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Genotypic Variation in the Response to Suboptimal Temperature at Different Plant Densities in Cut Chrysanthemum

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Abstract. **Energy efficiency of greenhouse cut chrysanthemum (*Chrysanthemum morifolium* Ramat.) may be increased by breeding. In addition to breeding for cultivars with a shorter reaction time at suboptimal temperatures, an alternative approach would be to develop cultivars that are heavier at suboptimal temperatures so that they could be grown at a higher plant density, enhancing the production per unit area. Therefore, the combined effect of temperature and plant density on growth and development of four cut chrysanthemum cultivars was investigated in three greenhouse experiments, carried out in different seasons. For growth-related traits, no interactions between temperature and cultivar were found, limiting the possibilities for breeding. At suboptimal temperatures, growth rate early in the cultivation period decreased as a consequence of a lower light interception resulting from a lower specific leaf area. Thus a higher dry mass production at lower temperature could only be explained by a longer cultivation time. Temperature also influenced external quality, but these effects were cultivar dependent. For instance, temperature affected the slope of the positive linear relationship between total dry mass and number of flowers, reducing number of flowers at low temperature for the same plant dry mass. It is concluded that there are possibilities for breeding for suboptimal temperature-tolerant cultivars.**

In The Netherlands, the greenhouse industry is an important user of energy, as it accounts for 7% of the national energy use (Oude Lansink and Bezlepkin, 2003). Most of this energy is used during the winter months for heating greenhouses to maintain high production levels year round. Cultivars that are better adapted to lower temperatures could contribute significantly to a reduction in energy use and consequently in CO_2 emission. For breeding of more energy-efficient cultivars, genotypic variation in temperature response is necessary.

Cut chrysanthemum is an important ornamental crop grown year round in heated Dutch greenhouses. Lowering the cultivation temperature results in a longer cultivation period due to a delay in flowering time (Lepage et al., 1984). Therefore, on an annual basis, a reduced number of stems per unit area can be harvested. One focus for the breeding of more energy-efficient chrysanthemum cultivars could be to create cultivars with less or no delay in flowering time at suboptimal temperatures. Within the chrysanthemum assortment, a large variation in temperature response for flowering time has been observed (De Jong, 1978; Van der Ploeg et al., 2007a). Additionally, as some cultivars produce heavier plants at suboptimal temperatures, another strategy is to breed for heavier plants at a suboptimal temperature regime (Van der Ploeg et al., 2007a). This would allow a higher planting density at lower temperatures, thus making up for the increase in cultivation time by producing more stems per unit area. However, this strategy would only be feasible if the growth rate of the crop is not decreased at suboptimal temperatures. Plant growth can be enhanced by increasing the efficiency in which light is converted into dry mass [i.e., light use efficiency (LUE)] and/or by increasing light interception due to a higher leaf area index (LAI). Thus far, little is known about the mechanisms responsible for increasing plant biomass at suboptimal temperatures, which has been observed in some cultivars.

Besides the effect of temperature on growth, temperature and plant density affect several external quality aspects. For example, increasing plant density strongly reduced the number of flowers per plant (Carvalho and Heuvelink, 2003; Lee et al., 2002b), while the effect of temperature on number of flowers and flower size is highly cultivar dependent (Lepage et al., 1984; Van der Ploeg et al., 2007a). Furthermore, a positive

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relationship between the number of flowers and the total biomass per plant was shown in cut chrysanthemum over a wide range of growing conditions (Carvalho and Heuvelink, 2003), but it is still unclear whether and how temperature affects this relationship.

The scope of this article is to study the combined effects of temperature and plant density on the growth and external quality aspects of cut chrysanthemum cultivars. To obtain a better insight into the variation in temperature response between cultivars, a detailed analysis is performed, focusing on the underlying morphological and physiological aspects.

Material and Methods

Experimental set up. Three experiments were conducted between Sept. 2003 and Feb. 2005 (Table 1). Each experiment was carried out in four greenhouse compartments (12.8 × 12.0 m) that were part of a multispan Venlo-type greenhouse (Wageningen University, The Netherlands, lat 52°N). Each compartment contained eight parallel soil beds (1.125 × 10.25 m) of which the outer two beds acted as borders. Block rooted cuttings of three or four cut chrysanthemum cultivars (Table 1) were obtained from two breeding companies (Fides Goldstock Breeding, Maasland, The Netherlands and Deliflor, Maasdijk, The Netherlands) and were planted at two or three plant densities on the dates indicated in Table 1. The selection of the four cultivars was based on their contrasting response to temperature, which was observed in a previous study for reaction time and biomass production (Van der Ploeg et al., 2007a).

Table 1. General information on the growth conditions of three greenhouse experiments conducted in different seasons to investigate the effect of temperature and plant density in a total of four cut chrysanthemum cultivars.

	Expt. 1	Expt. 2	Expt. 3
Year	2003	2004	2004–05
Planting date	10 Sept.	16 Jan.	3 Nov.
Duration of long days (d)	14	14	14
Duration of short days (d)	54–63	54–67	61–80
Outside global radiation ($mol \cdot m^{-2} \cdot d^{-1}$)[z]	32.2–35.0	22.5–28.1	9.7–11.5
Incident *PAR* LD ($mol \cdot m^{-2} \cdot d^{-1}$)[y]	16.1	5.1	6.0
Incident *PAR* SD ($mol \cdot m^{-2} \cdot d^{-1}$)[y]	7.4–8.3	7.4–8.3	3.7–4.1
Temperature LT/HT (°C)[x]	18.0/21.1	16.9/20.5	16.2/20.2
Plant density ($plants/m^2$)[w]	32, 48, 64	32, 48, 64	32, 64
Cultivar	Annecy Delianne Reagan Supernova	Annecy Delianne Reagan Supernova	Delianne Reagan Supernova

[z]Averaged over the complete cultivation period. Range of variation among cultivars.
[y]*PAR* = photosynthetically active radiation averaged over the complete long day (LD) or short day (SD) period. For the SD period, the range indicates variation among cultivars.
[x]Realized 24-h temperatures averaged over the complete cultivation period for low-temperature (LT) and high-temperature (HT) treatment.
[w]Per treatment.

Heating set point was 16 °C [low temperature (LT)] in two compartments and 20 °C [high temperature (HT)] in the other two compartments. Ventilation temperature set points were one degree above the heating set point. Plants were initially grown under long day (LD) conditions followed by short days (SD) until final harvest (Table 1). High-pressure sodium lamps providing 44 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (*PAR*) (Philips SON-T Agro; Philips, Eindhoven, The Netherlands) were kept on continuously during the day hours of the LD (0500–2400 HR) and SD period (0730–1700 HR). Plants were grown under ambient CO_2. Greenhouse climate was automatically recorded every 5 min using a commercial computer system. Irrigation was provided when needed and plant protection was applied according to an integrated pest management scheme using biological and chemical agents. No growth regulators were applied to the crop. The terminal flower bud was pinched as soon as it separated from the other crown buds (<5 mm).

Measurements. In all experiments, destructive measurements were carried out at planting, the start of SD, and at final harvest, using five plants per experimental unit (plot). Besides these three destructive measurements, some additional measurements were performed in different experiments. In Expts. 1 and 2, two extra periodic destructive measurements were conducted at about equal intervals during the SD. In Expt. 3, periodic destructive measurements were carried out every 4 to 6 d during the LD and every 10 to 14 d during the SD, resulting in three extra measurements during the LD and five during the SD. Between plots used for destructive measurements, always at least two rows of border plants were left to avoid disturbance of the light distribution. Final harvest of all five plants from the same plot occurred when at least three plants had at least three flowers fully open. This stage was reached at different times depending on the cultivar and temperature. Within a given treatment, data were collected on all plants at the same time. Stem, leaf, and flower fresh and dry mass and number of flowers (including flower buds >5 mm) were determined. To measure dry mass, the different plant organs were dried in a ventilated oven (105 °C) for at least 15 h. Total plant leaf area and individual flower area of the first lateral flower were measured with a leaf area meter (model 3100; LI-COR, Lincoln, NE).

Growth analysis and light use efficiency. In the early stage of the crop (i.e., at low LAI), absolute crop growth rate is proportional to LAI, and a common approach to account for the effects of plant size on growth rate during that stage is to evaluate weight increase on a logarithmic basis, calculating a relative growth rate (RGR). In closed canopies, most light is intercepted, hence it is more meaningful to consider absolute crop growth rate [AGR (Challa et al., 1995)]. Therefore, RGR was calculated for the LD period, whereas AGR was calculated over the SD period. The dry mass and leaf area observations collected during the LD period in Expts. 1 and 2 were used to calculate RGR, leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) according to the "classical approach" described by Hunt (1990). In Expt. 3, the growth analysis was conducted according to the "functional approach," as more measurements were available. The best fitting polynome

for the relation between the natural logarithms of total dry mass, leaf dry mass, and leaf area with time was calculated using the ordinary "least squares estimate." In all cases, polynomials of degree 2 were found to be necessary and sufficient. To exclude ontogenetic effects, all growth parameters in Expt. 3 were compared on the basis of a total dry mass (TDM) interval instead of a time interval. The TDM interval is 0.23 to 0.87 g because this was the largest possible interval in this experiment not requiring extrapolation of data.

Furthermore, data collected during the SD were used to calculate the absolute growth rate (AGR). In Expts. 1 and 2, AGR was calculated over the intervals between each of the four destructive harvests. The last interval up to the final harvest was omitted, as the final harvest date differed between cultivars and temperature treatments, resulting in different light sums. In Expt. 3, AGR was calculated over the first 20 d of SD and subsequent 23 d of SD as the ratio between total dry mass per square meter (TDM_a) and time.

A time course of LAI, based on linear interpolations between destructive leaf area measurements, was calculated for each treatment. Daily intercepted *PAR* was based on measured daily global radiation, assuming 47% *PAR* in global radiation, a greenhouse transmissivity of 49%, and a light extinction coefficient for a chrysanthemum crop of 0.72 (Lee et al., 2002a). LUE was defined as the slope of accumulated dry mass production during the SD (i.e., dry mass at the destructive measurements minus dry mass at the start of the SD period) and accumulated intercepted *PAR* over the SD period.

Statistical design and analysis. All experiments had a split-plot design with temperature as the main factor (allocated to the compartments) and cultivar × density as the split factor (i.e., 6 to 12 cultivar × density combinations, depending on the experiment, randomly distributed over the six beds within each compartment). Normality of data was checked, using the Kolmogorov-Smirnov test from SPSS (version 12.0; SPSS Inc., Chicago). Transformation of data was not necessary as all data were normally distributed. An analysis of variance was conducted and treatment effects were tested at 5% probability level. Mean separation was done by Student's *t*-test. The statistical software package Genstat 8 (VSN International, Hertfordshire, UK) was used.

To establish the effect of temperature and cultivar on the relationship between total biomass per plant and number of flowers a linear regression model was built using Genstat 8's General Linear Regression menu with cultivar, total dry mass per plant (TDM_p), and actual temperature as input. Due to the limited temperature range in these experiments, we chose not to use a quadratic temperature term. The terms of the model were chosen via stepwise regression.

Results

Climate data

The difference between realized temperature and set point temperature was rather large in Expt. 1 (on average 2 °C higher throughout the cultivation period; Table 1). Especially during the first weeks, it was difficult to maintain set point temperatures in this experiment. In contrast, in Expt. 2, desired temperatures were more difficult to maintain toward the end of the experiment, while in Expt. 3, set point temperatures could be maintained over the complete cultivation time. Consequently, the difference between HT and LT treatment varied between 3.1 and 4.0 °C, depending on the experiment (Table 1).

Reaction time

Reaction time varied greatly between experiments, temperature, and cultivar (Table 2), whereas plant density effects were relatively small; doubling the density from 32 to 64 plants/m^2 resulted in only a 2 to 4 d longer reaction time (data not shown). In Expt. 3, reaction time was longer than in the other two experiments for all cultivars. Furthermore, LT increased reaction time by 4 to 17 d compared with HT and made the difference between cultivars larger. 'Reagan Improved' was relatively temperature sensitive, especially in Expt. 3 where plants grown at LT were harvested 17 d later than plants grown at HT. On the other hand, 'Supernova' was in all experiments the least temperature sensitive.

Dry mass production

Total dry mass. The effect of temperature on TDM_a (Fig. 1) and TDM_p (Table 3) differed between experiments. In Expt. 1, no significant effect of temperature on TDM_a and TDM_p was found, while in Expts. 2 and 3, TDM_a and TDM_p were higher at LT (Fig. 1, B and D; Table 3). However, in Expt. 3, this temperature effect was strongest in 'Reagan Improved', which showed a 23% increase in TDM_p at LT compared with HT, while TDM_p in 'Delianne' only increased by 8% (Table 3). Plant density had a strong positive effect on TDM_a in all experiments, resulting in up to 36% higher TDM_a (Fig. 1C). In contrast, increasing plant density decreased TDM_p for all cultivars, but in Expts. 2 and 3, this negative effect was stronger in 'Reagan Improved' compared with the other cultivars (Table 4). Except for 'Reagan Improved', which showed a significantly higher yield, the other three cultivars did not differ from each other in terms of TDM_a and TDM_p (Fig. 1A; Table 3).

Growth rates. To get a better insight into the factors that influence TDM_a, the growth rates were analyzed, taking into account different phases of the cultivation period (Table 5). During the LD period, RGR was significantly affected by

Table 2. Effect of temperature [low temperature (LT), high temperature (HT)] and cultivar on the reaction time (days) averaged over three plant densities of cut chrysanthemum in three greenhouse experiments.

	Expt. 1			Expt. 2			Expt. 3		
	LT	HT	LT-HT	LT	HT	LT-HT	LT	HT	LT-HT
Cultivar	Reaction time (d)								
Annecy	59	54	5	63	54	9	–[z]	–	–
Delianne	62	56	6	62	57	5	73	63	10
Reagan Improved	63	57	6	67	60	7	80	63	17
Supernova	59	55	4	60	55	5	65	61	4

[z]–, not present in experiment.

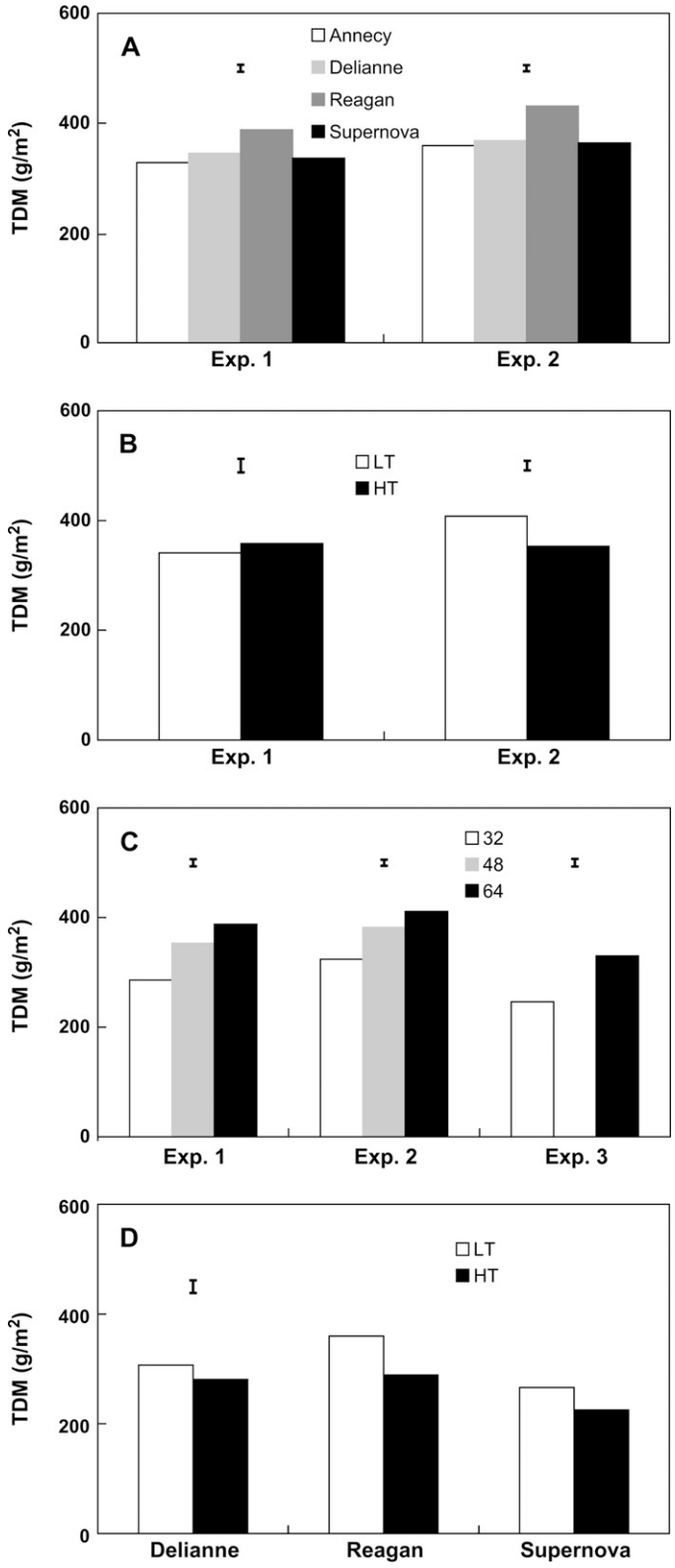

Fig. 1. Effect of the cultivar (**A** and **D**), temperature [low (LT) and high (HT) (**B** and **D**)], and plant density [32, 48, and 64 plants/m^2 (**C**)] on total dry mass per square meter (TDM_a) in a total of three greenhouse experiments with cut chrysanthemum. All the values are averaged overall the respective treatments and D represents Expt. 3 because a significant interaction between cultivar and temperature was observed. Vertical bars indicate LSD for each experiment.

temperature, plant density, and cultivar. LT and higher plant density decreased RGR up to around 8%. In all experiments, 'Supernova' had a significantly lower RGR than the other three cultivars.

The effect of temperature on AGR was not constant during the SD. In the first part of the SD period, AGR was significantly lower at LT, while in the second part of the SD period, temperature did not affect AGR (Table 5). In contrast, increasing plant density showed a consistently positive effect on AGR throughout the SD period, but this effect was less strong in the second part of the SD. Some significant differences in AGR were found among cultivars, but these were not consistent between experiments and over the SD period.

Leaf characteristics. LAR and SLA decreased up to 15% at LT, while LWR was hardly influenced by temperature (Table 6). In general, plant density had only a minor effect on these three parameters (<6%). Among the four studied cultivars, 'Annecy' showed a significantly higher LAR, SLA, and LWR.

Differences in the development of LAI occurred between temperatures, plant densities, and cultivars (Fig. 2). During LD and the first part of SD, LAI was lower at LT but differences between temperature treatments gradually disappeared during the SD period. In some cases, LAI was even higher at LT at the end of the experiment (Fig. 2). Furthermore, increasing plant density resulted in a higher LAI throughout all the cultivation period as a consequence of a higher LAI at planting. 'Annecy' was the cultivar with the higher LAI during the complete cultivation period in both experiments.

Light use efficiency. Temperature did not influence LUE and a significant plant density effect was only present in Expt. 3 (Table 7). However, LUE was significantly affected by cultivar: 'Annecy' had the lowest LUE, while highest LUE was recorded for 'Reagan Improved'. Moreover, LUE differed considerably among experiments, reaching its higher values in Expt. 3.

External quality

Flower characteristics. The effect of temperature on number of flowers (NoF) differed between experiments and between cultivars (Table 3). In most cases, NoF was not significantly affected by temperature, the only exceptions being 'Reagan Improved' in Expt. 2 and 'Supernova' in Expt. 3 where NoF was significantly lower at LT. In contrast, in all experiments and for all cultivars, increasing plant density decreased the NoF (Table 4). However, the magnitude of this effect was cultivar dependent. For instance, 'Reagan Improved' was especially sensitive to plant density: increasing density from 32 to 64 plants/m^2 decrease NoF by about 50%.

The influence of temperature on flower size also differed between experiments and cultivars (Table 3). Flower size of 'Delianne' and 'Supernova' was significantly increased at LT in all experiments. In contrast, in 'Reagan Improved', temperature did not affect flower size. Furthermore, in most cases, plant density showed a significant negative influence on flower size (Table 4), but this effect was less strong than the one described for NoF. Interestingly, flower size in 'Reagan Improved' was the least affected by plant density.

Relationship between NoF and TDM_p. Lower temperature significantly reduced the slope of the relationship between TDM_p and NoF (i.e., the same TDM_p resulted in lower NoF), especially in 'Reagan Improved' and 'Annecy', whereas 'Delianne' and 'Supernova' were clearly less temperature sensitive (Fig. 3). An overall linear regression model including

Table 3. Effect of temperature [low temperature (LT), high temperature (HT)] and cultivar on total dry mass per plant (TDM_p), number of flowers per plant (NoF), and individual flower area of cut chrysanthemum in three greenhouse experiments. Main effects of plant density are shown in Table 4.

Expt.		TDM_p (g/plant)			NoF			Flower area (cm^2)		
1	Temp	LT	HT	Mean	LT	HT	Mean	LT	HT	Mean
	'Annecy'	7.0	7.3	7.2 a[z]	16.5	17.3	16.9 c	19.3 a	18.2 a	18.8
	'Delianne'	7.4	7.5	7.5 a	11.8	12.4	12.1 b	50.5 e	40.1 d	45.3
	'Reagan Improved'	8.2	8.8	8.5 b	15.3	18.0	16.7 c	29.8 b	29.4 b	29.6
	'Supernova'	7.1	7.5	7.3 a	10.3	11.8	11.0 a	37.0 c	31.8 b	34.4
	Mean	7.4	7.8		13.5	14.9		34.1	29.9	
	$P > F$[y]									
	Temp (T)		0.101			0.089			0.016	
	Cultivar (C)		<0.001			<0.001			<0.001	
	T×D		0.634			0.809			0.117	
	T×C		0.619			0.076			<0.001	
	D×C		0.310			0.809			0.007	
	T×D×C		0.703			0.910			0.244	
2	Temp	LT	HT	Mean	LT	HT	Mean	LT	HT	Mean
	'Annecy'	8.5	7.2	7.8 a	16.9 b	17.8 bc	17.4	28.9 b	20.4 a	24.6
	'Delianne'	8.6	7.6	8.1 a	12.9 a	12.1 a	12.5	52.2 g	44.8 e	48.5
	'Reagan Improved'	10.1	8.9	9.5 b	17.4 b	18.5 c	17.9	35.1 c	35.1 c	35.1
	'Supernova'	8.6	7.3	7.9 a	12.3 a	12.6 a	12.4	48.2 f	39.6 d	43.9
	Mean	8.9 b	7.7 a		14.9	15.2		41.1	35.0	
	$P > F$									
	Temp		0.002			0.292			0.005	
	Cultivar		<0.001			<0.001			<0.001	
	T×D		0.568			0.057			0.234	
	T×C		0.784			0.015			<0.001	
	D×C		<0.001			<0.001			0.027	
	T×D×C		0.822			0.088			0.175	
3	Temp	LT	HT	Mean	LT	HT	Mean	LT	HT	Mean
	'Delianne'	6.8 d	6.3 bc	6.5	11.0 b	10.4 b	10.7	48.5 c	39.6 b	44.0
	'Reagan Improved'	8.1 e	6.6 cd	7.3	14.0 c	14.9 c	14.4	30.0 a	29.3 a	29.6
	'Supernova'	5.8 b	5.0 a	5.4	7.3 a	10.6 b	8.9	43.6 bc	33.4 a	38.5
	Mean	6.9	5.9		10.7	12.0		40.7	34.1	
	$P > F$									
	Temp		0.003			0.037			0.038	
	Cultivar		<0.001			<0.001			<0.001	
	T×D		0.530			0.377			0.078	
	T×C		0.008			<0.001			0.001	
	D×C		<0.001			<0.001			0.468	
	T×D×C		0.508			0.071			0.102	

[z]Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.
[y]Significant levels <0.05.

cultivar and TDM_p as regressors could explain 85% of the variation observed in NoF (data not shown). Adding temperature significantly ($P < 0.001$) improved the model (92% explained variance).

Discussion

Dry mass production. TDM_a in cut chrysanthemum is influenced by the length of the cultivation period (namely reaction time) and by the growth rate. However, higher TDM_a at lower temperatures (e.g., Expts. 2 and 3; Fig. 1) was merely the result of a longer reaction time, as growth early in the cultivation period was even reduced at LT. The increase in reaction time at suboptimal temperatures (Table 2) is probably due to delayed flower initiation and slower flower development (Karlsson et al., 1989a; Van der Ploeg et al., 2007a). Furthermore, plants flower later at lower light levels (Karlsson and Heins, 1986; Karlsson et al., 1989b), which explains our findings where Expt. 3 showed the longest reaction time and also the lowest light levels (Tables 1 and 2). Because plant density only increased reaction time slightly, it would not cause much further delay in harvesting at suboptimal temperature.

The lower RGR during LD and lower AGR during the first part of the SD observed in plants grown at LT (Table 5) were related to a reduced LAI (Fig. 2), resulting in a lower light interception. This reduction in LAI was caused by a lower LAR, due to a lower SLA (Table 6). The increase in leaf thickness (i.e., lower SLA) is a common response to lower temperatures,

Table 4. Effect of plant density on total dry mass per plant (TDM_p), number of flowers per plant (NoF), and individual flower area of four cut chrysanthemum cultivars in three greenhouse experiments. Main effects of temperature and cultivar are shown in Table 3.

Expt.		TDM_p (g/plant)			NoF			Flower area (cm^2)		
1	Density (plants/m^2)	32	48	64	32	48	64	32	48	64
	'Annecy'	8.5	7.4	5.6	21.2	17.0	12.5	21.8 b[z]	17.9 a	16.5 a
	'Delianne'	8.6	7.7	6.1	14.1	12.6	9.7	47.6 f	45.5 f	42.7 e
	'Reagan Improved'	10.1	8.6	6.7	22.3	16.8	10.9	29.6 c	30.2 c	29.0 c
	'Supernova'	8.5	7.4	5.9	13.6	11.2	8.3	35.4 d	33.4 d	34.3 d
	Mean	8.9 c[z]	7.8 b	6.1 a	17.8 c	14.4 b	10.4 a	33.6	31.8	30.6
	$P > F$[y]									
	Density (D)		<0.001			<0.001			<0.001	
	T×D		0.634			0.809			0.117	
	D×C		0.310			0.809			0.007	
	T×D×C		0.703			0.910			0.244	
2	Density (plants/m^2)	32	48	64	32	48	64	32	48	64
	'Annecy'	9.4 d	7.9 c	6.3 a	20.9 e	17.1 d	14.1 c	25.4 a	24.2 a	24.2 a
	'Delianne'	9.9 d	8.2 c	6.1 a	14.7 c	12.4 b	10.4 a	51.3 f	47.8 e	46.3 de
	'Reagan Improved'	11.9 e	9.6 d	7.1 b	24.1 f	17.7 d	12.0 b	35.5 b	35.6 b	34.1 b
	'Supernova'	9.4 d	8.2 c	6.2 a	14.8 c	12.7 b	9.8 a	46.2 d	44.1 d	41.3 c
	Mean	10.1	8.5	6.4	18.6	15.0	11.6	39.6	38.0	36.5
	$P > F$									
	Density		<0.001			<0.001			<0.001	
	T×D		0.568			0.057			0.234	
	D×C		<0.001			<0.001			0.027	
	T×D×C		0.822			0.088			0.175	
3	Density (plants/m^2)	32	48	64	32	48	64	32	48	64
	'Delianne'	7.7 d	—	5.4 b	12.5 d	—	8.9 b	46.3	—	41.8
	'Reagan Improved'	9.1 e	—	5.6 b	18.5 e	—	10.4 c	30.6	—	28.7
	'Supernova'	6.4 c	—	4.5 a	11.4 c	—	6.5 a	40.8	—	36.2
	Mean	7.7	—	5.1	14.1	—	8.6	39.2 b	—	35.5 a
	$P > F$									
	Density		<0.001			<0.001			<0.001	
	T×D		0.530			0.377			0.078	
	D×C		<0.001			<0.001			0.468	
	T×D×C		0.508			0.071			0.102	

[z]Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.
[y]Significant levels <0.05.
[x]—, not present in experiment.

which has been observed in several plant species (Van der Ploeg et al., 2007a, 2007b). However, because chrysanthemum has a determinate growth pattern (Pearson et al., 1995), delayed flower initiation at LT resulted in a longer time for the formation of new leaf area. This explains why the difference in LAI between temperature treatments gradually disappeared during the SD. Therefore, final LAI was equal at both temperature levels or even higher at LT, depending on the experiment and the cultivar (Fig. 2). LUE was unaffected by temperature (Table 7). This agrees with previous findings where chrysanthemum crop photosynthesis shows a rather flat temperature optimum (Körner, 2003). Therefore, LUE is not a limiting factor for growing chrysanthemum under lower temperature regimes. Nevertheless, LUE was higher in Expt. 3, confirming that at lower light levels (Table 1), plants convert light more efficiently into biomass (Karlsson et al., 1987; Lee et al., 2002a).

In all experiments, the longer cultivation period at LT compensated at least partly for the lower growth rate during early cultivation. However, TDM_a only increased significantly at LT in Expts. 2 and 3 (Fig. 1). In Expt. 1, light levels at the end of experiment were relatively low; thus, an extension of the cultivation period only contributed for a relatively small part to TDM_a. However, in Expts. 2 and 3, light levels increased toward the end of the cultivation period. Extra cultivation time at the end of the experiment contributed strongly to TDM_a. Another aspect that might explain differences between experiments is the difference in realized temperatures, as in Expt.1 there was only 3.1 °C between the LT and HT compartments, whereas for Expts. 2 and 3, this increased to 3.6 and 4.0 °C, respectively.

External quality. As expected, reducing temperature or increasing plant density to improve energy efficiency had consequences on the flower characteristics, but these effects were strongly cultivar dependent. In most cases, NoF was hardly influenced by temperature, whereas plant density strongly reduced NoF in all cultivars. This negative effect of plant density was previous observed and it is related to a reduction in assimilate availability (Carvalho and Heuvelink, 2003). A positive linear relationship between NoF and TDM_p was described in the past for 'Reagan Improved' (Carvalho and

Table 5. Effect of temperature [low temperature (LT), high temperature (HT)], plant density, and cultivar on relative growth rate during the long day period (RGR LD) and absolute growth rate during the first (AGR SD_1) and second (AGR SD_2) part of short day (SD) period of cut chrysanthemum in three greenhouse experiments.

	RGR LD ($g \cdot g^{-1} \cdot d^{-1}$)[z]			AGR SD_1 ($g \cdot m^{-2} \cdot d^{-1}$)[y]			AGR SD_2 ($g \cdot m^{-2} \cdot d^{-1}$)[x]		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Temp									
LT	0.104 a[w]	0.082 a	0.076 a	5.39 a	3.29 a	2.40 a	5.24	7.05	3.57
HT	0.111 b	0.088 b	0.083 b	6.46 b	3.63 b	2.84 b	5.42	6.87	3.58
Density (plants/m^2)									
32	0.110 b	0.086 b	0.083 b	5.01 a	2.81 a	2.36 a	4.64 a	6.57	3.13 a
48	0.109 ab	0.085 ab	—[v]	5.92 b	3.42 b	—	5.46 b	6.87	—
64	0.105 a	0.082 a	0.077 a	6.30 b	3.84 c	2.87 b	5.58 b	6.81	3.90 b
Cultivar									
Annecy	0.110 bc	0.099 c	—	6.02	3.69 b	—	4.93 a	6.60	—
Delianne	0.113 c	0.093 c	0.084 b	5.79	3.22 a	2.70	4.92 a	7.04	3.62
Reagan Improved	0.108 b	0.083 b	0.082 b	6.06	3.64 b	2.64	6.28 b	6.94	3.74
Supernova	0.101 a	0.075 a	0.072 a	5.83	3.30 a	2.52	5.17 a	7.26	3.36
$P > F$[u]									
Temp (T)	0.023	0.009	0.011	0.026	0.043	0.039	0.239	0.568	0.933
Density (D)	0.014	0.021	<0.001	<0.001	<0.001	0.002	0.002	0.115	<0.001
Cultivar (C)	<0.001	<0.001	<0.001	0.711	0.002	0.592	0.001	0.251	0.319
T×D	0.673	0.773	0.975	0.673	0.318	0.551	0.589	0.291	0.591
T×C	0.114	0.222	0.957	0.864	0.833	0.577	0.861	0.356	0.445
D×C	0.988	0.547	0.224	0.722	0.106	0.307	0.875	0.367	0.730
T×D×C	0.876	0.938	0.320	0.571	0.695	0.508	0.818	0.820	0.246

[z]Calculated according to classical approach in Expts. 1 and 2 and according to the functional approach in Expt. 3.
[y]Absolute growth rate calculated over the first 20 d of the SD period.
[x]Absolute growth rate calculated over the period between 20 d after start of SD until 47, 41, or 43 d after start of SD for Expt. 1, Expt. 2, and Expt. 3, respectively.
[w]Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.
[v]—, not present in experiment.
[u]Significant levels <0.05.

Table 6. Effect of temperature [low temperature (LT), high temperature (HT)], plant density, and cultivar on leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) over the long day period of cut chrysanthemum in three greenhouse experiments.

	LAR ($cm^2 \cdot g^{-1}$)[z]			SLA ($cm^2 \cdot g^{-1}$)[z]			LWR[z]		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Temp									
LT	184 a[y]	235 a	238 a	280	335 a	349 a	0.656	0.699 b	0.683 b
HT	194 b	272 b	252 b	287	392 b	373 b	0.676	0.691 a	0.676 a
Density (plants/m^2)									
32	188	251 a	243 a	280 a	359 a	356 a	0.673	0.698	0.684 b
48	185	250 a	—[x]	279 a	358 a	—	0.665	0.695	—
64	193	259 b	247 b	293 b	374 b	366 b	0.659	0.692	0.675 a
Cultivar									
Annecy	209 c	299 b	—	291 c	389 c	—	0.721 c	0.769 d	—
Delianne	180 a	237 a	255 b	260 a	341 a	356 b	0.691 b	0.694 c	0.716 c
Reagan Improved	172 a	241 a	222 a	279 b	364 b	339 a	0.617 a	0.664 b	0.656 a
Supernova	193 b	237 a	258 b	306 d	362 b	387 c	0.631 a	0.654 a	0.667 b
$P > F$[w]									
Temp (T)	0.038	0.002	0.002	0.741	0.001	<0.001	0.179	0.008	0.022
Density (D)	0.142	0.025	0.019	0.010	0.002	<0.001	0.108	0.179	<0.001
Cultivar (C)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
T×D	0.596	0.753	0.679	0.330	0.794	0.486	0.724	0.484	0.413
T× C	0.149	0.488	0.077	0.874	0.475	0.138	0.105	0.152	0.832
D×C	0.927	0.193	0.341	0.913	0.160	0.377	0.998	0.639	0.507
T×D×C	0.829	0.595	0.135	0.963	0.836	0.145	0.803	0.082	0.289

[z]Calculated according to classical approach in Expts. 1 and 2, and according to the functional approach in Expt. 3.
[y]Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.
[x]—, not present in experiment.
[w]Significant levels <0.05.

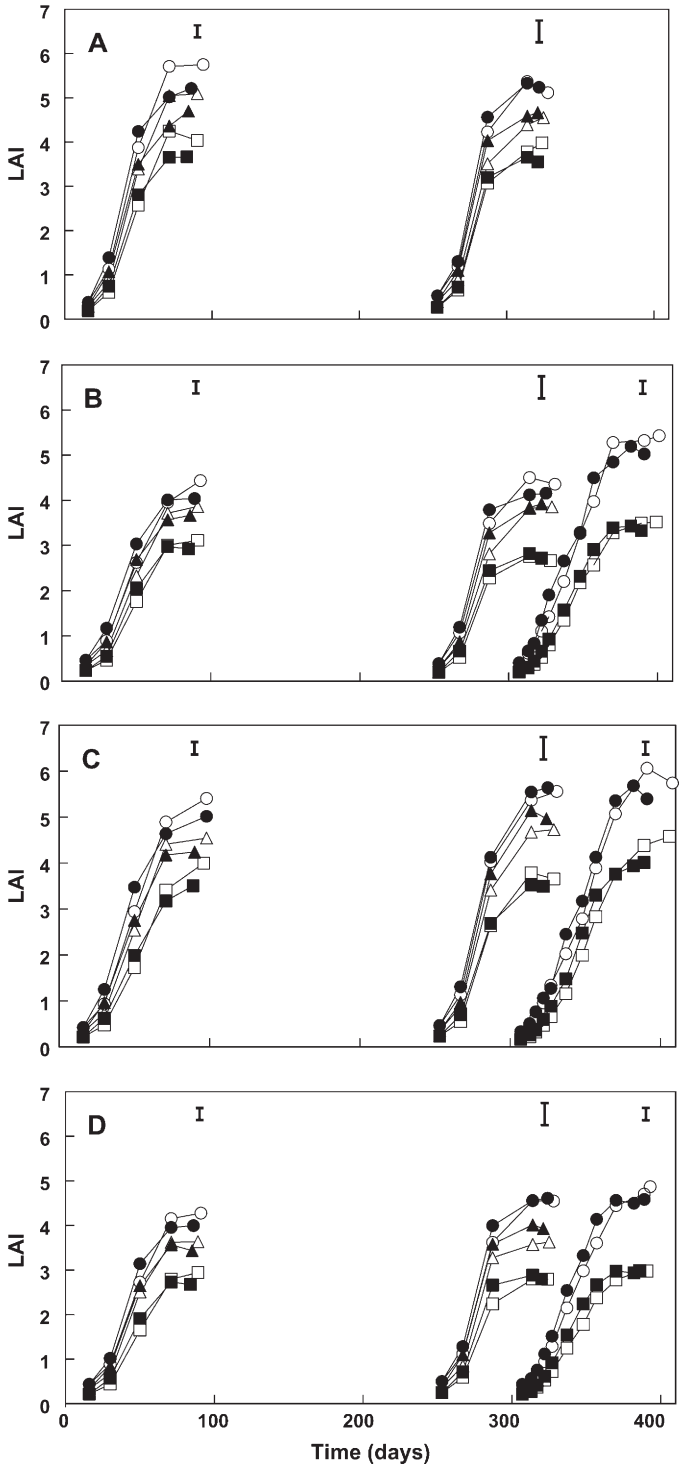

Fig. 2. Time patterns of leaf area index (LAI) for cut chrysanthemum cultivar Annecy (**A**), Delianne (**B**), Reagan (**C**), and Supernova (**D**) grown at three plant densities [32 (■, □), 48 (▲, △), and 64 plants/m^2 (○, ●)] and at two temperatures [low (□, △, ○) and high (■, ▲, ●)] in three greenhouse experiments. Dates are expressed as days after 1 Jan. (1 = 1 Jan.): Expt. 2 = 16–97, Expt. 1 = 253–330, Expt. 3 = 307–401. Vertical bars indicate LSD at the final harvest date of each experiment.

Heuvelink, 2003). This study goes one step further to show that this relationship is not only influenced by temperature but also extremely cultivar dependent (Fig. 3). Furthermore, the extent to which temperature affects this relationship is cultivar dependent. For instance, in 'Delianne', this relationship was the least sensitive to lower temperature, offering good prospects for breeding. The difference in response between the cultivars might be related to a different optimum temperature for flower initiation (Van Ruiten and De Jong, 1984) as well as to flower type. The less temperature sensitive cultivars 'Delianne' and 'Supernova' formed less second-order lateral flowers, both at HT and LT, therefore they did not benefit from an extension in the cultivation period at LT that resulted in higher assimilate availability and consequently in higher TDM_p.

Lowering temperature can be beneficial for 'Delianne' and 'Supernova', which responded with larger flowers (Table 3), while flower size in 'Reagan Improved' was unaffected by temperature. The positive effect of lower temperature on flower size is probably mainly an effect of a longer flower growth period at lower temperatures (Nothnagl et al., 2004). Strangely, this did not happen in 'Reagan Improved' in spite of being the cultivar with the largest temperature effect on reaction time. However, the large increase in reaction time in Expt. 3 at LT (17 d) was likely due to a delay in flower initiation because the time to visible bud (data not shown) was also increased at LT. In general, the effect of plant density on flower size was considerably smaller than the effect on NoF (Table 4). This is in agreement with Carvalho et al. (2005) who observed that flower size in 'Reagan Improved' was only influenced by assimilate supply at very low light levels.

Table 7. Effect of temperature [low temperature (LT), high temperature (HT)], plant density, and cultivar on light use efficiency (LUE) during the short day (SD) period of cut chrysanthemum in three greenhouse experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.

	Light use efficiency (g·MJ^{-1})[z]		
	Expt. 1	Expt. 2	Expt. 3
Temp			
LT	3.42	4.00	5.66
HT	3.60	4.10	5.50
Density (plants/m^2)			
32	3.32 a[y]	3.94	5.47
48	3.69 b	4.23	—[x]
64	3.53 ab	3.98	5.69
Cultivar			
Annecy	3.24 a	3.87 a	—
Delianne	3.54 b	4.08 b	5.67 b
Reagan Improved	3.83 c	4.18 c	5.79 b
Supernova	3.43 b	4.06 b	5.28 a
$P > F$[w]			
Temp (T)	0.063	0.072	0.279
Density (D)	0.010	0.342	0.065
Cultivar (C)	<0.001	<0.001	0.002
T×D	0.620	0.064	0.102
T×C	0.498	0.194	0.124
D×C	0.620	0.064	0.102
T×D×C	0.892	0.173	0.847

[z]Calculated for the SD period.
[y]Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5% level.
[x]—, not present in experiment.
[w]Significant levels <0.05.

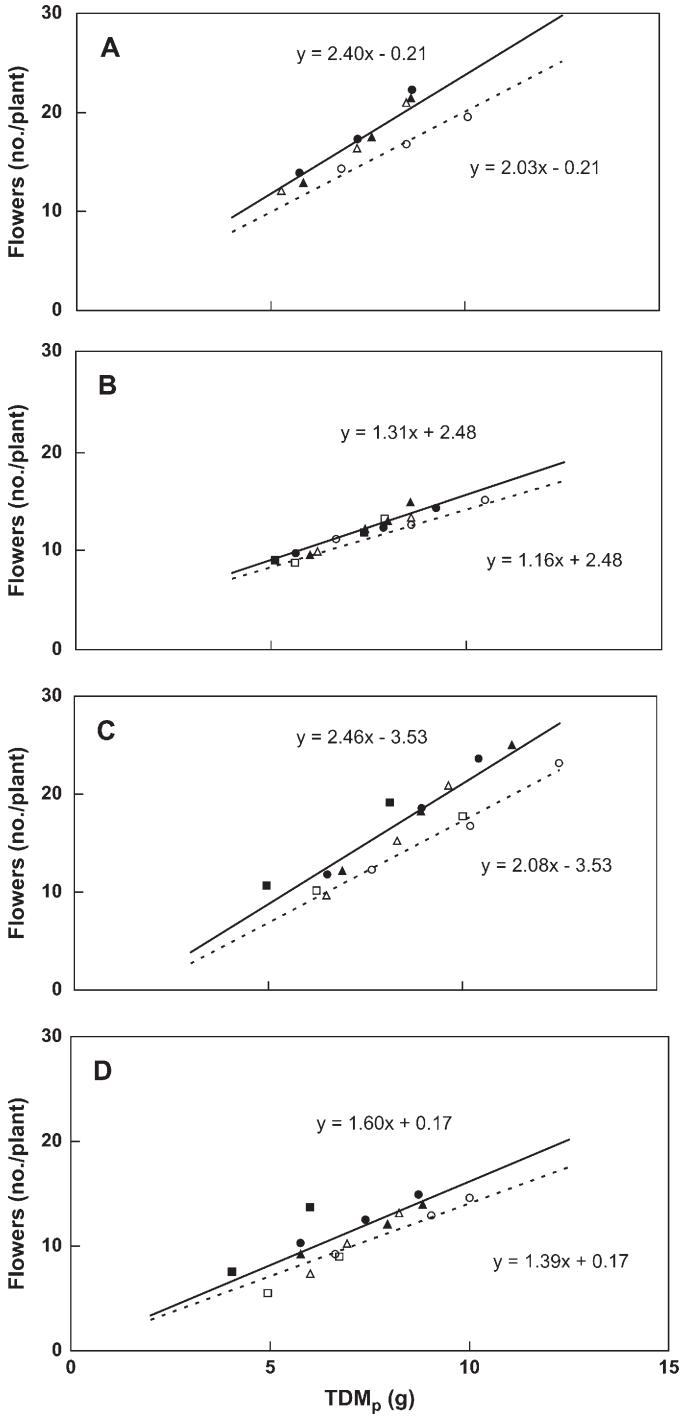

Fig. 3. Relationship between total number of flowers per plant and total dry mass per plant (TDM_p) for four cut chrysanthemum cultivars [Annecy (**A**), Delianne (**B**), Reagan (**C**), and Supernova (**D**)] grown at high temperature (closed symbols) and low temperature (open symbols) in Expt. 1 (▲, △), Expt. 2 (○, ●), and Expt. 3 (■, □). Lines represent prediction by the regression model for 16 °C (dotted line) and 20 °C (solid line) realized temperature.

Implications for breeding for suboptimal temperature tolerance. Lowering the temperature to reduce energy use in cut chrysanthemum cultivation results in a longer duration of the cultivation period, which has negative consequences in the annual yield. On other hand, from the growth point of view, LT could be applied during the second phase of the SD period, as AGR during the last part of the cultivation is unaffected by temperature. Because flower development is less temperature sensitive than flower initiation (Karlsson et al., 1989a; Van der Ploeg et al., 2007a; Van Ruiten and De Jong, 1984), we expect that lowering the temperature during the last phase of cultivation would only increase reaction time slightly. This small increase in reaction time could be compensated with a slightly higher plant density. However, currently it is still not feasible to adjust the temperature to the stage of the cultivation because in the same greenhouse compartment, different stages of development are cultivated simultaneously (Carvalho et al., 2005).

Besides the negative effects that a combination of lower temperature and higher plant density have on reaction time and yield, some adverse effects on plant quality were also found. For instance, even if the same TDM_p could be reached, this would result in a lower number of flowers at LT. On the contrary, lowering the temperature can increase flower size in some cultivars, although higher plant density could reduce this effect slightly. Therefore, due to the counteractive effects of temperature on the flower characteristics, the growth conditions must be adjusted taking the market demand into account. Another disadvantage of growing cut chrysanthemum at higher plant densities is the reduction of the crop uniformity in terms of plant weight, especially under low light (winter) conditions (Langton et al., 1999).

Reaction time is one of the traits that showed the largest differences in temperature response between cultivars. Therefore, it is obviously an important characteristic to be used by breeders. To improve yield at LT, the breeders should focus on reducing the temperature effect on SLA. In this study, SLA and consequently growth in the first half of the cultivation were reduced at suboptimal temperatures (Tables 5 and 6). Unfortunately, no variation in temperature response was found for growth and underlying components within these four cultivars. However, it could be possible that within a wider range of cultivars, more variation is present. Nevertheless, it is important to keep in mind that these four cultivars were already selected out of a sample of 25 cultivars based on the widest range of temperature response for several characteristics including growth. An alternative option for breeding less temperature-sensitive cultivars would be to counteract the lower SLA by increasing partitioning of assimilates to the leaves at lower temperatures (i.e., increasing LWR), thus compensating for the negative effects of SLA on LAR. However, it is also important to consider the possible implications that this strategy would have on other plant aspects, like external quality attributes.

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