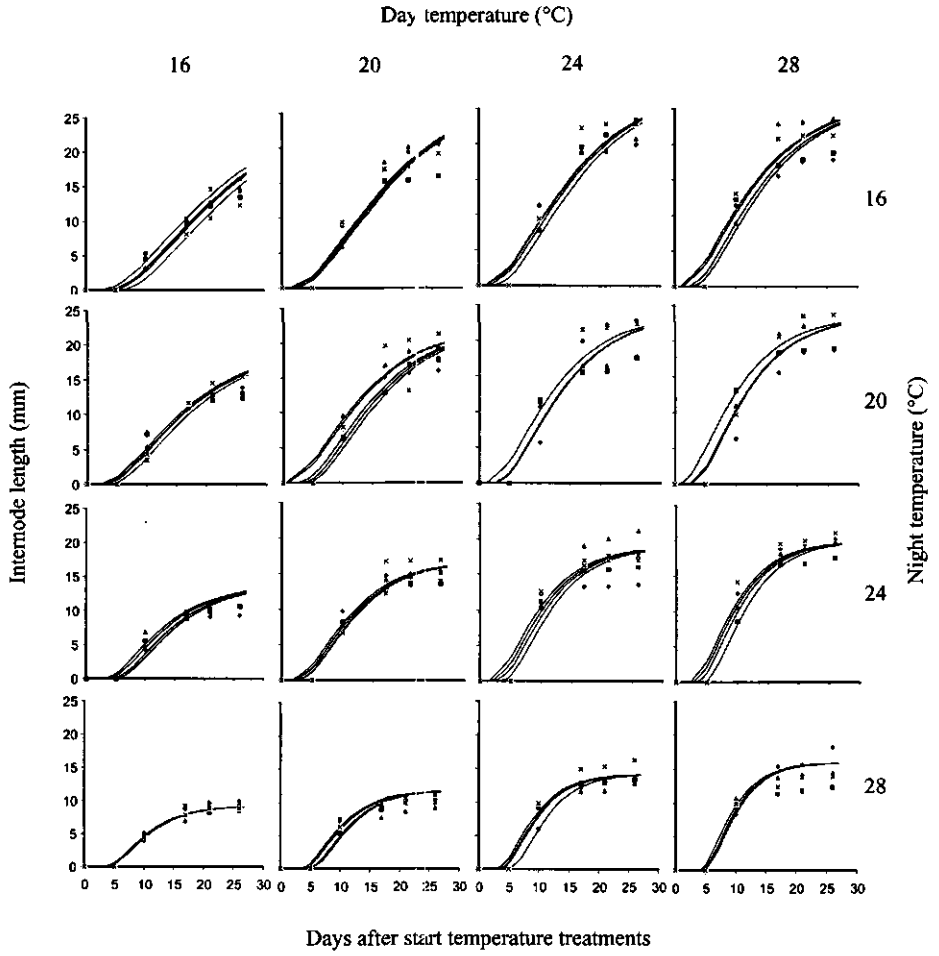

FIG. 1. Experimental (symbols) and fitted (solid lines) internode elongation over time for five plants for each combination of day and night temperature.

To validate the model formulation, the model parameters estimated on the data set of the first replication (Table 1) were applied to the data set of the second replication in a separate analysis. In this analysis only the t_{shift} values were estimated. The scatterplot, showing observed against expected internode length data for all temperature treatments, had a R^2_{adj} of 92 % (Fig. 2). A comparison between the distribution of the estimated start day of elongation, t_{shift}, per tray and per replication (Fig. 3) showed that the distributions per replication were more closely related than

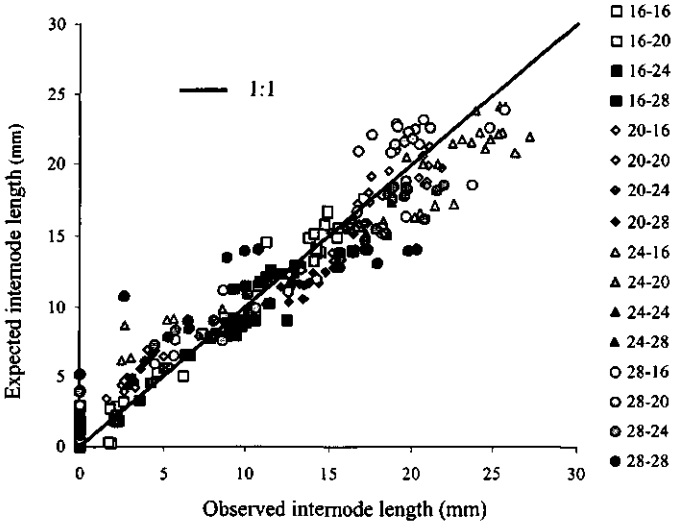

FIG. 2. Observed and expected internode length for chrysanthemum of replication 2. Symbols represent the different combinations of day and night temperatures (DT-NT).

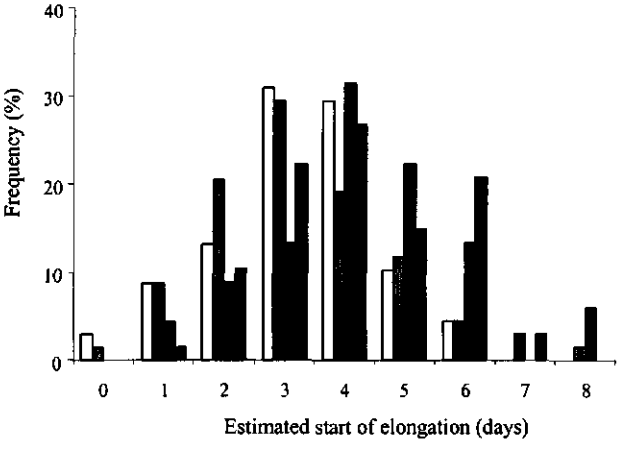

FIG. 3. Distribution of t_{shift}, the estimated point in time when elongation starts, encountered during the analysis of the data sets per tray and per replication. White and light grey bars indicate the distribution of t_{shift} for tray 1 and tray 2 of replication 1, and the dark grey and black bars those for tray 1 and tray 2 of replication 2.

those per tray. The difference between the t_{shift} distributions of the two replications was about one day, indicating that internodes from the first replication started growing, on average, one day earlier. Indeed, for the first replication, the length of internode nine was generally greater at the time when measurements of internode ten began (data not shown).

Simulation of the internode length

After calibration of the model parameters on the internode length data, it was possible to simulate final internode length as function of DT and NT (Fig. 4). The maximum internode length was achieved by combining a DT of 22 °C and the lowest NT (16°). Extrapolating the simulation to temperature ranges just below the experimental temperature range resulted in the maximum internode length after 30 days being achieved by a combination of 22 °C DT and 13 °C NT (data not shown).

Internode length development as function of DIF (°C) and the mean 24 h temperature, MT_{24h} (°C), is shown at 10 (Fig. 5A and B), 20 (Fig. 5C and D) and 30 days (Fig. 5E and F). Over the range of MT_{24h}, the maximum difference between the minimum and maximum internode length within one DIF was 9 mm at 10 days (Fig. 5B). At 20 days, this maximum difference within one DIF decreased to about 4 mm (Fig. 5D), and increased again at 30 days to about 6 mm (Fig. 5F).

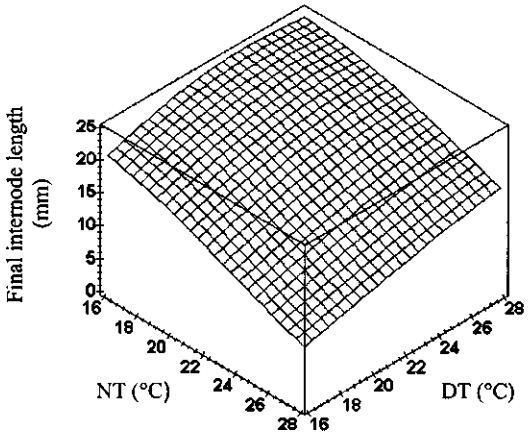

FIG. 4. Simulation of the internode length at 30 days after the start of the temperature treatments, as function of day temperature (DT) and night temperature (NT).

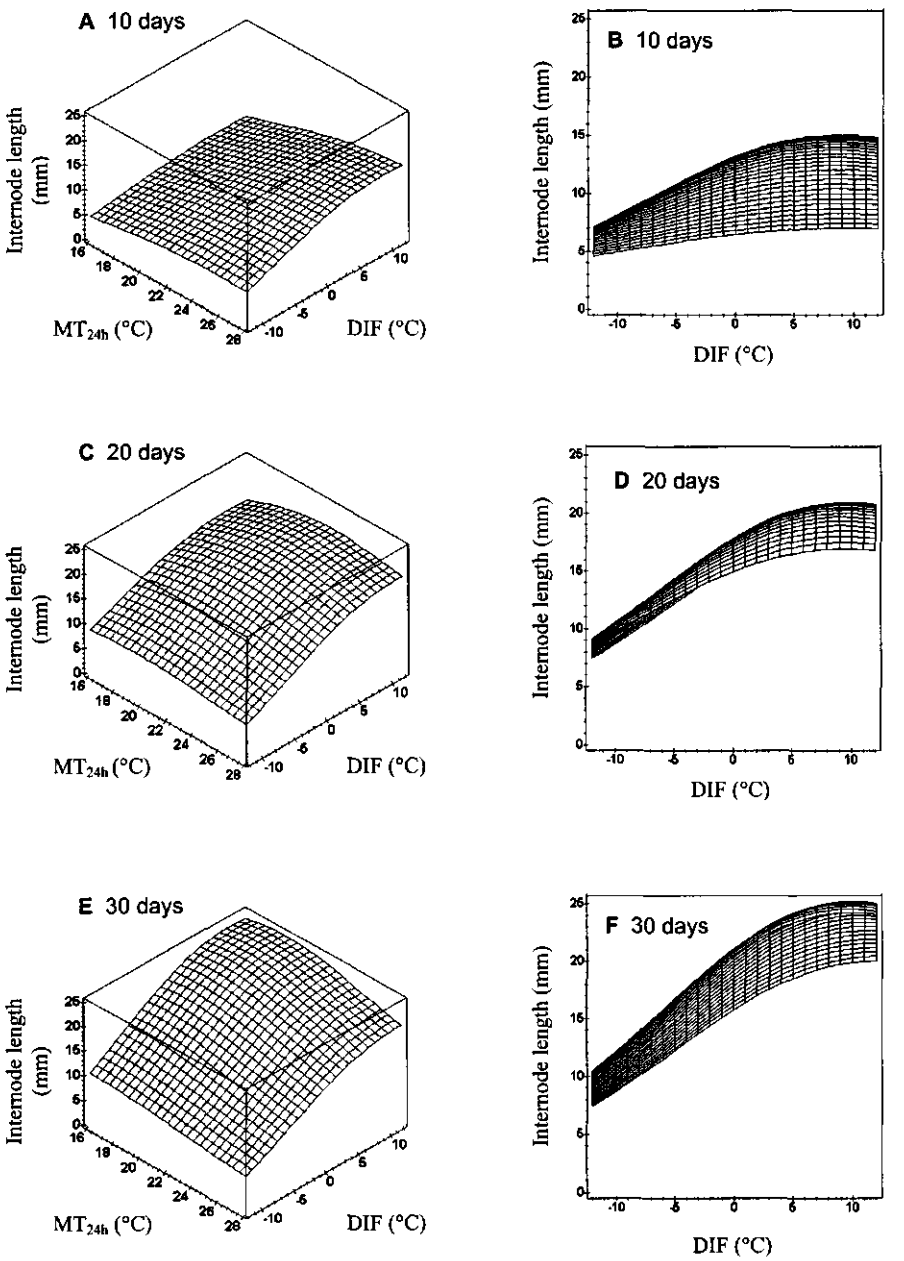

FIG. 5. Simulations of the internode length as function of DIF and the mean 24 h temperature, MT_{24h}, at 10 (A and B), 20 (C and D) and 30 days (E and F) after the start of the temperature treatments. Right panels (B, D and F) show a different view of the simulation.

The differences between minimum and maximum internode lengths at 10, 20 and 30 days are rather small as the range of internode lengths observed over DIF and MT_{24h} was about 18 mm at 30 days (Fig. 5E and F). However, at 10 days, the range in internode lengths over DIF and MT_{24h} was about 9 mm and this difference was also encountered within one DIF (Fig. 5B). So, at 10 days, no clear DIF response is observed, which was also found by Langton and Cockshull (1997a). However, at 20 and 30 days, the range in internode lengths over DIF and MT_{24h} increased rapidly, whereas the variation in the internode length within one DIF was much smaller (4 mm at 20 days, Fig. 5D; 6 mm at 30 days, Fig. 5F) than the variation in the internode length at 10 days. Thus, when the internode elongation is observed for more than 20 days, the variation in internode length is primarily explained by DIF.

Discussion

Regression models

Karlsson *et al.* (1989c) used a regression-based approach to describe final internode length as a quadratic function of mean 24 h temperature and DIF. Pearson *et al.* (1995) described final internode length by using a regression-based approach for internode elongation based on the weighted 24 h mean temperature. Both descriptions lack biological significance as plants do not react primarily to DIF or weighted 24 h mean temperature with regard to internode elongation. In Chapter 3.1 the same regression-based approach was used and the final internode length was described as a quadratic combination of DT and NT. This approach was successful for the temperature range examined, but gave poor results when extrapolated to slightly higher or lower temperatures. The advantage of the proposed model is that it is process-based, indicating that only physiologically viable parameters are included; this means that only actual DT, NT and time are included. As these processes are also likely to dominate at slightly higher and slightly lower temperatures, extrapolation to temperatures just outside the experimental range is more likely to yield plausible results.

The Richards function

The observed sigmoidal behaviour over time encountered for internode elongation (Fig. 1) has been described before. Modelling efforts of this sigmoidal behaviour have centred on the Richards function (Berghage and Heins, 1990; Larsen, 1990; Karlsson

and Heins, 1994; Jacobson and Willits, 1998). This empirical equation showed flexibility in describing final internode elongation of different internodes and as function of photosynthetically active radiation in a glasshouse. Difficulties arise when small differences in the data produce large differences in the predicted parameters, making interpretation of the relationships between parameters and the resulting growth difficult (Jacobson and Willits, 1998). Jacobson and Willits (1998) used an approach where the coefficients of the Richards function were assumed to be independent of environmental factors, and included growth factors to modify the Richards function. Although growth factors were linked to physiological parameters, the application was carried out on an *ad hoc* basis. This implicates that for a batch, as defined by the products of one harvest, one glasshouse and one cultivar (Schouten *et al.*, 2002), these coefficients and growth factors may be fitted satisfactorily, but that the applicability of the model over batches of chrysanthemum is likely to be limited. The proposed internode elongation model is based on plausible physiological processes occurring in chrysanthemum elongation and is therefore of a more fundamental nature. As these processes occur at the same rate in other batches of the same cultivar and growth conditions, transferability of model parameters is possible. This was shown by applying the model parameters estimated on the data set of replicate 1 to describe the internode length elongation of chrysanthemums of replicate 2 (cross validation, Fig. 2). Application of the same growth conditions on chrysanthemums of other cultivars should be possible, as the same processes are likely to determine internode elongation. The only difference would be the value of the kinetic parameters, but the model structure would be identical. Application of a process-based model for colour development in cucumbers was shown to be transferable to different cultivars (Schouten *et al.*, 2002). Furthermore, as the processes underlying the internode elongation model also occur in other plant species, the model structure might be applicable for a host of other plant species that show a clear DIF response.

A limitation of the proposed internode length model is that it was formulated and tested for chrysanthemums grown in growth chambers, with rigorously defined light and temperature conditions, and not in a commercial glasshouse. However, this model may be the basis for an expanded version that incorporates dynamically changing temperature and light conditions. For instance, incorporation of dynamically changing temperature conditions is a matter of approaching the statistical analysis differently by applying rate sums for all the reaction rates constants in the model (Tijskens and Verdenius, 2000).

5.2

Interactive effects of duration of long-day period and plant density on external quality: a case study

Abstract

A greenhouse experiment was conducted during summer to quantify the interactive effects of duration of the long-day (LD) period and plant density on chrysanthemum plant height, flower characteristics and total aerial plant fresh mass (TFM). *Chrysanthemum* 'Reagan Improved' was grown under three durations of the LD period (2, 9 and 16 days) combined with three plant densities (48, 64 and 80 plants m^{-2}).

Plant height was strongly influenced by the duration of the LD period, whereas plant density had a marginal effect only. Decreasing LD period from 16 to 2 days resulted in 25 % shorter plants, but a marketable height was always reached (> 65 cm). In general, a decrease in duration of the LD period and/or an increase in plant density had a negative effect on the total number of flowers and flower buds per plant (NoF) and on TFM. For example, plants that received 16 days of LD period and were grown at 48 plants m^{-2} were the heaviest (91.5 g $plant^{-1}$) and obtained the highest NoF (28 flowers $plant^{-1}$). In contrast, 2 days of LD period and 80 plants m^{-2}, resulted in 57 % lighter plants with 67 % less flowers. However, a similar TFM could be obtained using several combinations of plant density and duration of the LD, without affecting either NoF or individual flower size. A photosynthesis-driven crop growth model was validated and used to quantify this trade-off, when aiming at a certain TFM. It was shown that such trade-off is dependent on the planting date throughout the year. A module to predict NoF, based on total dry mass per plant, was validated and measured NoF was accurately predicted.

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Introduction

Cut chrysanthemum is a short-day (SD) plant, which is produced year-round in greenhouses. To permit the initiation of sufficient leaves so as to provide an adequate stem length, cuttings are kept vegetative under a long-day (LD) period for several days after planting. This is followed by a SD period that induces flower initiation. In winter LD treatment is achieved with supplementary light, while in summer blackout screens are used to create the SD conditions (Horridge *et al.*, 1984). However, in Northern Europe the product quality varies greatly throughout the year (Heuvelink *et al.*, 2001), as a consequence of the strong variation in the daily light integral (Lee *et al.*, 2002b). Plant height, total number of flowers and flower buds per plant (NoF), flower size and flower position are major aspects of cut chrysanthemum external quality (Chapter 2). Because cut chrysanthemum is sold in different weigh classes (Trip *et al.*, 2000), total aerial plant fresh mass (TFM, g $plant^{-1}$) is also a critical aspect for its commercialisation (Langton *et al.*, 1999). To achieve higher yield and to reduce these seasonal fluctuations in quality, chrysanthemum growers adjust plant density and duration of the LD period according to the growing season (Spaargaren, 2002). For instance, in The Netherlands a crop planted in week 45, in a greenhouse with 49 $\mu mol\ m^{-2}s^{-1}$ of supplementary assimilation light, receives 20 LDs and is planted at 44 plants m^{-2}. In the same greenhouse a crop planted in week 23 receives only 10 LDs and its plant density is increased to 65 plants m^{-2} (Fig. 1, Chapter 1). To which extent the manipulation of these growth conditions affects external quality is hardly studied. Since cut chrysanthemum is planted on a weekly basis (Spaargaren, 2002), the use of an explanatory model is a valuable tool to generalise this knowledge and as part of a decision support system (Challa, 1997).

The objective of this case study is to investigate the possibility of a trade-off, between duration of the LD period and plant density, when aiming at a certain external quality. Therefore, a greenhouse experiment was conducted in summer combining three durations of the LD period (2, 9 and 16 days) with three plant densities (48, 64 and 80 plants m^{-2}). In the second part of the paper the obtained data set is used to validate an explanatory photosynthesis-driven crop growth model for cut chrysanthemum (Lee *et al.*, 2002a). This model was further used to simulate total dry mass per m^{-2} for eight different dates throughout the year (four planting dates and four start of SD period dates), in order to analyse how general the results obtained with the experiment are and to extrapolate this knowledge to other growing seasons. Moreover, the linear relationship between NoF and total aerial plant dry mass (TDM g $plant^{-1}$) developed in Chapter 4.1 is validated.

Materials and methods

Plant material and growth conditions

An experiment was carried out in one compartment (12.8 m × 12.0 m) of a multispan Venlo-type glasshouse at Wageningen University, The Netherlands (lat. 52°N). Block-rooted cuttings of *Chrysanthemum* 'Reagan Improved' (Fides Goldstock Breeding, Maasland, The Netherlands) were planted on three different planting dates (9, 16 and 23 May 2001), in eight parallel soil beds (1.125 m × 10.25 m).

Plants were grown under natural light conditions during the LD period (around 16 h light per day for 2, 9 or 16 days). SD period was achieved by closing the blackout screen for 13 h a day, from 25 May to harvest. No supplementary light was applied and temperature was set at 18.5 °C day temperature and 19.5 °C night temperature. Outside global radiation, greenhouse temperature and CO_2 concentration were automatically recorded each 5-min using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Incident daily photosynthetically active radiation (PAR) averaged over the whole cultivation period was 18.2 mol $m^{-2}d^{-1}$. Mean 24 h greenhouse temperature was 22.2 °C and mean CO_2 concentration, between 1000 and 1600 h, was 346 µmol mol^{-1}.

Destructive measurements were carried out at planting and harvesting dates. Initial stem and leaves fresh and dry mass (ventilated oven, 105 °C for at least 15 h) were measured on 20 plants, at each planting date. Harvest stage was defined as the moment when the first row of disc florets had reached anthesis, in at least three inflorescences per plant. Since flower development rate differed slightly among treatments, harvest was spread over 6 days. Final plant height (i.e. stem length added to the peduncle's length from the uppermost lateral flower), stem length, number of leaves on the main stem, number of flowers, number of flower buds (>5 mm) and flower position (15 cm from top) were recorded. TFM and TDM were also calculated. Individual flower dry mass and individual flower area (LI-COR Model 3100 Area Meter, USA) was determined for the fully open flowers only. Measurements were done on five plants per experimental plot, leaving two border rows on each side of the bed and between different treatments. No root measurements were performed.

General model description

A photosynthesis-driven crop growth model (Fig. 1) for cut chrysanthemum CHRYSIM1.0 (Lee *et al.*, 2002a) was used to simulate total dry mass per m^2.

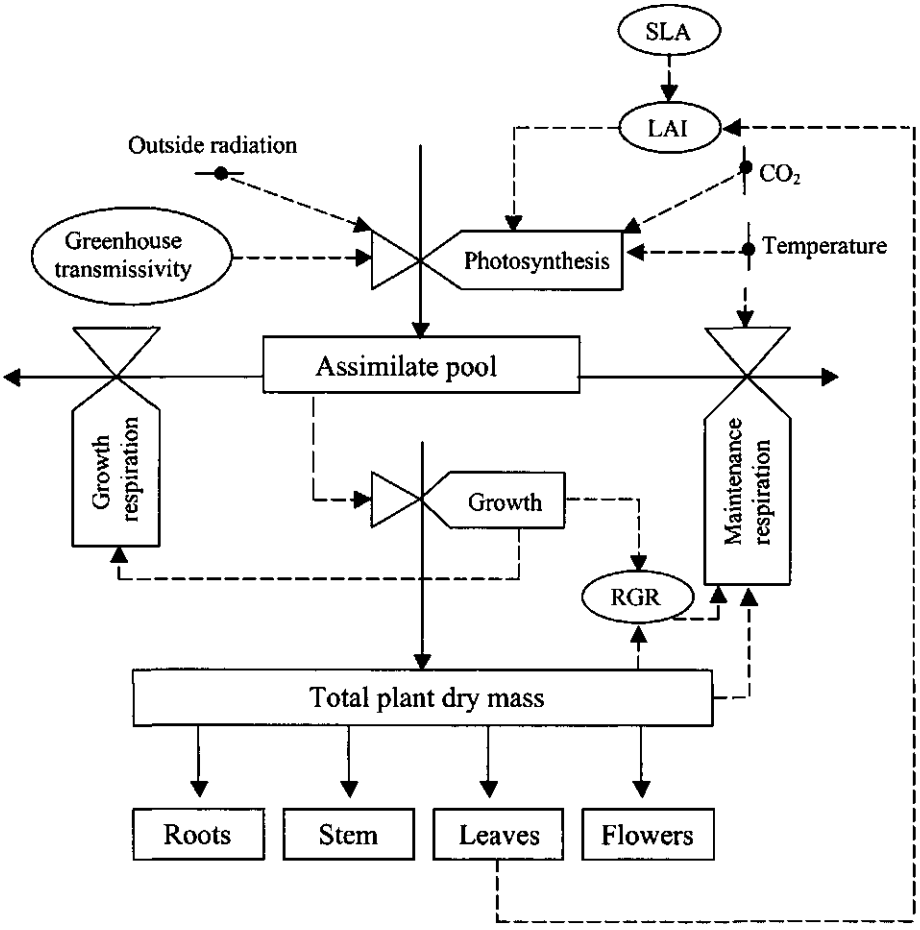

FIG. 1. Simplified relational diagram describing plant growth and dry mass partitioning of an ornamental crop growing in a greenhouse. Boxes are state variables, circles are parameters and valves are rate variables. Solid arrows represent carbon flow and dashed arrows represent information flow. Abbreviations: LAI = leaf area index, RGR = relative growth rate, SLA = specific leaf area.

Crop growth rate (g $m^{-2}d^{-1}$) was computed from crop gross assimilation rate (P_{gd}, g CH_2O $m^{-2}d^{-1}$) minus maintenance respiration rate (R_m, g CH_2O $m^{-2}d^{-1}$), multiplied by a conversion efficiency of assimilates to structural dry mass. P_{gd} depends primarily on the incident PAR and on the crop leaf area index (LAI). R_m is a function of temperature, plant dry mass and simulated relative crop growth rate (Heuvelink, 1995b). In CHRYSIM1.0 the maximum endogenous photosynthetic capacity of a leaf was assumed to be 1 mg CO_2 $m^{-2}s^{-1}$ and all the leaves were

considered to have identical photosynthetic properties. For both model validation and utilisation LAI and specific leaf area (SLA) were calculated. LAI was simulated as leaf dry mass multiplied by SLA. SLA was calculated as a function of the year, but the values used in CHRYSIM1.0 (Lee *et al.*, 2002a) were adjusted to 80 %, based on the measured SLA. Dry mass partitioning between stem, leaves and flowers was simulated as a function of crop development stage and three main developmental stages were distinguished. Stage 0 corresponds to the planting date, Stage 1 is the start of the SD and Stage 2 is the harvest. Between these stages, development increased linearly with time. Dry mass partitioning to the roots was assumed to be constant (10 % of the TDM), based on experiments with plants grown in expanded clay grit (Lee *et al.*, 2002a). Further details on the model description are the same as for Lee *et al.* (2002a).

Model validation and utilisation

CHRYSIM1.0 and the module to predict NoF (NoF = 1.938TDM – 2.34; Chapter 4.1) were both validated using the greenhouse experiment referred above. To validate CHRYSIM1.0, measured daily outside global radiation, inside greenhouse temperature and CO_2 concentration were inputs to the model. A greenhouse transmissivity of 49 % for diffuse radiation was measured and used as input. Observed initial stem and leaf dry mass per plant were also input to the model.

Total dry mass per m^2 was simulated using the same combinations of LD period and plant density as in the present greenhouse experiment. For each growing season, two types of simulations were performed, using a fixed planting date (start of SD dependent on the treatment) or a fixed date of start of the SD period. The former type reflects the grower's situation, since the grower plants in a certain date and has to decide how many LDs should be given to the crop. The latter type synchronises the start of SD, to represent the greenhouse experiment situations. An additional simulation per season was carried out, using the reference commercial growth conditions for a crop grown under a supplementary assimilation light level of 4000 lux (i.e. 49 μmol $m^{-2}s^{-1}$) (DLV consultancy group, Wageningen, The Netherlands). Values for daily outside global radiation were taken from Breuer and Van de Braak (1989), representing the average annual data at De Bilt (52°N, The Netherlands). This so-called 'selected year' results in the same average irradiance as the one observed over 30 years (1951-1980) at De Bilt, but with natural variation. The use of supplementary assimilation light was dependent on the global radiation (switch on at 200 and off at 300 W m^{-2}). Average daily greenhouse temperature varied between 19°C in winter and 21°C in summer. CO_2 concentration ranged from 400 μmol mol^{-1} in summer up to

1000 μmol mol^{-1} in winter. Temperature and CO_2 concentration were considered constant during 24 h. Day length was 20 h for LD and 11.5 h for SD period. Greenhouse transmissivity for diffuse radiation was assumed to be 70 %, as it is more representative for commercial conditions. Initial organ dry mass per plant was input to the model: 0.09 g for the stem, 0.16 g for the leaves and 0.03 g for the roots. Duration of the response time, i.e. time from start of SD period to harvest, from plants grown under the reference commercial growth conditions was obtained from DLV consultancy group. For the non-reference growth conditions that information was adjusted based on experimental data. Thus, it was assumed that when LD period increased by 1 week, for the same plant density, the duration of the SD period decreased by 1 day. Total dry mass per m^2 was converted into total plant fresh mass by dividing by plant density and dry matter content. Based on experimental data (Carvalho, unpublished), total dry matter content was adjusted to the season (0.11-0.15) and to the duration of the LD period (increasing 1 % when LD period increased by 1 week). Graphics of the annual variation of the climatic data, SLA and response time, as well as the dry mass partitioning as a function of the development stage are given by Lee *et al.* (2002a).

Statistical design and analysis

Nine treatments, resulting from the combination of three durations of the LD period (2, 9 and 16 days) with three plant densities (48, 64 and 80 plants m^{-2}), were allocated to six beds and the two outer beds were used as border. The experimental set-up was a complete randomised block design with three replications. Each replication consisted of two consecutive soil beds. Analysis of variance and linear regression analysis was conducted and treatment effects were tested at 5 % probability level. Mean separation was done using Student's *t*-test ($P = 0.05$). The statistical software package Genstat 5 (IACR-Rothamsted, UK) was used.

Results

Greenhouse experiment

Plants that received 16 LDs and were grown at 48 plants m^{-2} were the first ones to be harvested, hence showing the shortest response time (53 days). In contrast, plants that received 2 LDs and were grown at 80 plants m^{-2} had the longest response time (58 days). Nevertheless, the total cultivation period of the later combination was still 9

days shorter than the former, due to the shorter duration of the LD period (data not shown).

Plant height had a significant positive linear relationship ($P < 0.001$) with duration of the LD period (Fig. 2A). Plants that received 16 days of LD period were 34 % taller than plants that received 2 LDs. This positive effect on plant height was mainly a result of increased number of internodes (34 % more internodes) and only marginally due to higher average internode length (6 % longer internodes) (data not shown). Plant height increased with plant density up to an optimum density of 72 plants m^{-2} ($P = 0.033$). However, at this density plants were only 7 % taller than plants grown at 48 plants m^{-2} (Fig. 2B). Stem length was on average 9-11 cm shorter than the plant height (which includes the peduncle length of the uppermost flower) and showed a similar response to both duration of the LD period and plant density (data not shown).

NoF was significantly ($P = 0.019$) influenced by the interaction between duration of the LD period and plant density (Fig. 3A). In general, extending the duration of the LD period and decreasing plant density resulted in more flowers per plant. However, the absolute effect of duration of the LD period on NoF was larger at 48 plants m^{-2} compared to at higher plant densities. Furthermore, in plants that received 16 LDs there was a strong reduction in NoF when plant density increased from 48 to 64 plants m^{-2}, but a further increase up to 80 plants m^{-2} showed no significant effect on NoF. As a result of this interaction a similar NoF could be obtained with different combinations of these growth conditions. For example, a plant with 16 flowers, including flower buds, could be obtained in a crop receiving16 LDs at 80 plants m^{-2}, 9 LDs at 60 plants m^{-2} or 2 LDs at 52 plants m^{-2} (Fig. 3A).

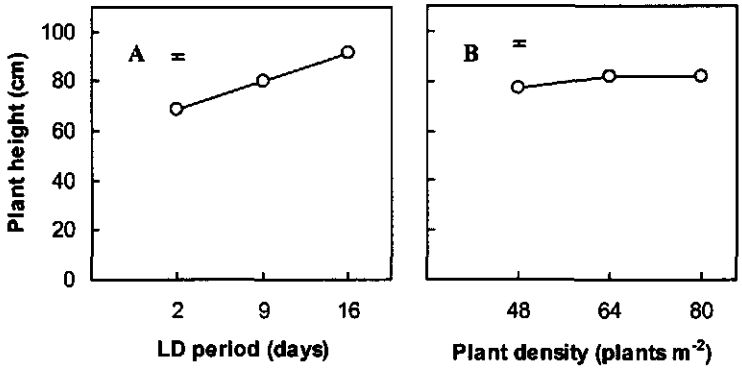

FIG. 2. Plant height as a function of duration of the LD period (A) and plant density (B) at harvest of *Chrysanthemum* 'Reagan Improved'. Regression lines: A, $y = 1.67x + 65.4$; B, $y = -0.0093x^2 + 1.34x + 34.5$. Vertical bars indicate $LSD_{16, 0.05} = 2.5$.

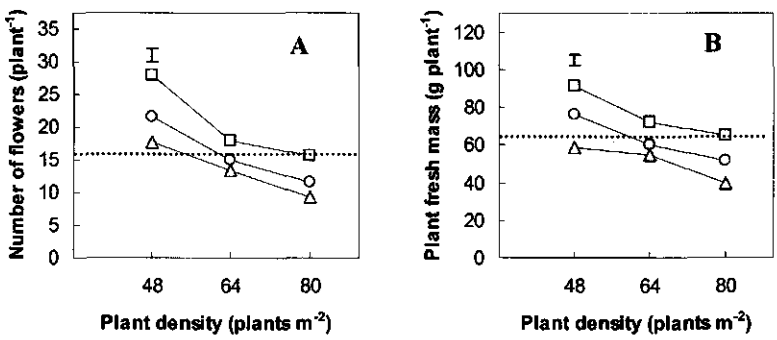

FIG. 3. Total number of flowers and flower buds per plant, NoF (A), and total plant fresh mass, TFM (B), as a function of duration of the LD period (△ 2 days, ○ 9 days and □ 16 days) and plant density at harvest of *Chrysanthemum* 'Reagan Improved'. Vertical bars indicate $LSD_{16,0.05}$ = 2.15 (A) and 6.04 (B). Dashed line represents an example of a trade-off between the growth conditions to obtain a given NoF (16 flowers plant^{-1}) and TFM (65 g plant^{-1}).

The percentage of flowers in a bud stage was not significantly influenced by the duration of the LD period (P = 0.271), but plant density showed a significant negative linear effect (P = 0.002). Thus, plants grown at 48 plants m^{-2} had 15 % of their flowers in a bud stage, whereas plants grown at the highest plant density had 5 % of flower buds only. Similarly, the percentage of flowers located at the first 15 cm from the top of the plant was not influenced by the number of LDs (P = 0.878). A significant positive linear relationship was, however, found between this flower percentage and plant density (P = 0.007). Plants grown at higher plant densities had their flowers more concentrated at the top of the plant (e.g. 78 % top flowers, for 80 plants m^{-2}), whereas reducing plant density flowers became more distributed over the main stem (e.g. 69 % top flowers, for 48 plants m^{-2}).

In contrast with the previous quality aspects, individual flower size was not affected by the duration of the LD period (P = 0.145: flower area; P = 0.432: flower dry mass) nor by plant density (P = 0.995: flower area; P = 0.404: flower dry mass). Flower area and dry mass of the fully open flowers was on average 29 ± 0.5 cm^2 flower^{-1} and 0.21 ± 0.003 g flower^{-1}, respectively.

A significant interaction (P = 0.017) between duration of the LD period and plant density was observed for TFM (Fig. 3B). The effect of this interaction on TFM was very close to the one observed for NoF, suggesting a positive relationship between these two variables (Fig. 3). Actually, when NoF was plotted against TFM a positive

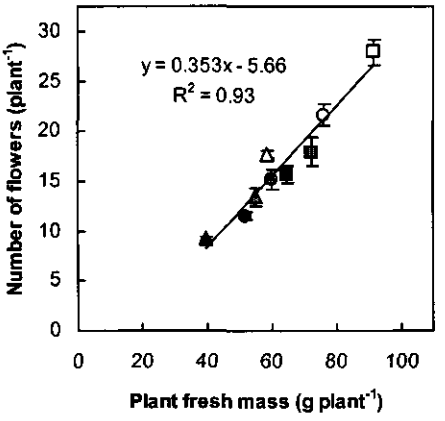

FIG. 4. Relationship between total number of flowers and flower buds per plant, and total plant fresh mass at harvest of *Chrysanthemum* 'Reagan improved'. Each symbol represents the average from the combination of three durations of the LD period (△, ▲, ▲ 2 days; ○, ●, ● 9 days and □, ■, ■ 16 days) and three plant densities (△, ○, □: 48 plants m^{-2}; ▲, ●, ■: 64 plants m^{-2} and ▲, ●, ■: 80 plants m^{-2}). Line represents linear regression. Vertical bars indicate s.e.m. (n = 3).

linear relationship was found (Fig. 4). Plants that received 16 days of LD period and were grown at 48 plants m^{-2} were the heaviest (91.5 g $plant^{-1}$) and obtained the highest NoF (28 flowers $plant^{-1}$). The opposite treatment combination, i.e. 2 days of LD period and 80 plants m^{-2}, resulted in plants with only 39.4 g and around 9 flowers per plant including buds.

Model simulations

Simulated and measured TDM showed a good agreement for the nine studied combinations of LD period and plant density (Fig. 5A). The slope of the regression line, which relates these variables, was 1.08 indicating an average overestimation of 8 %. However, this slope did not differ significantly from 1.0. Predicted dry mass varied between 96 % and 139 % of the measured value, where the highest overestimation was observed for combinations including 2 days of LD period. This was a result of a general overestimation of the LAI (leaf area index), which had a relatively stronger impact on a crop with low LAI. Simulated NoF was also very close to the measured values. Predicted NoF varied between 79 % and 111 %, resulting in an average underestimation of 4 % (Fig. 5B).

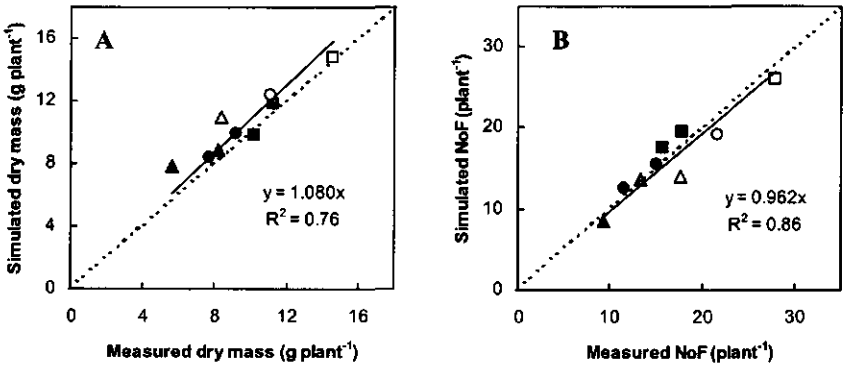

FIG. 5. Simulated and measured total plant dry mass (A) and total number of flowers and flower buds per plant (B) at harvest of *Chrysanthemum* 'Reagan improved'. Symbols' legend in Fig. 4. Solid line represents linear regression and dashed line represents 1:1 relationship.

Simulated TFM for the different seasons (Fig. 6 and 7) responded similarly to duration of the LD period and plant density as the TDM (not shown). Nevertheless, the lines were closer to each other for TFM as dry matter content is 1 % higher when LD period increases by 1 week. Similarly to the greenhouse experiment higher number of LDs and lower plant density resulted in higher simulated TFM, being the LD period effect larger at low plant density (Fig. 6 and 7). This trend was always observed except for the crops planted in 22 September (Fig. 6C) and crops with a common start of SD on 5 January (Fig. 7D), where no effect of the duration of the LD period on predicted TFM was found. Furthermore, the negative effect of plant density on simulated TFM was larger in spring and summer (Fig. 6A and B) compared to plants grown in autumn or winter (Fig. 6C and D). Therefore, although different combinations of number of LDs and plant densities resulted in the same TFM, this trade-off was dependent on the season.

Simulations performed on the reference commercial growth conditions showed a rather constant TFM for the spring and summer crops, with several possibilities to achieve an identical TFM (Fig. 6A and B). For instance, a crop planted in 22 March that received 2 LDs and was grown at 53 plants m^{-2} would result in a TFM of 86 g $plant^{-1}$, just like a crop that received 11 LDs and was grown at 65 plants m^{-2} (i.e., under the reference growth conditions) (Fig. 6A). In autumn, but especially in the winter crops, a drastic reduction of predicted TFM was observed for the reference growth conditions. No possibilities of trade-off, within the simulated combinations, were feasible for a crop planted in 22 September (Fig. 6C) or a crop with a common

start of SD on 5 January (Fig. 7D). In contrast, for a crop planted in 22 December this possibility was present, but all the simulated combinations of LD period and plant density resulted in a lower TFM than the reference growth conditions (Fig. 6D).

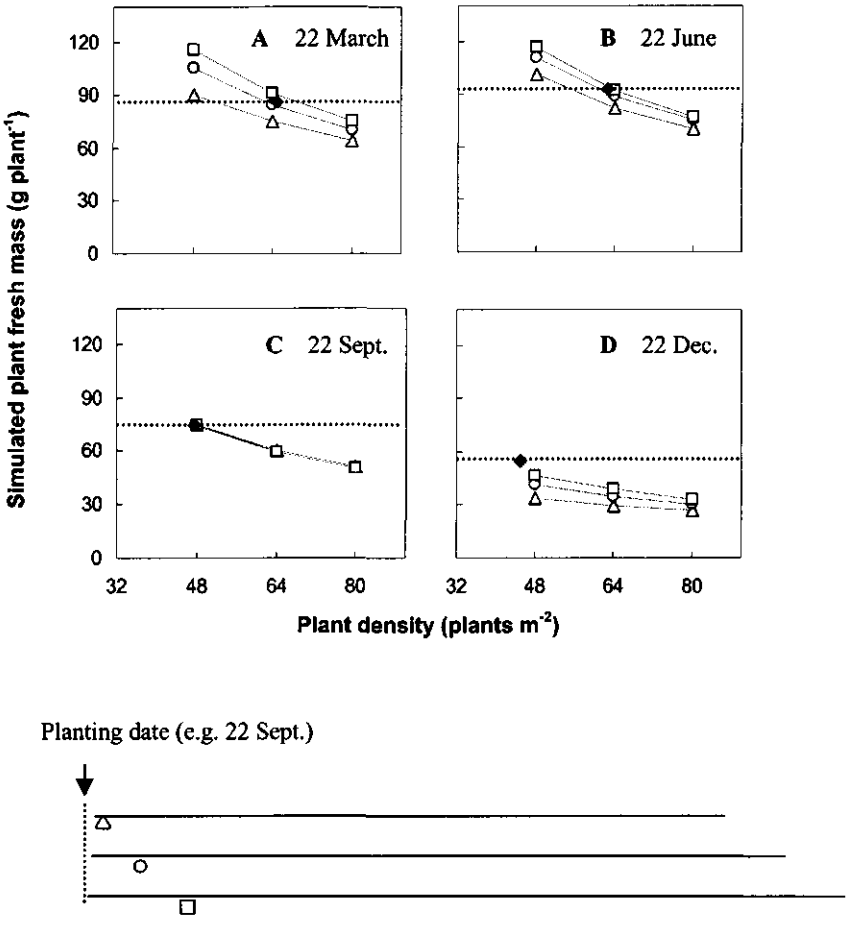

FIG. 6. Simulated total plant fresh mass (TFM), as a function of duration of the long-day (LD) period (△ 2 days, ○ 9 days and □ 16 days) and plant density at harvest of *Chrysanthemum* 'Reagan Improved', for four planting dates (A-D). Black diamonds represent simulations under reference commercial growth conditions: A, 11 days LD and 65 plants m^{-2} (1343 mol m^{-2}, cumulative incident PAR); B, 10 days LD and 62.5 plants m^{-2} (1449 mol m^{-2}); C, 15 days LD and 47.5 plants m^{-2} (649 mol m^{-2}); D, 19 days LD and 45 plants m^{-2} (469 mol m^{-2}). Dashed lines represent the trade-off between the growth conditions to obtain a similar TFM.

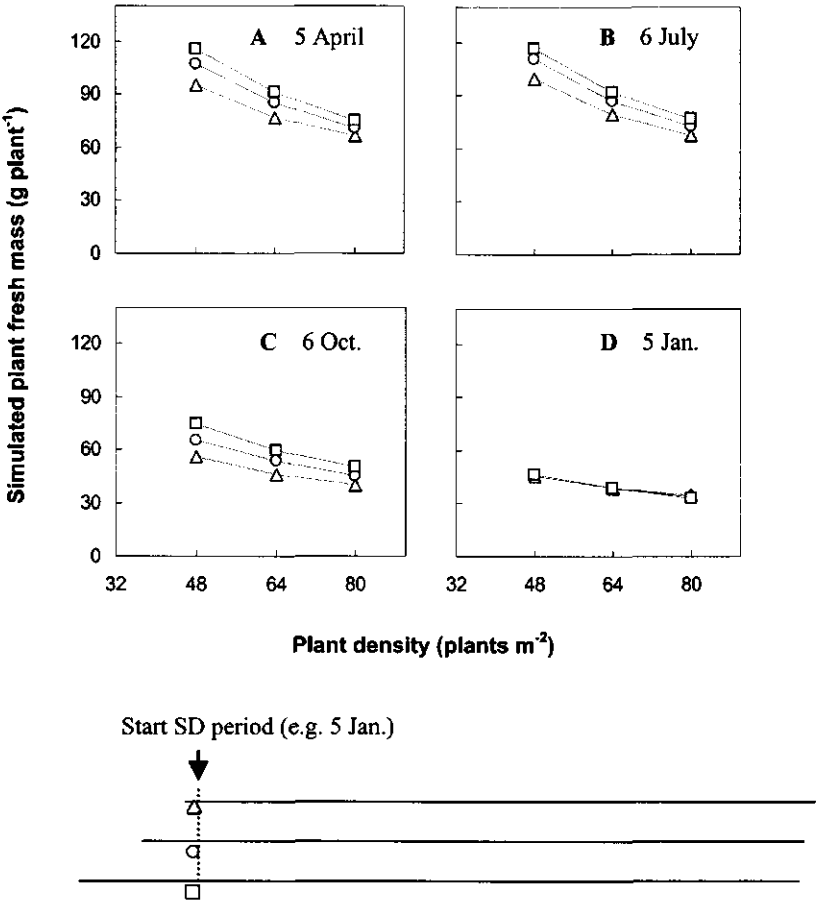

FIG. 7. Simulated total plant fresh mass, as a function of duration of the LD period (△ 2 days, ○ 9 days and □ 16 days) and plant density at harvest of *Chrysanthemum* 'Reagan Improved', for four dates of starting of the short-day period (A-D).

Discussion

Decreasing the number of LDs, which results in a shorter cultivation period, or increasing plant density are possible ways to increase annual yield in cut chrysanthemum. This study clearly demonstrates that such changes can, however, strongly affect several external quality aspects, except for the individual flower size. For example, plants grown under a shorter duration of the LD period or a higher plant

density had fewer flowers per plant (Fig. 3A) and lower TFM (Fig. 3B). These results are consistent with previous studies (Lee *et al.*, 2002b and Chapter 4.1). Nevertheless, it is interesting to realise that although plants grown at higher plant densities had less flowers, they had relatively more flowers with marketable value, i.e., lower percentage of flowers in a bud stage and higher percentage of flowers located at the top 15 cm of the plant (data not shown). In contrast, flower size was not significantly affected by the studied cultivation measures. The constant average value of 0.21 g $flower^{-1}$, obtained for a fully open flower, is in agreement with the module for flower size prediction proposed in Chapter 4.1. The observed positive linear relationship between NoF and TFM (Fig. 4) was also described in Chapter 4.1, but for TDM. Both relationships reflect the positive effect of higher assimilate availability on NoF, and this can be successfully used to predict NoF (Fig. 5B).

As TFM shows a positive linear relationship with cumulative incident PAR per plant (Lee *et al.*, 2002b) a higher simulated TFM was obtained during spring and summer compared to autumn and winter (Fig. 6). This relationship also explains why plants were heavier when the number of LDs increased, as this resulted in a longer cultivation period. In the September planting, however, the extension of the cultivation period had only a slight contribution to the cumulative incident PAR, because this extension was at the end of the cultivation, which occurred in a period of low light intensity (Fig. 6C). Interestingly, an identical situation was observed in crops with a common start of SD on 5 January. Actually, the two types of simulations had in common the moment where the extension of the cultivation period occurred, i.e. in December, but in the latter this happened at the beginning of the cultivation period (Fig. 7D). Furthermore, the positive effect of LD duration on TFM was larger at low plant density (Fig. 3B; Fig. 6). This is due to a higher light interception per plant at low plant density. Consequently additional LDs represent per plant more additional light than at high plant density.

The negative effect of plant density on TFM was gradually smaller at higher densities. This can be explained by the fact that when plant density increases from 48 to 64 plants m^{-2} this represents a relative increase of 33 %, whereas this is only 25 % when plant density increases from 64 to 80 plants m^{-2}. The linear relationship between cumulative incident PAR and TFM also explains the larger negative effect of plant density on TFM during spring and summer than in autumn and winter.

From this work it can be concluded that a cut chrysanthemum grower can achieve a similar quality using different combinations of the two studied cultivation measures if an adequate trade-off between them is chosen. For instance, plants that received 16 LDs and were grown at 80 plants m^{-2} resulted in a TFM of approximately 65 g and 16

flowers per plant, which would also be obtained using the combination of 9 days of LD period and 60 plants m^{-2} (Fig. 3), without affecting individual flower size. However, this trade-off is very much dependent on the light intensity during the cultivation period, being more flexible at higher light levels (Fig. 6). Furthermore, the strong positive effect of LD period on plant height must also be taken into account (Fig. 2A). This positive effect was mainly due to higher number of internodes as a result of later flower initiation. Therefore, the manipulation of LDs can be an effective method to control plant height. This is especially interesting in summer as an alternative method to the temperature manipulation (Hendriks and Ueber, 1995).

Conclusions

In this study it is shown that a crop simulation model is particularly useful as the trade-off, between duration of the LD period and plant density, is strongly dependent on the moment where the extension of the cultivation period occurs. The present biomass production model, combined with the modules to predict flower number and size, can be used as a tool to define optimal combinations of these growth conditions throughout the year, when aiming at a certain chrysanthemum quality.

6

General Discussion

The development of models on product quality has been an important and challenging issue in the greenhouse simulations, over the last few years (Challa, 2002). Nevertheless, only few models for ornamental crops are available (Marcelis *et al.*, 1998) and these are mainly focused on the growth and development, rather than on product quality (Gary *et al.*, 1998). Lee (2002) developed and validated a photosynthesis-driven growth model, for the prediction of dry mass production in year-round cut chrysanthemum (CHRYSIMv1.0). Since the visual quality aspects have a large influence on the selling price of chrysanthemum, this explanatory model could highly benefit from the incorporation of 'modules' to predict the main external quality aspects.

To predict the main external quality aspects of cut chrysanthemum a good quantification and understanding of the effects of the above-ground growth conditions is needed. The influence of some growth conditions on specific external quality aspects of chrysanthemum has been well documented, especially for pot chrysanthemum. This is particularly the case for stem length control, based on day and night temperature manipulation, and for the photothermomorphogenic effects on time to flowering (Chapter 2). Although this information could also be partly used for cut chrysanthemum, an integrated view on the factors involved in chrysanthemum external quality was still missing. In Chapter 2 an attempt was made to integrate the available knowledge on this topic. From this literature study it became clear that the quality aspects were rarely the focus in studies on chrysanthemum. The information was widely spread and often a clear understanding of the processes behind a quality aspect was lacking, and the results could not easily be generalised. Furthermore, some contradictions were also found when comparing different studies. Part of these contradictions could be attributed to the use of different cultivars in different studies, but others needed additional research. For instance, the conflicting results about validity of the DIF concept (difference between day and night temperature) to predict internode length needed to be clarified (Chapter 3.1). Moreover, the physiological background of this concept needed further investigation (Chapter 5.1). In contrast with the influence of temperature on stem length, only limited information was available on the flower characteristics, i.e. on number of flowers per plant and flower size of chrysanthemum. The effects of plant density and duration of the LD period on various quality aspects was also a weak point in chrysanthemum research. From Chapter 2 it became clear that assimilate availability (increased by higher light intensity, higher CO_2 concentration and lower plant density) positively affects several visual quality aspects.

Several experiments were conducted and important functional relationships were developed (Chapter 3 and 4). These experiments provided a basis for modelling several chrysanthemum external quality attributes. A special focus was given to the prediction of plant height, number of flowers and flower size, as they were considered the most important external quality attributes that needed further investigation. In the present study plant height and stem length showed exactly the same results since the former was equal to the latter plus around 8 to 11 cm from the length of the flower peduncles at harvest stage (data not shown). Therefore we refer only to the plant height, which is most sound in terms of visual quality. The module, however, was build for stem length. A photosynthesis driven crop growth model for predicting total dry mass in cut chrysanthemum (Lee *et al.*, 2002a) was validated and further used to address some practical questions (Chapter 5.2).

Modelling external quality of cut chrysanthemum: achievements and limitations

Plant height

Although several growth conditions have been described to have an effect on chrysanthemum stem elongation (Chapter 2), in the present study it was found that only the temperature (Chapter 3.1) and the length of the LD period (Chapter 4.1) had a major influence on plant height. Increased assimilate availability by higher light intensity, higher CO_2 concentration and lower plant density only increased plant height with a maximum of 10 % (Chapter 4.1). Hence, it is concluded that stem elongation is not principally a matter of assimilate availability. This conclusion is supported by the absence of a seasonal effect on stem length, even though incident photosynthetically active radiation (PAR) increased by more than a factor four from winter to summer crops (Table 1, Chapter 4.1).

Lee (2002) suggested that applying the concept of specific stem length (i.e. average stem length per g of stem dry mass, cm g^{-1}; e.g. Kropf and Van Laar, 1993) could be an interesting approach for modelling chrysanthemum stem length based on photosynthesis and dry mass partitioning into the stem. The major advantage of using such approach would be the possibility of directly including this information into a photosynthesis-driven model. Nevertheless, this approach is not interesting in the case of cut chrysanthemum as assimilate availability has only a minor effect on stem length. Stem length of cut chrysanthemum should be, therefore, modelled separately

from crop photosynthesis. A module for stem length could be based on the prediction of the number of internodes and the internode length (Fig. 1).

Due to the determinate growth pattern of cut chrysanthemum, new internodes are formed only until the start of flower initiation. The longer the duration of the LD period the taller the plants, as more time exists to form new internodes (Chapter 4.1 and 5.2). This explains the positive linear relationship between plant height and LD period observed in Chapter 5.2. Temperature was also found to have a strong positive effect on the number of internodes, due to its effect on the internode appearance rate (IAR) (Chapter 3.1). This relationship was described as a quadratic response of IAR to day temperature, with an optimum at 25.7 °C (Table 2, Chapter 3.1).

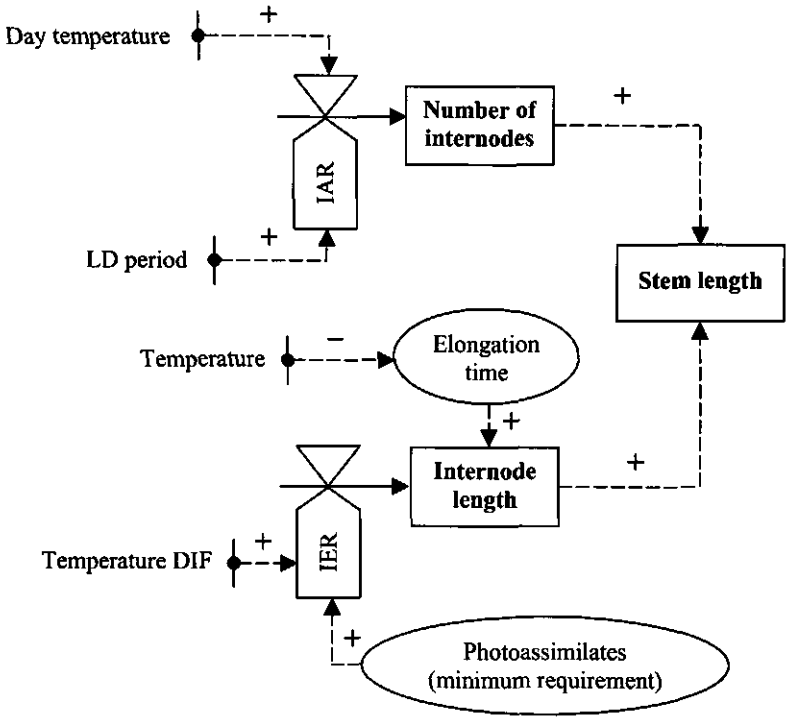

FIG. 1. Relational diagram of the module describing stem length in cut chrysanthemum. Boxes are state variables, circles are parameters and valves are rate variables: (+) indicates positive influence, (–) indicates negative influence. Solid arrows represent mass flow and dashed arrows represent information flow. Abbreviations: DIF = difference between day and night temperature; IAR = internode appearance rate; IER = internode elongation rate; LD = long-day.

Number of flowers per plant

A positive linear relationship between the number of flowers per plant, including flower buds (NoF), and the total aerial plant dry mass (TDM) was found (Chapter 4.1, Fig. 3; NoF = 1.938 TDM – 2.34). This module adequately described NoF, for highly variable growth conditions, i.e. daily incident PAR (from 4.2 to 18.3 mol $m^{-2}d^{-1}$), CO_2 concentration (from 345 to 623 μmol mol^{-1}) and duration of the LD period (from 0 to 21 days). The variation in temperature was smaller, but still between 19.1 and 22.6 °C (Table 1, Chapter 4.1). This relationship was further validated using an independent data set (Chapter 5.2). Simulated and measured TDM showed a good agreement for the nine studied combinations of LD period (2, 9 and 16 days) and plant densities (48, 64 and 80 plants m^{-2}), with an average underestimation of 4 % only (Chapter 5.2, Fig. 5B). Therefore, as NoF is closely related to the assimilate availability, a photosynthesis driven crop growth model to predict TDM (Lee, 2002) can be extended with this module for predicting NoF (Fig. 2).

Attention should, however, be paid when using this module to predict NoF at low light intensity combined with a relatively high temperature. This was the case in Chapter 3.2, where it was found that temperature had a strong positive effect on NoF but only a minor influence on TDM. When temperature increased from 17 to 21 °C, plants showed 47 % higher NoF whereas TDM only increased by 7 % (Table 2, Chapter 3.2). For this reason the regression module for NoF gave a good prediction for the 17 °C temperature treatment (5 % overestimation), but it underestimated NoF in the 21 °C treatment with 23 %. The reason that temperature had a large impact on the NoF in Chapter 3.2, and only a minor effect in Chapter 4.1 may be related to the different range of temperatures. However, it is most likely due to an interaction between irradiance and temperature, as in plants grown under high light levels NoF is less temperature sensitive (Chapter 4.1). Several studies reported that increased photosynthetic photon flux can partly reduce the unfavourable temperature conditions (e.g. Karlsson and Heins, 1986; Karlsson *et al.*, 1989b) Therefore, a positive effect of temperature on NoF at low light conditions should be included in this module, in particular for temperature in the last phase of the SD period (phase III). In Chapter 3.2 such a linear relationship between temperature and NoF is given. However, to establish such function more accurately this interaction between light and temperature needs further investigation, using a wider range of light and temperatures.

In Chapter 5.2 a similar relationship using total aerial fresh mass (TFM) instead of TDM to predict NoF was shown, which can be more interesting for growers. This module can also be made more practical, from the viewpoint of product quality, when

excluding the flower buds as they do not have a commercial value, because buds do not develop into flowers during vase life (data not shown).

An important conclusion from the present study is that when plant assimilate status becomes more favourable (e.g. wider spacing and/or higher light intensity), chrysanthemum will react with producing more flowers and flower buds, rather than larger flowers (Chapter 4.1 and 4.2). It has clearly been demonstrated that the reason for this reaction is not that flowers would not be able to grow bigger, as actual flower dry mass is only 42 % of potential flower dry mass (Chapter 4.2).

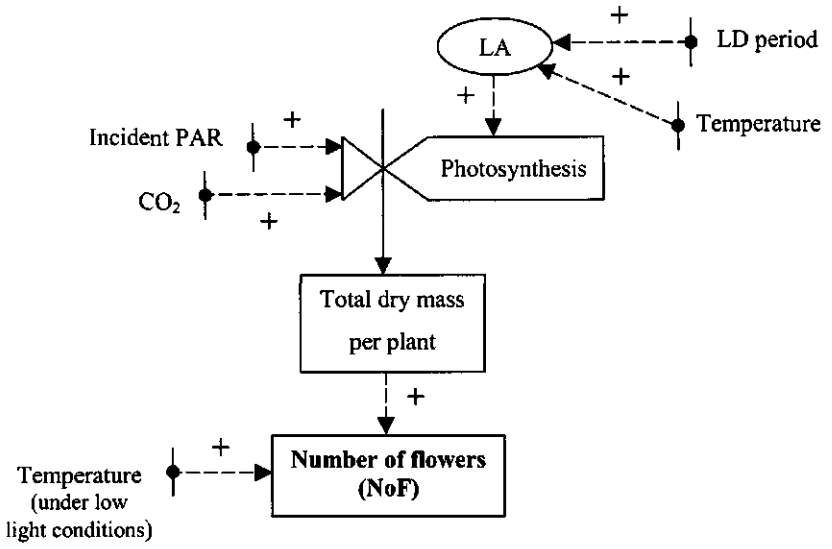

FIG. 2. Relational diagram of the module describing number of flowers and flower buds (NoF) in cut chrysanthemum: (+) indicates positive influence. Boxes are state variables, circles are parameters and valves are rate variables. Solid arrows represent carbon flow and dashed arrows represent information flow. Abbreviations: LA = leaf area; PAR = photosynthetically active radiation.

Individual flower size

In general, for the prediction of the individual flower dry mass or individual flower area, light intensity does not need to be taken into account, unless incident PAR during the SD period is under a certain threshold value: 7.5 mol $m^{-2}d^{-1}$ for 'Reagan Improved' (Fig. 3). For light values below this threshold a positive linear relationship between flower size and light level was observed, but above threshold value flower size remained constant (Chapter 4.1, Fig. 4). Temperature during the SD period is an important model input. In Chapter 3.2 a temperature regression model is presented including the distinct effects of temperature, according to the phase of the SD period. Nevertheless, similarly to the above described interaction between the irradiation and temperature on the NoF effect, it was found that higher temperatures during the SD period had only a negative effect on flower size at low irradiance levels (Chapter 3.2). Further research is needed to find out if a threshold value for light exists, under which high temperatures have a negative effect.

When modelling flower size in spray-type cut chrysanthemum flower position was found to be irrelevant, except when comparing flowers located on the first and second order axillary shoots (Chapter 4.2). Although further research is needed to determine whether a constant ratio between size of first and second order flowers exists, it was observed that the latter had around 60 % of the dry mass and area of the former (Chapter 4.2, Fig. 2B and 3B). If such constant ratio would be general, size of the second order flowers could be predicted from the size of the first order ones. Further investigation is also needed on the quantification of the effects of the growth conditions on the number of second order flowers. Light intensity and plant density certainly affect this aspect, as they are known to play an important role in chrysanthemum branching (Chapter 2).

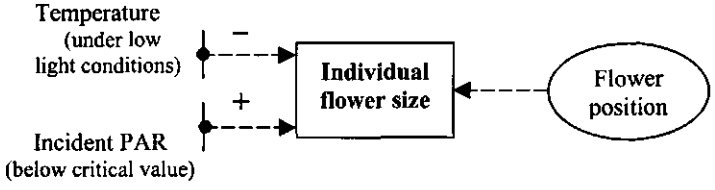

FIG. 3. Relational diagram of the individual flower size of a fully open flower of cut chrysanthemum: (+) indicates positive influence, (–) indicates negative influence. Boxes are state variables and circles are parameters. Dashed arrows represent information flow. Abbreviations: PAR = photosynthetically active radiation.

Flower position and colour

Flower position within the plant was studied based on the flower distance (i.e. distance between the highest and the lowest flower on the stem) and on the percentage of open flowers located at the top 15 cm. Only the effects of temperature, plant density and duration of the LD period were evaluated. Except for the plant density no relevant effects were found on the flower position (Chapter 5.2). At reduced plant density, flowers became more distributed over the main stem. Therefore, it seems that flower position is closely related to the light quality, as high plant density results in a canopy that filters much of the red light, while allowing larger amounts of far-red light to pass through (Heins and Wilkins, 1979). This reduction in red:far-red ratio stimulates primary stem elongation and inhibits lateral branching (Heins and Wilkins, 1979). Furthermore, high densities result in a lower assimilate availability per plant and therefore less lateral buds break (Chapter 4.1). Therefore, in a high density crop only buds at the apical part of the stem will break and grow; hence flower distance will decrease and percentage open flowers will increase with plant density.

Flower colour was found to have a negative linear relationship with the temperature applied during flower development phase (phase III, Chapter 3.2). Variation in other growth conditions showed no substantial differences in visual flower colour at harvest (no measurements conducted). Although it was not quantified, monoflower plants of 'Reagan Improved' showed a darker pink colour compared to the control spray type. This is possibly related to the higher carbohydrate content of the monoflower plants (Chapter 4.2), which are used for the conversion into anthocyanins (pigments responsible for the pink colour) (Weiss, 2000).

Flower dry mass partitioning

Temperature is the most important climatic factor influencing dry mass partitioning in crops, since irradiance and CO_2 concentration primarily affect source activity (Marcelis and De Koning, 1995). Interestingly, in the present work temperature (17-21 °C) hardly affected flower mass ratio (FMR, i.e. the ratio between total flower dry mass and total aerial plant dry mass). The reason why chrysanthemum did not respond to temperature is related to the compensation effect described in Chapter 3.2, i.e. at higher temperature plants show higher NoF but of smaller size (Table 2). When applying a wider temperature range in climate rooms (15-24 °C), a negative linear effect of temperature in the first part of the SD period (phase II) on FMR was described and explained in Chapter 3.2. Over that temperature range FMR decreased from 0.20 to 0.16 (Chapter 3.2, Fig. 7).

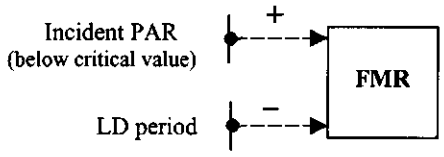

FIG. 4. Relational diagram of the module describing flower mass ratio (FMR) in cut chrysanthemum: (+) indicates positive influence, (–) indicates negative influence. Boxes are state variables, circles are parameters and valves are rate variables. Solid arrows represent carbon flow and dashed arrows represent information flow. Abbreviations: LD = long-day; NoF = number of flowers and flower buds; PAR = photosynthetically active radiation.

When modelling dry mass partitioning towards the flowers much attention should be paid to the duration of the LD period (Fig. 4). Increased number of LDs linearly reduced FMR, as plants were for longer time growing vegetatively only (Chapter 4.1). Despite incident PAR had shown a positive effect on FMR when comparing different seasons, within the same season reducing incident PAR from 17 to 7.3 mol $m^{-2}d^{-1}$ had a very minor effect (Table 3, Chapter 4.1). Therefore, it seems that incident PAR mainly influences FMR at low PAR.

Besides the effects of the growth conditions mentioned above, FMR showed a saturation response to number of flowers per plant, in plants were the flower number was imposed (Chapter 4.2). This agreed with observations for many other crops where partitioning to sink organs (e.g. fruits) showed a saturation response to the number of those sink organs on the plant (e.g. Heuvelink and Buiskool, 1995; Marcelis, 1993a).

Practical application

From this study it is clear that external quality in cut chrysanthemum is a rather complex phenomenon as several growth conditions can affect many quality aspects. The simplification of this system, by the use of prediction models, is therefore very useful to define the 'optimum greenhouse climate' to reach a certain quality at harvest. In Chapter 5.2 a case study was conducted and it was shown that the use of an explanatory model that also incorporates some quality aspects, is extremely valuable in terms of decision support as several options are possible to obtain a given quality and these options are very much dependent on the planting week.

As the daily light integral plays an important role in several quality aspects, special attention should be paid to this factor. For instance, when chrysanthemum was planted in September (autumn crop) quality was drastically reduced in terms of TFM, NoF and even a reduction in flower size was observed (Chapter 4.1 and 5.2). The use of supplementary assimilation lamps in the autumn and winter crops is, therefore, an important tool to reduce the natural variation in the external quality. However, economic calculations are necessary to determine whether their use is profitable and which light intensity should be installed (e.g. Roelofs *et al.*, 2001). Our study can supply the yield and quality predictions needed for such economic evaluations. An example of that, only focused on TFM, was given already by Lee *et al.* (2002a).

Although this research was strongly based on one cultivar (Reagan Improved) it seems likely that other cultivars will show a similar behaviour. This was already observed for both other cultivars studied ('santini' type), which are substantially different from 'Reagan Improved' in their morphology (smaller plants, with higher number of flowers of small size), but reacted much the same (Chapter 4.1 and 4.2). For instance, 'Goldy' and 'Lupo' also showed a linear relationship between NoF and TDM. The model is explanatory and built up from individual modules, such that it is relatively easy to adapt it to other cultivars. Most likely, the model structure will not change, but several model parameters are certainly cultivar specific. These parameter values may be determined from a limited number of experiments (much less than needed in this thesis). For example, if we know, based on the present work, that the relationship between TDM and NoF is linear, only two extreme conditions, resulting in a low and in a high TDM are needed for each new cultivar to determine this relationship. The model could include cultivar correction factors, which are set to one for 'Reagan Improved' and adapted for other cultivars by calculating the ratio between a parameter for a given cultivar and 'Reagan Improved'. The advantage of this approach is the limited number of experiments that it will require to adjust the modules to other cultivars.

It should be stressed that, unlike many previous studies, this work covers different seasons and a wide range of growth conditions within experiments. Therefore, results are more general and to some extent also validated for cultivars very different from 'Reagan Improved'.

Future research

Major limitations to this research are: (1) lack of information on the effects of growth conditions on the uniformity at harvest; (2) limited validation and no sensitivity analysis.

Obtaining a uniform crop at harvest, in terms of timing and quality, is an important aim for growers, as this will allow a single (machine) harvest and product uniformity is much desired. For example, when using the trade-off between length of the LD period and plant density (Chapter 5.2) to optimise cultivation schedules, effects on uniformity should be taken into account. Increasing plant density, especially in autumn and winter when light is limiting, is known to reduce the uniformity within a chrysanthemum crop (Langton *et al.*, 1999). In Chapter 2, the effect of other growth conditions on uniformity at harvest was reviewed. This can be used as a starting point to further study this important phenomenon.

Future work should be directed at: (1) validation of the model at commercial chrysanthemum farms; (2) integrating this study with complementary research fields, such as economics and postharvest physiology; (3) extending these results to other cultivars. To prove the value of the present work for practice, extensive validation with commercial data is needed. This may result in modifications to the model. After proven validity under commercial conditions, the present model can be included in a decision support system for chrysanthemum growers.

In order to calculate an optimum greenhouse environmental strategy to minimise costs and to produce a plant of a certain quality (Karlsson *et al.*, 1983), or to decide on certain investments (e.g. supplementary light) the results from this study should be complemented with an economical analysis. This would allow to quantify the additional costs, of producing a higher quality chrysanthemum. It would also be very interesting to analyse the postharvest performance (vase life) of a 'high external quality chrysanthemum' and relate it to its growth conditions. Recently, the relationship between growth conditions and vase life in cut chrysanthemum is being studied (U. Van Meeteren, personal communication). A detailed study using a large range of cultivars to calibrate and validate the current modules is particularly important because many new cultivars are introduced in the market every year.

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Summary

For many years the emphasis in floricultural research laid with quantity rather than quality. Nowadays, since the prices are often determined on the basis of visual quality aspects, the so-called external quality, chrysanthemum growers aim to provide a high and constant product quality throughout the year. The external quality of cut chrysanthemum is usually evaluated in terms of stem and leaf morphology and flower characteristics. The priority within the external quality attributes depends on the particular market for the product.

Chrysanthemum cultivation is one of the most controlled and intensive crop production systems in horticulture. This quantitative short-day plant can only be cultivated year-round in greenhouses by controlling several growth conditions. However, many combinations of these conditions are possible, according to the growth strategy being employed. To produce year-round high quality chrysanthemum is a constant challenging problem for the grower, as the seasonal variations in daily light integral will produce large seasonal fluctuations in yield and quality. Therefore, in order to choose the optimal strategy adjusted to the planting week, it is necessary to know how the growth conditions influence plant quality. Thus, the factors involved in chrysanthemum external quality need to be carefully analysed and effectively combined to achieve the production of flowers with the maximum ornamental value year-round, while maintaining a high yield and an acceptable low energy input. Considering the complexity of cut chrysanthemum production, with its many options for control and its range of product quality attributes, management of such a system would be expected to highly benefit from the use of simulation models. For instance, the explanatory models are a valuable tool to integrate knowledge and to assist in the decision support. The development of such models for product quality is still a weak feature in crop modelling research, since priority has been given to simulation of productivity. To develop an explanatory model for the external quality of cut chrysanthemum, detailed knowledge about its growth and morphological development is needed.

The main aim of the present study was to quantify and understand the effects of the aboveground growth conditions on the external quality of cut chrysanthemum at harvest. Special attention has been paid to the integration of this knowledge and its incorporation into an explanatory model to predict the main external quality aspects of cut chrysanthemum. The focus was on the effects of the climate conditions (temperature, light intensity and CO_2 concentration) and cultivation practices (duration of the long-day period and plant density) on plant height (stem length), number of flowers and flower size.

Chapter 2 presents an overview of the growth conditions involved in the different chrysanthemum external quality aspects, and identifies the gaps in literature. A synthesis of the available models that have been built to predict some external quality attributes of chrysanthemum is also given.

The DIF concept states that internode length is dependent upon the DIFference between day (DT) and night (NT) temperature, and is independent of the mean 24 h temperature. This controversial proposition was investigated by means of an experiment described in Chapter 3.1. *Chrysanthemum* 'Reagan Improved' was grown in growth chambers at all 16 combinations of four DT and four NT (16, 20, 24 and 28 °C) with a 12 h daylength. The length of internode 10, the number of internodes and the stem length were measured

periodically. The experiment ended when internode 10 had reached its final length in all temperature combinations employed (27 days). A significant positive linear relationship between DIF and the length of the fully developed internodes was observed over the range of temperatures studied (16-28 °C). It was also found that internode lengths recorded in early stages of development do not bear a close relationship to the final internode lengths, which explained contradictions in literature. In addition to being dependent on the developmental stage of the internodes, the effectiveness of DIF was related to the range of temperatures. It was shown that the DIF concept is valid only within a temperature range where the effects of DT and NT are equal in magnitude and opposite in sign (18-24 °C). Therefore, it was concluded that the response of internode length to temperature is strongly related to DIF, but this response is simply the result of independent and opposite effects of DT and NT. Internode appearance rate, as well as stem length formed during the experiment, showed an optimum response to DT.

Chapter 3.2 is the description of an in depth study on the sensitivity of several flower characteristics to temperature, with the aim of obtaining a better understanding of the underlying physiological processes of flower initiation and development. An attempt has been made to analyse the effects of temperature, applied at different phases of the cultivation period, on each of the studied flower characteristic. Plants were grown in glasshouse compartments at two constant temperatures (17 and 21 °C), and in growth chambers at 32 temperature combinations (from 15 to 24 °C). In the growth chamber experiment the temperature treatments were based upon a division of the cultivation period into three consecutive phases: from planting until the end of the long-day (LD) period (phase I; 18 and 24 °C), from the start of the short-day (SD) period until the visible terminal flower bud (phase II; 15, 18, 21 and 24 °C), and finally from the visible terminal flower bud until harvest (phase III; 15, 18, 21 and 24 °C). Of the characteristics investigated only the flower position within the plant was independent of temperature. The number of flowers and flower buds per plant (NoF), individual flower size and colour (pink) were strongly affected by temperature. It is shown that the temperature effect was largely dependent on the cultivation phase and on the flower characteristic itself. In general, flower characteristics were less influenced by temperature applied during the LD period, compared to the SD period. A higher temperature increased NoF, mainly by increasing the number of flower buds. NoF was affected positively by temperature mainly during phase III, whereas individual flower size increased with temperature during phase II but decreased with temperature during phase III. Lower temperatures during phase III significantly enhanced flower colour intensity. It was concluded that it is not possible to ascribe to each phase of the cultivation a common optimum temperature for all the flower quality aspects. Hence, to define the most suitable temperature in each cultivation phase it is necessary to decide which quality attribute is to be maximised.

The effects of the assimilate availability on the NoF, individual flower size and plant height is described in Chapter 4.1. Seven greenhouse experiments were conducted in different seasons using the cultivar 'Reagan Improved' (spray type). One extra experiment was carried out to extend this study to two other cultivars ('Goldy' and 'Lupo': 'santini' type), focusing on their response to plant density. Assimilate availability, measured as total plant dry mass (TDM, g plant^{-1}), increased with higher light intensity, higher CO_2 concentration, lower plant density or longer duration of the LD period. In contrast, variation in the growth conditions

produced hardly any effect on flower mass ratio (FMR), and only an increased duration of the LD period had a negative linear effect on the partitioning towards the flowers. The season also had an effect on chrysanthemum FMR: when planted in September (lowest light levels during the SD period), FMR was reduced compared to the other seasons. It is concluded that within a wide range of growth conditions chrysanthemum invests the additional assimilates, diverted to the generative organs, in increasing NoF rather than in increasing flower size. Individual flower size was only affected by assimilate availability when average daily incident photosynthetically active radiation during the SD period was lower than 7.5 mol $m^{-2}d^{-1}$, resulting in lighter and smaller flowers. When incident photosynthetically active radiation (PAR) during the SD period was higher than this threshold value, a constant flower size was observed for the fully open flowers (0.21 ± 0.10 g $plant^{-1}$ and 25 ± 2 cm^2 $plant^{-1}$). Excluding the positive linear effect of the duration of LD period, assimilate availability had no relevant influence on plant height (< 10 % increase). Irrespective of the growth conditions and season, a positive linear relationship between NoF and TDM was observed (NoF = 1.938TDM – 2.34; R^2 = 0.90). The parameters of this relationship are cultivar-specific. The generic nature of these results is discussed is this chapter. The functional relationships developed for predicting NoF and flower size were incorporated as 'modules' in a photosynthesis-driven growth model for cut chrysanthemum (Chapter 5.2).

The influence of assimilate availability on flower size can also be tested by manipulating sink-source ratio. This allows the estimation of the potential flower size, which is defined as the size reached under conditions of non-limiting assimilate availability. In Chapter 4.2 sink-source ratio was manipulated by flower bud removal (leaving one, two or four flowers and a control), by the presence or absence of axillary shoots, and by varying the light intensity. To investigate whether flower size is dependent on flower position within the stem, the apical terminal flower, the apical lateral flowers (from first order axillary shoots) and the first flower locate in a second order axillary shoot were compared. The results indicated that in treatments where a limit on number of flowers was imposed, individual flower dry mass and area increased significantly under conditions of lower competition for assimilates (for example, by decreasing sink-source ratio by either leaving fewer flowers per plant, removing axillary shoots or using supplementary assimilation light). The effect of flower position on flower size, in both the disbudded and control plants, was found to be only important when comparing flowers located on the first order axillary shoots with flowers on the second order axillary shoots, the latter being 40 % smaller than the former. Monoflower plants without side shoots represented the potential flower size, and their flower was up to 2.4 times as heavier and 76 % as larger in area as the control flower in 'Reagan Improved'. The 'santini' cultivars also produced their maximum flower size on the monoflower plants, but the increase in size relative to the control plants was cultivar specific. Higher leaf starch content and lower specific leaf area (thicker leaves) were observed in the monoflower treatments, reflecting an abundance of assimilates. Plant dry mass was only reduced at the lowest sink strength treatment (monoflower plants without axillary side shoots), whereas FMR showed a saturation response to the number of flowers per plant with a maximum value of 0.22.

The data obtained in the previous chapters were further explored to model and validate some external quality attributes. In Chapter 5.1 a process-based model was developed to describe internode elongation in time as a function of temperature. This model was calibrated

with the data from Chapter 3.1, and it was built based on three plausible physiological processes occurring in chrysanthemum elongation: (1) the accumulation of elongation requirements during the day, (2) elongation during the night using the accumulated elongation requirements, and (3) the limitation of the internode length due to low turgor pressure unable to counteract cell wall elasticity. Simulated and measured internode length showed a good agreement (R^2 = 0.91). The presented model may be extended to include variable light conditions and other plant species that show elongation control by DIF.

In Chapter 5.2 a case study is presented on the interactive effects of duration of the LD period (2, 9 and 16 days) and plant density (48, 64 and 80 plants m^{-2}) on several external quality aspects. An existing photosynthesis-driven crop growth model for cut chrysanthemum (Lee *et al.*, 2002) was validated and used to simulate total dry mass for the nine treatments. The possibility of a trade-off between the cultivation measures was analysed, while aiming to maintain a certain quality at harvest. It was concluded that a similar total plant fresh mass could be obtained using several combinations of plant density and number of LDs without affecting either NoF or individual flower size. This trade-off is, however, very dependent on the planting date of the crop, which emphasises the need for a crop simulation model as a decision support tool. Furthermore, special attention should be paid to plant height when choosing a combination of the cultivation measures studied, since this is strongly and positively influenced by the duration of the LD period. The modules developed in Chapter 4.1, for number of flowers and flower size were validated and the measured values were accurately predicted.

The main achievements and limitations of this study are discussed in Chapter 6, and suggestions for future research are presented.

Samenvatting

Gedurende vele jaren is er in het gewaskundig onderzoek naar siergewassen meer gelet op productiehoeveelheden (aantallen stuks) dan op productkwaliteit. Nu de prijzen voor een groot deel bepaald worden door visuele kwaliteitsaspecten, de zogenaamde externe kwaliteit, streven telers van snijchrysanten een hoge en gedurende het jaar constante productkwaliteit na. Bepalend voor de externe kwaliteit van snijchrysanten zijn morfologische aspecten van stengel en bladeren en bloemkenmerken. De prioriteit binnen deze externe kwaliteitsaspecten hangt af van het marktsegment waarvoor geteeld wordt.

De kasteelt van snijchrysanten is één van de meest intensieve en beïnvloedbare gewasproductiesystemen in de tuinbouw. Deze kwantitatieve kortedag plant wordt jaarrond geteeld in kassen door een aantal groeicondities te beïnvloeden waarbij, afhankelijk van de teeltstrategie, vele combinaties mogelijk zijn. Het is voor de telers een uitdaging om het hele jaar door chrysanten van hoge kwaliteit te telen, vanwege het sterke seizoensverloop in dagelijkse hoeveelheid licht die leidt tot sterke seizoensverschillen in opbrengst en kwaliteit. Daarom is het belangrijk om te weten hoe teeltcondities de productkwaliteit beïnvloeden, zodat de optimale strategie bepaald kan worden afhankelijk van de week waarin geplant wordt. Daartoe moeten de factoren die betrokken zijn bij de externe kwaliteit van snijchrysant geanalyseerd en gecombineerd worden om een maximale sierwaarde te bereiken, terwijl een laag energiegebruik en een hoge productiviteit behouden blijven. Juist vanwege de complexiteit van de teelt van snijchrysanten, met zijn vele mogelijkheden voor teeltsturing en de verschillende kwaliteitskenmerken van het eindproduct, kan management van zo'n systeem veel profijt hebben van het gebruik van simulatiemodellen. Een verklarend model is een waardevol instrument om kennis te integreren en het kan een belangrijke rol spelen bij het ondersteunen van beslissingen. De ontwikkeling van verklarende modellen voor productkwaliteit is nog steeds een zwak punt in het onderzoek aan gewasgroei- en productiemodellen, omdat prioriteit gegeven is aan de simulatie van de opbrengst in kwantitatieve zin. Om een verklarend model voor de externe kwaliteit van snijchrysanten te ontwikkelen, is gedetailleerde kennis over groei en ontwikkeling (morfologische processen) nodig.

Het doel van deze studie was het kwantificeren en begrijpen van de effecten van bovengrondse teeltcondities op de externe kwaliteit van chrysanten bij de oogst. Speciale aandacht is besteed aan het integreren van kennis en het inbouwen hiervan in een verklarend model om de belangrijkste externe kwaliteitsaspecten van snijchrysanten te voorspellen. De nadruk werd gelegd op de effecten van kasklimaatsomstandigheden (temperatuur, lichtintensiteit en CO_2-concentratie) en teeltomstandigheden (lengte van de langedag periode en plantdichtheid) op de hoogte van de plant (taklengte), het aantal bloemen en de bloemgrootte.

Hoofdstuk 2 geeft een overzicht van de teeltomstandigheden, die betrokken zijn bij verschillende externe kwaliteitsaspecten, en identificeert hiaten in de literatuur. Ook wordt een overzicht gegeven van de beschikbare modellen, die gemaakt zijn om sommige externe kwaliteitsaspecten van chrysant te voorspellen.

Het DIF concept ('DIFference'; verschil tussen dag (DT) en nacht (NT) temperatuur) stelt dat een gelijke internodiumlengte bereikt wordt bij hetzelfde verschil tussen DT en NT,

onafhankelijk van de gemiddelde etmaaltemperatuur. De controverse over de geldigheid van dit concept is onderzocht in Hoofdstuk 3.1. *Chrysanthemum* 'Reagan Improved' werd geteeld in klimaatkamers bij alle 16 combinaties van vier DT en vier NT (16, 20, 24 en 28 °C) en bij een daglengte van 12 uur. De lengte van het tiende internodium, het aantal internodia en de lengte van de stengel werden periodiek gemeten. Het experiment eindigde na 27 dagen, zodra het tiende internodium zijn uiteindelijke lengte had bereikt bij alle temperatuurcombinaties. Binnen het gehanteerde temperatuurbereik van 16 tot 28 °C werd een significante positieve lineaire relatie tussen DIF en internodiumlengte gevonden als de internodia hun uiteindelijke lengte hadden bereikt. Bovendien werd gevonden dat de internodiumlengte in de eerste stadia van uitgroei geen duidelijk verband liet zien met de uiteindelijke internodiumlengte, wat contradicties in de literatuur kan verklaren. Behalve het uitgroeistadium van de internodia speelt ook het gebruikte temperatuurbereik een belangrijke rol in het al of niet geldig zijn van het DIF concept. Er is aangetoond dat het DIF concept alleen geldig is binnen een temperatuursbereik waar het effect van DT en NT even groot is, maar tegengesteld van aard (18-24 °C).Daarom wordt geconcludeerd dat de reactie van de uiteindelijke internodiumlengte van chrysant op temperatuur sterk gerelateerd is aan DIF, maar dat dit simpelweg het resultaat is van onafhankelijke en tegenovergestelde effecten van DT en NT. Internodiumverschijningssnelheid, en ook de lengte van de stengel gevormd tijdens het experiment, vertoonden een optimum reactie op DT.

In Hoofdstuk 3.2 werd een diepgaande studie uitgevoerd naar de temperatuurgevoeligheid van verschillende bloemkenmerken om een beter inzicht te krijgen in de onderliggende fysiologische processen. Er is een poging ondernomen om de effecten van temperatuur tijdens verschillende fases van de teelt te scheiden voor elk van de onderzochte bloemkenmerken. De planten werden geteeld in zowel kascompartimenten, bij twee constante temperaturen (17 en 21 °C), als in klimaatkamers, bij 32 temperatuurscombinaties (van 15 tot 24 °C). In het laatstgenoemde experiment werden temperatuursbehandelingen toegepast die de teeltperiode in drie opvolgende fasen verdeelden: langedag (LD) periode (fase I; 18 en 24 °C), begin van de kortedag (KD) periode tot het zichtbaar worden van de terminale bloemknop (fase II; 15, 18, 21 en 24 °C) en het einde van fase II tot de oogst (fase III; 15, 18, 21 en 24 °C). Het aantal bloemen per plant (inclusief bloemknoppen; NoF), individuele bloemgrootte en -kleur werden sterk beïnvloed door temperatuur, alleen de positie van bloemen binnen de plant werd niet beïnvloed. Er werd aangetoond dat de temperatuursinvloed veel verschilde tussen de fasen van de teelt en voor de diverse bloemkenmerken. Over het algemeen kan gezegd worden dat de bloemkenmerken minder beïnvloed werden door de temperatuur tijdens de LD periode dan tijdens de KD periode. Een hogere temperatuur verhoogde het NoF, grotendeels veroorzaakt door een hoger aantal bloemknoppen. NoF werd vooral positief beïnvloed door temperatuur tijdens fase III, terwijl de individuele bloemgrootte toenam met temperatuur gedurende fase II maar afnam met temperatuur tijdens fase III. De kleurintensiteit (roze) van de bloem werd significant verhoogd door lage temperaturen tijdens fase III. Er wordt geconcludeerd dat het niet mogelijk is om één optimale temperatuur te bepalen voor alle kwaliteitsaspecten tezamen omdat geen consistent betere temperatuur gevonden werd binnen de onderzochte fases van de teelt. Om de optimale temperatuur binnen ieder fase te definiëren kan dus het kwaliteitsdoel niet uit het oog verloren worden en moeten prioriteiten vastgesteld worden.

De effecten van de beschikbaarheid van assimilaten op NoF, de grootte van individuele bloemen en de plantlengte wordt beschreven in Hoofdstuk 4.1. In verschillende seizoenen zijn er zeven kasexperimenten met het ras 'Reagan Improved' (troschrysant) uitgevoerd. Er is één extra experiment gedaan om deze studie uit te breiden naar twee andere rassen ('Goldy' en 'Lupo'; Santini-rassen), toegespitst op de effecten van plantdichtheid. De beschikbaarheid van assimilaten, gemeten als totaal drooggewicht van de plant (TDM, g plant^{-1}), nam toe met een hogere lichtintensiteit, een hogere CO_2-concentratie, een lagere plantdichtheid en een langere duur van de LD periode. De gewichtsfractie bloemen (FMR) werd daarentegen nauwelijks beïnvloed, behalve door de duur van de LD periode, die een negatief lineair effect had op de verdeling naar de bloemen. Ook het seizoen had een effect op de FMR van chrysant: bij een plantdatum in september (laagste lichtniveaus gedurende de KD periode), was de FMR lager dan in de andere seizoenen. Er wordt geconcludeerd, dat over een breed bereik van groei-omstandigheden, chrysant de extra assimilaten, die naar de generatieve organen gaan, gebruikt voor het maken van meer bloemen in plaats van grotere bloemen. De grootte van de individuele bloemen werd alleen beïnvloed door de beschikbaarheid van assimilaten, wanneer de gemiddelde dagelijkse opvallende fotosynthetisch actieve straling (PAR) gedurende de KD periode lager was dan 7,5 mol $m^{-2}d^{-1}$, met als resultaat lichtere en kleinere bloemen. Wanneer de opvallende PAR gedurende de KD periode hoger was dan deze drempelwaarde, werd er een constante bloemgrootte waargenomen voor volledig open bloemen (0,21 ± 0,10 g plant^{-1} en 25 ± 2 cm^2 plant^{-1}). Uitgezonderd het positieve effect van de lengte van de LD periode, had de beschikbaarheid van assimilaten geen significante invloed op de plantlengte (minder dan 10 % toename). Onafhankelijk van de groeiomstandigheden en het seizoen is er een positieve lineaire relatie gevonden tussen NoF en TDM (NoF = 1,938TDM-2,34; R^2 = 0,90). De parameters in deze vergelijking zijn cultivar afhankelijk. De generieke aard van deze resultaten wordt bediscussieerd in dit hoofdstuk. De functionele relaties voor NoF en bloemgrootte werden als 'modules' ingebouwd in een door fotosynthese gestuurd groeimodel voor snijchrysant (Hoofdstuk 5.2).

De invloed van de beschikbaarheid van assimilaten op de bloemgrootte kan getest worden door manipulatie van de sink-source verhouding. Op deze wijze kan ook de potentiële bloemgrootte bepaald worden, dat is de grootte die bereikt wordt onder omstandigheden waarin de beschikbaarheid van assimilaten niet limiterend is. In Hoofdstuk 4.2 worden proeven weergegeven, waarin de sink-source verhouding beïnvloed is door het verwijderen van bloemknoppen (één, twee of vier bloemen laten zitten en een controlebehandeling zonder bloemknopverwijdering), door wel of niet laten zitten van de axillaire scheuten, en door de lichtintensiteit te variëren. Om te onderzoeken of de bloemgrootte afhankelijk is van de positie van de bloem aan de stengel, werden de terminale bloem, laterale bloemen van de eerste orde axillaire scheuten en tweede orde bloemen vergeleken. De resultaten laten zien dat wanneer het aantal bloemen is opgelegd, de drooggewichten en het oppervlak van individuele bloemen significant toenemen onder omstandigheden waarbij de concurrentie om assimilaten laag is (bijvoorbeeld onder afnemende sink-source verhouding door minder bloemen per plant, door het verwijderen van axillaire scheuten of door het gebruik van assimilatiebelichting). Een effect van de positie van de bloem op de bloemgrootte, in zowel geplozen planten als controle planten, werd alleen waargenomen wanneer de bloemen aan de eerste orde axillaire scheuten werden vergeleken met die aan de tweede orde scheuten; de laatste zijn 40 % kleiner dan de

eersten. Planten met één bloem en geen zijscheuten vertoonden de potentiële bloemgrootte en hun bloemen waren 2,4 maal zwaarder en hadden een 76 % groter oppervlak dan in de controle voor 'Reagan Improved'. Deze maximale bloemgrootte is echter cultivar afhankelijk. In de behandelingen met één bloem werd een hogere zetmeelconcentratie in het blad en een lager specifiek bladoppervlak (dikker blad) gevonden, hetgeen op een overvloed aan assimilaten duidt. Het drooggewicht van de plant werd alleen verlaagd bij de behandeling met de laagste sinksterkte, terwijl FMR een verzadigingsrespons vertoonde met het aantal bloemen per plant.

De gegevens die zijn verkregen in de voorafgaande hoofdstukken, zijn verder gebruikt om enkele uitwendige kwaliteitseigenschappen te modeleren en om dit model te valideren. In Hoofdstuk 5.1 is een op processen gebaseerd model ontwikkeld, om de lengtegroei van internodiën te beschrijven in de tijd, als functie van de temperatuur. Dit model is gecalibreerd met data uit Hoofdstuk 3.1 en is gebouwd op basis van drie aannemelijke fysiologische processen die een rol spelen bij de lengtegroei van chrysant: (1) de ophoping, gedurende de dag, van stoffen die nodig zijn voor lengtegroei, (2) lengtegroei gedurende de nacht, gebruikmakend van deze opgehoopte stoffen, en (3) de beperking van de internodiumlengte als gevolg van lage turgor druk, die de elastische krachten van de uitrekkende celwand niet kan overwinnen. Gesimuleerde en gemeten internodiumlengte kwamen goed overeen (R^2 = 0.91). Het gebruikte model kan worden uitgebreid naar variabele lichtomstandigheden en naar andere plantensoorten, waarvan de strekking wordt bepaald door DIF.

In Hoofdstuk 5.2 wordt onderzoek gepresenteerd naar de interactie van het effect van de LD periode (2, 9 en 16 dagen) en de plantdichtheid (48, 64 en 80 planten m^{-2}) op een aantal uitwendige kwaliteitsaspecten. Een bestaand door fotosynthese gestuurd gewasgroeimodel voor snijchrysant (Lee *et al.*, 2002) is gevalideerd en gebruikt om het totale drogestofgewicht voor de negen behandelingen te simuleren. De mogelijkheid van een uitruileffect tussen de twee factoren is geanalyseerd, met als doel behoud van een bepaalde kwaliteit. Geconcludeerd kan worden dat een gelijk totaal plant gewicht bereikt kan worden gebruikmakend van verscheidene combinaties van plantdichtheid en aantal dagen LD zonder NoF of de individuele bloemgrootte te beïnvloeden. Deze uitruil is echter sterk afhankelijk van de plantdatum, wat de noodzaak benadrukt van een gewassimulatiemodel als een beslissingsondersteunend hulpmiddel. Verder moet er speciale aandacht worden besteed aan de planthoogte bij het kiezen van een combinatie van de bestudeerde factoren, daar deze sterk en positief beïnvloed wordt door de duur van de LD periode. De modules die in Hoofdstuk 4.1 zijn ontwikkeld, betreffende bloemaantal en grootte, zijn gevalideerd en de gemeten waarden werden nauwkeurig voorspeld.

De belangrijkste resultaten en beperkingen van de studie worden besproken in Hoofdstuk 6. Tevens worden hier aanbevelingen gedaan voor toekomstig onderzoek.

Resumo

Durante muitos anos a investigação conduzida no sector da floricultura privilegiou a quantidade em detrimento da qualidade. Actualmente, uma vez que os preços são frequentemente determinados com base em aspectos de qualidade visual, também designados por qualidade externa, os produtores de crisântemo de corte desejam obter uma produção elevada e uma qualidade constante ao longo de todo o ano. A qualidade externa do crisântemo de corte é geralmente avaliada em função da morfologia do caule e das folhas, bem como em função de várias características das flores. O estabelecimento de prioridades de entre os atributos visuais depende do mercado a que o produto se destina.

No domínio da horticultura o cultivo do crisântemo em estufas é um dos sistemas de produção mais controlado e intensivo. Esta planta de resposta quantitativa aos dias curtos só pode ser cultivada ao longo de todo o ano em estufas e através do controlo de várias condições de crescimento. Existe contudo a possibilidade de efectuar várias combinações das mesmas, de acordo com a estratégia de cultivo pretendida. Produzir crisântemo de elevada qualidade durante todo o ano é um desafio constante para os produtores uma vez que a variação sazonal do integral de luz diário conduz a uma forte variação sazonal da quantidade e qualidade do produto final. Deste modo, para escolher a estratégia de crescimento mais adequada, ajustada à semana de plantação, é necessário saber de que forma as condições de crescimento afectam a qualidade das plantas. Como tal, as condições de crescimento envolvidas na qualidade externa do crisântemo deverão ser cuidadosamente analisadas e eficazmente combinadas de modo a obter uma produção com o máximo valor ornamental durante todo o ano, mantendo uma produtividade elevada e um 'input' energético aceitável. Tendo em consideração a complexidade da produção de crisântemo de corte, com as múltiplas opções de controlo e a vasta gama de atributos de qualidade visual, a gestão de um sistema desta natureza irá largamente beneficiar da aplicação de modelos de simulação. Por exemplo, os modelos explanatórios são ferramentas valiosas na integração de conhecimento e no apoio à tomada de decisão. O desenvolvimento deste tipo de modelos, vocacionados para a qualidade do produto, tem recebido pouca atenção na área da modelação das culturas, uma vez que tem sido atribuída prioridade à simulação da produtividade. Para desenvolver um modelo explanatório de previsão da qualidade externa do crisântemo, é necessário um conhecimento detalhado acerca do seu crescimento e desenvolvimento morfológico.

Esta tese teve como objectivo principal quantificar e compreender a influência dos factores climáticos e das práticas de cultivo na qualidade externa final do crisântemo de corte. Neste estudo prestou-se uma atenção especial à integração da informação obtida, para posterior incorporação num modelo explanatório de previsão dos principais aspectos da qualidade externa do crisântemo. Este trabalho centrou-se na análise dos efeitos dos factores climáticos (temperatura, intensidade luminosa e concentração de CO_2) e das práticas de cultivo (duração dos dias longos e densidade de plantação) no comprimento do caule, número de flores e tamanho das mesmas à altura da colheita.

O capítulo 2 apresenta uma perspectiva geral das condições de crescimento, envolvidas nos diferentes aspectos de qualidade externa do crisântemo, e identifica as falhas encontradas

na literatura existente. É também apresentada uma síntese dos modelos disponíveis, que foram desenvolvidos para prever alguns dos aspectos de qualidade do crisântemo.

O conceito de DIF (amplitude térmica diária) define que o comprimento dos entrenós depende da DIFerença entre a temperatura diurna (TD) e a temperatura nocturna (TN), sendo por sua vez independente da temperatura média diária. O carácter controverso deste conceito foi investigado no Capítulo 3.1. Plantas da cultivar *Chrysanthemum* 'Reagan Improved' foram colocadas em câmaras de crescimento e submetidas a dezasseis combinações de quatro TD e quatro TN (16, 20, 24 e 28 °C), com um fotoperíodo de 12 h. O comprimento do entrenó 10, o número de entrenós e o comprimento do caule foram medidos periodicamente. A experiência terminou quando o entrenó 10 atingiu o seu comprimento final em todas as combinações de temperatura utilizadas (27 dias). Para o intervalo de temperaturas estudado (16-28 °C), o comprimento final dos entrenós e o DIF e apresentaram uma relação significativa do tipo linear e positiva. Foi igualmente observado que o comprimento dos entrenós em estádios iniciais de desenvolvimento não se encontrava fortemente relacionado com o seu comprimento final, o que explica as contradições encontradas na literatura. Paralelamente, constatou-se que a eficácia do conceito do DIF, para além de depender do estádio de desenvolvimento dos entrenós, está também relacionada com o intervalo de temperaturas aplicadas. Como tal, foi demonstrado que este conceito só é válido para um intervalo de temperaturas no qual a TD tem um efeito oposto à TN, mas de igual magnitude (18-24 °C). Finalmente, concluiu-se que a resposta do comprimento dos entrenós à temperatura está fortemente relacionada com o DIF, mas que esta resposta é simplesmente o resultado dos efeitos independentes e opostos da TD e TN. A taxa de aparecimento dos entrenós, bem como o comprimento do caule (obtido durante a experiência), apresentaram um óptimo térmico em resposta à TD.

Para melhor entendimento dos processos fisiológicos inerentes à iniciação e desenvolvimento floral, no Capítulo 3.2 foi conduzido um estudo exaustivo acerca da sensibilidade térmica de várias características das flores. Neste estudo procurou-se analisar o efeito da temperatura em diferentes fases do período de cultivo nas características das flores. As plantas foram colocadas em diferentes compartimentos de uma estufa e submetidas a duas temperaturas constantes (17 e 21 °C), ou colocadas em câmaras de crescimento recebendo 32 combinações de temperaturas (no intervalo de 15 a 24 °C). Na experiência conduzida em câmaras de crescimento, as temperaturas foram aplicadas com base na divisão do período de cultivo em três fases consecutivas: da plantação ao fim do período de dias longos (DL) (fase I; 18 e 24 °C), do início dos dias curtos (DC) até ao aparecimento do botão floral terminal (fase II; 15, 18, 21 e 24 °C) e finalmente desde o aparecimento do botão floral terminal até à colheita das plantas (fase III; 15, 18, 21 e 24 °C). Entre as características investigadas apenas a posição relativa das flores no caule se revelou independente da temperatura. Em contrapartida, o número total de flores e botões florais por planta (NF), o tamanho individual das flores e a sua cor foram significativamente influenciados pela temperatura. Foi observado que o efeito da temperatura depende fortemente da fase de cultivo e da característica investigada. Em regra, as características das flores são menos influenciadas pela temperatura aplicada durante os DL, do que pela temperatura durante o período de DC. As temperaturas mais elevadas aumentaram o NF, sobretudo devido ao aumento do número de botões florais. A temperatura apresentou um efeito positivo no NF especialmente durante a fase III, enquanto

que o tamanho individual das flores aumentou com a temperatura durante a fase II, diminuindo em seguida com a temperatura durante a fase III. Por sua vez a intensidade da cor das pétalas (cor-de-rosa) aumentou significativamente em resposta às baixas temperaturas durante a fase III. Concluiu-se que não é possível atribuir a cada uma das fases de cultivo uma temperatura óptima comum que se ajuste a todos os aspectos de qualidade das flores. Consequentemente, para escolher a temperatura mais adequada a cada fase de cultivo, é necessário pré-definir qual o atributo de qualidade que se pretende maximizar.

Os efeitos da disponibilidade de fotoassimilados no NF, no tamanho individual das flores e na altura das plantas estão descritos no Capítulo 4.1. Um total de sete experiências em estufas foram levadas a cabo em diferentes estações do ano, usando a cultivar 'Reagan Improved' (tipo spray). Paralelamente, de forma a estender esta análise a outro tipo de cultivares ('Goldy' e 'Lupo': tipo 'santini'), uma experiência adicional foi conduzida com vista a estudar a resposta das mesmas à densidade de plantação. A disponibilidade de fotoassimilados, medida através do peso seco total da planta (PST, g planta^{-1}), respondeu positivamente ao aumento da intensidade luminosa, ao aumento da concentração de CO_2, à diminuição da densidade de plantação, ou à maior duração do período de DL. No entanto, a variação das condições de crescimento quase não afectou a proporção de fotoassimilados destinada às flores. Registou-se apenas um efeito linear negativo na distribuição de fotoassimilados para as flores aquando do aumento do período de DL. A estação do ano teve igualmente um efeito relevante na distribuição de assimilados. Para crisântemos plantados em Setembro (níveis de baixa radiação durante o período de DC), a distribuição de fotoassimilados para as flores diminuiu comparativamente às outras estações do ano. Concluiu-se que num largo intervalo de condições de crescimento, o crisântemo investe os fotoassimilados adicionais, destinados aos órgãos generativos, no incremento do NF e não no aumento do tamanho das mesmas. Curiosamente, o tamanho individual das flores só foi afectado pela disponibilidade de fotoassimilados quando a média diária da radiação incidente fotossinteticamente activa durante o período DC foi inferior a 7.5 mol $m^{-2}d^{-1}$, resultando em flores mais leves e mais pequenas. Para valores inferiores a este ponto de referência verificou-se que as flores completamente abertas no momento da colheita apresentavam um tamanho constante (0.21 ± 0.10 g planta^{-1} e 25 ± 2 cm^2 planta^{-1}). Excluindo o efeito linear e positivo da duração do período de DL, a disponibilidade de fotoassimilados teve uma influência reduzida na altura da planta (< 10 % de aumento). Independentemente das condições de crescimento e da estação do ano, observou-se uma relação linear positiva significativa entre o número total de flores e botões florais, e o peso seco total da planta (NF = 1.938PST – 2.34; R^2 = 0.90). Contudo, os parâmetros desta relação são específicos de cada cultivar. O carácter geral destes resultados é discutido neste capítulo. As relações lineares obtidas para prever o NF e o tamanho das flores foram integradas como 'módulos' de um modelo de crescimento explanatório (fotossinteticamente orientado) para o crisântemo de corte (Capítulo 5.2).

O efeito da disponibilidade de fotoassimilados no tamanho das flores pode ser igualmente testada através da manipulação do rácio 'sink-source'. Esta manipulação permite estimar o tamanho potencial das flores, definido como o tamanho atingido em condições não limitantes de disponibilidade de fotoassimilados. No Capítulo 4.1 o rácio 'sink-source' foi manipulado através da remoção de botões florais (deixando um, dois ou quatro flores e uma testemunha), através da presença ou ausência de rebentos axilares e através da variação da intensidade

luminosa. De forma a investigar se o tamanho das flores está relacionado com a sua posição no caule, comparou-se a flor apical terminal, as flores apicais laterais (localizadas nos rebentos principais) e a flor localizada no primeiro rebento secundário. Os tratamentos em que o número de flores havia sido imposto, o peso seco e a área de cada flor aumentaram significativamente em condições de baixa competição por fotoassimilados (por exemplo, através de um elevado rácio 'sink-source' resultante da remoção de um grande número de flores, da remoção dos rebentos axilares e do uso suplementar de radiação fotossinteticamente activa). O tamanho das flores em plantas manipuladas e em plantas testemunha, foi apenas influenciado pela sua posição no caule quando se compararam diferentes flores localizadas em rebentos principais com a flor localizada no primeiro rebento secundário, em que a última era 40 % mais pequena que as primeiras. Plantas com uma só flor (monoflor) e sem rebentos laterais evidenciaram o tamanho potencial da flor, que atingiu um peso seco e uma área, respectivamente, 240 % e 76 % superior aos valores observados em flores das plantas testemunha da cultivar 'Reagan Improved'. As flores das cultivares 'santini' também obtiveram um tamanho máximo em plantas monoflor, embora o aumento relativo comparativamente às plantas testemunho fosse dependente da cultivar. Um aumento no teor de amido nas folhas e uma menor área foliar específica (SLA) (folhas mais grossas) foram observados em plantas do tipo monoflor, o que revela que estas plantas estiveram perante uma situação de abundância de fotoassimilados. O peso seco total das plantas foi apenas reduzido no tratamento em que o rácio 'sink-source' era mais elevado, isto é em plantas do tipo monoflor e sem rebentos axilares, enquanto que a distribuição de fotoassimilados destinada aos órgãos generativos aumentou com o número de flores por planta até ao valor máximo de 22 %.

Os dados obtidos nos capítulos anteriores foram posteriormente utilizados para modelar e validar alguns atributos de qualidade visual. No Capítulo 5.1 desenvolveu-se um modelo para descrever o processo dinâmico de alongamento dos entrenós em função da temperatura. O modelo foi calibrado com os dados do Capítulo 3.1 e foi construído com base em três processos fisiológicos fundamentais envolvidos no crescimento em altura do crisântemo: (1) a acumulação de 'requisitos de alongamento' durante o dia, (2) o alongamento nocturno utilizando os requisitos acumulados e (3) a limitação física da expansão dos entrenós devido à baixa pressão osmótica incapaz de contrapor a elasticidade das paredes celulares. O comprimento simulado e observado dos entrenós estavam fortemente correlacionados (R^2 = 0.91). Este modelo poderá ser alargado no futuro, de forma a incluir condições de radiação variáveis, assim como incluir outras espécies de plantas em que o crescimento em altura esteja relacionado com a amplitude térmica diária (DIF).

O Capítulo 5.2 apresenta um caso prático acerca do efeito interactivo da duração do período de DL (2, 9 e 16 dias) e da densidade de plantação (48, 64 e 80 plantas m^{-2}), em diversos atributos de qualidade externa. Um modelo de crescimento, previamente desenvolvido para crisântemo de corte com base no processo fotossintético (Lee *et al.*, 2002), foi validado e usado para simular o peso seco total das plantas dos nove tratamentos estudados. Analisou-se a possibilidade de combinar diferentes práticas de cultivo visando manter uma determinada qualidade à colheita. Concluiu-se que as plantas poderiam apresentar um peso fresco idêntico, recorrendo à combinação de diferentes densidades de plantação com diferentes períodos de DL, sem afectar nem o NF nem o tamanho individual das flores.

Contudo, este 'trade-off' depende da data de plantação do crisântemo, o que reforça a necessidade de desenvolver um modelo de simulação de apoio à tomada de decisão. Além disso, aquando da escolha da combinação das práticas de cultivo mais adequada deverá ser dada uma atenção especial ao comprimento do caule, uma vez que esta apresenta uma relação linear positiva significativa com a duração do período de DL. Os módulos desenvolvidos no Capítulo 4.1, para o número e tamanho das flores foram validados e os valores observados foram correctamente estimados.

Os principais resultados e limitações deste estudo são discutidos no Capítulo 6, onde são igualmente sugeridos temas para futuras investigações.

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Susana.

Curriculum Vitae

Susana Maria Pinto de Carvalho was born on January 1st 1973 in Braga, Portugal. In 1991 she started studying Agricultural Engineering (five years degree program) at the University of Trás-os-Montes e Alto Douro, Vila Real, Portugal. During the last three years of the high school, and subsequently till her graduation, she received a merit-scholarship from Calouste Gulbenkian Foundation, Lisbon. In 1997 she obtained her Diploma de Licenciatura in Agricultural Engineering, which received the prize of the Engineer António de Almeida Foundation for the highest final classification in that year in Agricultural Engineering. Between March 1997 and September 1998 she was a teacher assistant of the Ecology and Cell Sciences courses at the Biological and Environmental Engineering Department, University of Trás-os-Montes e Alto Douro. From November 1997 to September 1998 she followed a Postgraduate Program on Sustainable Agriculture and Horticulture at Instituto Superior de Agronomia, Technical University of Lisbon. In October 1998 she moved to The Netherlands to begin a four years PhD research project at Horticultural Production Chains Group, Department of Plant Sciences, Wageningen University. This project was financed by Fundação para a Ciência e a Tecnologia (Portugal) and this thesis is the outcome of the project.

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