Variation Between Cut Chrysanthemum Cultivars in Response to Suboptimal Temperature

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ABSTRACT. To breed for more energy-efficient cut chrysanthemum (*Chrysanthemum morifolium* Ramat.) cultivars it is important to know the variation of the temperature response existing in modern cultivars. In a greenhouse experiment with 25 chrysanthemum cultivars, a significant variation was observed in temperature response (16 °C or 20 °C) for reaction time, total dry weight produced, stem length, and flower size and number. To study this genetic variation in temperature response over a larger range of temperatures (15 °C to 24 °C), four contrasting cultivars (Annecy, Delianne, Reagan, and Supernova) were selected in a second experiment. Furthermore, a third experiment was performed in which the cultivation period was split into three phases and the influence of temperature in each of these phases was studied for the four selected cultivars. Dry weight production in all cultivars was very sensitive to temperature during the long day period. Relative growth rate showed an optimum response to temperature, with the optimum around 24 °C. Net assimilation rate also showed an optimum response to temperature, whereas leaf area ratio increased linearly with temperature. Compared with these temperature effects during the long day, the effect of temperature on absolute growth rate during the short day was, depending on the cultivar, relatively small or even absent. The reaction time, on the other hand, was very temperature sensitive, showing an optimum that was cultivar dependent. The temperature response of the total dry weight production during the whole cultivation period was, therefore, very cultivar dependent. Furthermore, depending on the cultivar, stem length increased with temperature, especially during long day, as a result of both increasing internode number and average internode length. The response of both flower size and number to temperature was also highly cultivar specific. The possibilities of using this genetic variation for breeding are discussed.

Several crop species (e.g., cut chrysanthemum) can only be grown year-round in colder climates when cultivated in heated greenhouses. This implies that production during autumn and winter will require high energy inputs, and therefore energy efficiency (i.e., the number of marketable stems produced per unit of energy input) is low. Lowering the temperature set point in the greenhouse could be a way to increase this energy efficiency. This may, however, have adverse effects on crop growth and quality. It is therefore important that new cultivars are better adapted to cultivation at lower temperatures. Chrysanthemum cultivation can be divided into a long-day (LD) period, during which the plants grow vegetatively, and a short-day (SD) period. During this latter period, flower initiation and further development takes place. The number of days from the start of SD to harvest is referred to as the reaction time, and this trait is very sensitive to temperature, showing a definite temperature optimum (De Jong, 1978). Adaptation to lower temperatures can be achieved by breeding cultivars with a broader temperature optimum for reaction time. Another option is to create cultivars with a higher biomass production at suboptimal temperatures, so that a longer reaction time can

be compensated by a higher plant density or shorter LD period. For breeding new, more energy-efficient cultivars, genetic variation for these traits in the current cultivar range is extremely valuable.

The genetic variation in temperature response for time to flowering has been well studied in cut chrysanthemum (e.g., Cockshull et al., 1981; De Jong, 1978, 1984). This research has resulted in cultivars that were capable of flowering at lower temperatures (Larsen and Persson, 1999), whereas the temperature optimum of these cultivars did not change. Therefore, growers did not reduce temperature set points to guarantee a shorter cultivation period. Despite the importance of the response of plant growth to temperature, relatively few studies have focused on the genetic variation within chrysanthemum accessions for this trait. It is also important to know how chrysanthemum growth and development react to temperature in different stages of the cultivation period. For example, temperature is known to be a critical factor for flower initiation (Wilkins et al., 1990), whereas for further flower development the optimum temperature becomes lower as the plants mature (Karlsson et al., 1989a). Although a higher temperature during the LD period increased the number of flowers in the cultivar Reagan, most flower characteristics (e.g., size, color) were more sensitive to temperature during the SD period (Carvalho et al., 2005). However, because these studies were done with a single cultivar, it is difficult to generalize these results. More information is also fundamental for a proper analysis of possible differences between cultivars.

The aim of the current study was to evaluate the variation in growth and development for temperature responses among chrysanthemum cultivars and to obtain a better insight into the

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underlying physiological processes. Furthermore, we tried to identify phases in the cultivation period during which temperature is crucial. To study the variation for temperature response on various characteristics related to growth performance an experiment was carried out during which 25 cut chrysanthemum cultivars were grown at two temperature regimes. From these 25 cultivars, four contrasting cultivars were selected for an in-depth study in a second experiment under a wider range of temperatures (15 °C to 24 °C). Finally, a third experiment was carried out during which different temperatures were applied to three different phases of the cultivation period for the four selected chrysanthemum cultivars.

Materials and Methods

EXPERIMENTAL SETUP. Expt. 1 (Table 1) was carried out in four compartments (12.8 × 12.0 m) of a multispan Venlo-type greenhouse (Wageningen University, Wageningen, The Netherlands, lat. 52 °N). Each compartment contained eight parallel soil beds (1.125 × 10.25 m), of which the outer two were used as border. Rooted cuttings in peat blocks (4.2 × 4.2 cm) of 25 chrysanthemum cultivars were obtained from two breeding companies [Fides Goldstock Breeding, Maasland, The Netherlands (cultivars Feeling Green, Grand Pink, Greenbird, Mundial, Reagan Improved, Shining, Spoetnik, Supernova, Tiger, Universe, Voyager, and Woodpecker) and Deliflor, Maasdijk, The Netherlands (cultivars Anastasia, Annecy, Beverly, Biarritz, Bradford, Cayenne, Delianne, Dublin, Granada, Hastings, Managua, Orinoco, and Zembla)]. These cuttings were planted on 27 Nov. 2002 at a density of 48 plants/m². The 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 set 1 °C above the heating set point. During the first 3 weeks of the cultivation period, the plants were grown under LD conditions, followed by SD until flowering. The apical flower bud was removed during an early stage. Supplementary irradiance [44 μmol·m⁻²·s⁻¹ photosynthetic active radiation (*PAR*)] was provided by high-pressure sodium lamps (HPS; SON-T Agro; Philips, Eindhoven, The Netherlands), which were kept on continuously during the day period of the LD (19 h) and SD (9 h 30 min). Plants were grown under ambient CO₂. Greenhouse climate was automatically recorded every 5 min using a commercial computer system (Hoogendoom, Vlaardingen, The Netherlands).

Expts. 2 and 3 (Table 1) were conducted in four artificially lit climate rooms (4.50 × 3.25 × 2.20 m). Cuttings of four chrysanthemum cultivars (Annecy, Delianne, Reagan Improved, and Supernova) were planted in 14-cm-diameter pots containing peat-based commercial potting compost [85%

peat, 15% clay (Lentse Potgrond no. 4; Lentse Potgrond, Lent, The Netherlands)] at a plant density of 69 plants/m². Plants were placed on side-by-side trolleys. During the first 2 weeks, plants were grown under LD conditions, followed by SD until flowering. Assimilation lights (380 μmol·m⁻²·s⁻¹ *PAR*, 1 HPI-T plus: 1 HPS SON-T Agro; Philips) were continuously on for 8 h followed by 11 h (LD) or 3 h (SD) of incandescent light (12 μmol·m⁻²·s⁻¹ *PAR*, purely photoperiodic). Plants were grown under ambient CO₂ and at constant vapor pressure deficit (0.58 kPa). Plants were watered by hand as required. Fertilization was done on a weekly basis (2 g·L⁻¹, 19N–2.6P–16.6K–0.6Mg with micronutrients; Kristalon; Hydro Agri, Vlaardingen, The Netherlands). In Expt. 2, the temperatures in the four climate rooms were set at 15, 18, 21, and 24 °C. Within each climate room there were two blocks of each cultivar. This experiment was repeated. In Expt. 3 the temperatures in two climate rooms were set at 16 °C (LT), and in the other two at 20 °C (HT). Both at the start of SD and at the moment the apical bud became visible [visible bud (VB)], plants from half the trolleys were placed in the other temperature treatment and the other half remained at the same temperature, dividing the cultivation period in three phases (phase I, LD; phase II, start SD till VB; phase III, VB till final harvest). This resulted in a total of 32 treatments (i.e., eight temperature treatments for each of the four cultivars).

MEASUREMENTS. In all, experiments destructive measurements were carried out at planting, the start of the SD, and at final harvest. In Expt. 2, one more harvest was conducted during the LD and four more destructive measurements were conducted during SD at about equal intervals (10–12 d). In Expt. 1, at each destructive measurement five plants per plot were harvested, leaving two rows of border plants between cultivars and harvests. In Expt. 2 and Expt. 3, three plants per plot were measured at each harvest, except for the final harvest, when six plants per plot were taken. Final harvest of all plants 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. After each destructive measurement the plants were rearranged such that plant density remained equal and a row of border plants around the measurement plants was maintained. Stem, leaf, and flower fresh and dry weight (ventilated oven, 105 °C for at least 15 h), number of leaves on the main stem, number of flowers (including flower buds larger than 5 mm), and stem length were determined. Total plant leaf area and individual flower area of the first lateral flower (model 3100 area meter; LI-COR, Lincoln, Nebr.) were determined. Internode appearance rate (IAR) was determined as the slope of the regression line

Table 1. Basic information on three cut chrysanthemum experiments described in this paper.

Expt.	Location	Cultivars (n)	Realized temperature (°C) ^z	Plant density (plants/m ²)	Incident <i>PAR</i> (mol·m ⁻² ·d ⁻¹)
1	Greenhouse	25	16.5 (LT), 20.1 (HT)	48	4.2–5.6 ^y
2	Climate room	4	15.0, 18.0, 21.0, 24.0	69	10.9
3	Climate room	4	16.0 (LT), 20.0 (HT) ^x	69	10.9

Realized actual air temperature and incident photosynthetic active radiation (*PAR*) are averages over the whole growing period.

^zLT = low temperature, HT = high temperature.

^yRange of variation among cultivars.

^xCultivation is divided in three phases [phase I, long-day (LD) period; phase II, start of short day (SD) to visible bud (VB); phase III, VB to flowering] and at the end of each phase, one half of the plants were moved to the other temperature.

between leaf number and time for observations during the LD and the first measurement in SD.

With the dry weight and leaf area observations collected in Expt. 2, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) were calculated over the LD period according to the “classic approach” described by Hunt (1990), using the measurements at planting [leaf area index (LAI) > 0.31] and at the end of the LD period (LAI < 1.9). Furthermore, the absolute growth rate (AGR) was calculated as the slope of total dry weight (TDW) against time during the SD period (for all treatments $R^2 > 0.95$; LAI > 2.2).

STATISTICAL DESIGN AND ANALYSIS. Expt. 1 and Expt. 2 had a split-plot design with temperature as the main factor and cultivar as the split factor. Expt. 3 was analyzed as a complete randomized design with two replications. Normality of data were checked, using the Kolmogorov-Smirnov test in SPSS (version 12.0.1; SPSS, Chicago). When data were not normally distributed, a square root transformation was obtained and normality of these transformed data was checked. In all cases, normality was produced by this transformation. An analysis of variance (ANOVA) was conducted and treatment effects were tested at a 5% probability level, except for the main temperature effects in Expt. 3, which were tested at a 1% probability level (high *df* for residual). Mean separation was done by Student's *t* test ($P = 0.05$). The statistical software package Genstat (version 8; VSN International Ltd., Hemel Hempstead, UK) was used. In Expt. 2, the effect of temperature was separated in a linear and a quadratic component. Based on the outcome of the ANOVA, a linear regression model was built with temperature, (temperature)², and cultivar as regressors. If a quadratic effect of temperature was found, the linear component was also put in the model.

Results

TEMPERATURE VARIATION AMONG 25 CULTIVARS. In Expt. 1 a significant interaction was observed between cultivar and temperature for all analyzed characteristics, which reflects the contrasting behavior of these 25 cut chrysanthemum cultivars (Table 2). Reaction time was always longer at LT, but the magnitude of this effect varied greatly between cultivars. Flowering in ‘Supernova’ was delayed by only 4 d whereas flowering for ‘Reagan’ was delayed by 13 d (Table 2). Eleven

cultivars had a significantly higher TDW at flowering when cultivated at LT compared with HT, whereas for the other 14 cultivars there was no significant difference between TDW produced under HT and LT (data not shown). For quality attributes, such as flower number and size, a large variation in temperature response was observed (Table 2).

From the 25 cultivars studied in Expt. 1, four cultivars (Annecy, Delianne, Reagan, and Supernova) were selected based on their contrasting behavior in response to temperature in reaction time and biomass production. Additionally, these cultivars showed differences in temperature response to leaf area, leaf number, stem length, flower number, and flower size (Table 2). In general, ‘Delianne’, which reacted similarly to ‘Granada’, and ‘Reagan’ were more sensitive to temperature. On the other hand, ‘Annecy’ and ‘Supernova’ were less sensitive and only flower number was significantly decreased at LT, although reaction time was more affected by temperature for ‘Annecy’ (11 d delayed at LT compared with HT).

Temperature response in four selected cultivars

REACTION TIME. The minimum of the reaction time (Fig. 1A) was cultivar dependent ($P < 0.001$). The cultivar Supernova had the lowest temperature minimum and the shortest reaction time at 15 °C, whereas ‘Reagan’ had the highest optimum and the longest reaction time at 15 °C. The overall regression model could explain 98% of the variation in reaction time.

When grown at LT during phases II and III, all cultivars showed a delay in flowering, varying between 2 to 4 d (Table 3). However, for all four cultivars, the delay at LT was similar in both phases of the SD, even though phase III was about twice as long.

TOTAL DRY WEIGHT. Total dry weight (TDW) showed a significant interaction ($P = 0.002$) between cultivar and temperature (Fig. 1B). ‘Reagan’ was very sensitive to temperature, showing a minimum around 20 °C. ‘Delianne’ responded similarly, although less pronounced. In contrast, for ‘Supernova’ the TDW was only significantly increased at 24 °C, and ‘Annecy’ did not show any significant response to temperature within the studied temperature range (15 °C to 24 °C). An overall regression model showed that temperature could explain 84% of the variation observed in TDW for the four cultivars.

The temperature effect on TDW depends on the phase of the cultivation (Table 4). During the LD period (phase I) plants

Table 2. Range of variation for temperature response among 25 cut chrysanthemum cultivars grown at low temperature [LT (16.5 °C)] and high temperature [HT (20.1 °C)] for reaction time, total dry weight (TDW), leaf area (LA), number of internodes (NoI), stem length, individual flower area (FA), number of flowers (NoF), and flower mass ratio (FMR) in Expt. 1.

	Difference (LT – HT) ^z				
	Median		Maximum		Minimum
Reaction time (d)	8	Reagan	13 (21)	Supernova	4 (7)
TDW (g/plant)	0.84	Granada	2.64 (44)	Annecy	–0.58 (NS)
LA (cm ² /plant)	23	Granada	192 (17)	Grand Pink	–165 (–12)
NoI (no./plant) ^y	–1.4	Reagan	3.3 (9)	Grand Pink	–5.8 (–11)
Stem length (cm)	–10	Anastacia	13.8 (19)	Shining	–30 (–35)
FA (cm ² /flower)	5.2	Bradford	12.7 (35)	Feeling Green	–0.8 (NS)
NoF (no./plant) ^y	–1.3	Reagan	2.3 (19)	Annecy	–4.0 (–23)
FMR (g·g ^{–1})	0.009	Spoetnik	0.051 (34)	Feeling Green	–0.028 (NS)

Numbers in parentheses are the relative increase at LT (as a percentage) when the differences were found to be significant.

^{NS}Difference between LT and HT nonsignificant.

^zF probability of cultivar × temperature interaction (<0.009 for all parameters).

^yAnalysis of variance based on square root transformed data.

Fig. 1. Reaction time (A), total plant dry weight (TDW) (B), and absolute growth rate (AGR) during the short-day period (C) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an $R^2_{adj} = 0.98$ (A), $R^2_{adj} = 0.84$ (B), and $R^2_{adj} = 0.83$ (C). Vertical bars denote SE of regression = 0.90 (A), SE of regression = 0.82 (B), and SE of regression = 0.012 (C).

were most sensitive to temperature. Plants grown at HT during phase I (Table 4) were heavier than plants grown at LT during this phase ($P < 0.001$). Furthermore, the effect of temperature during phase II was dependent on the temperature during the previous phase ($P = 0.023$). When plants were cultivated at HT during phase I, the temperature during phase II did not have a significant influence on TDW. However, when plants were grown at LT during phase I, plants had a higher TDW when cultivated at HT during phase II than plants cultivated at LT

Table 3. Duration of each of the cultivation phases and the delay in reaction time measured at final harvest at low temperature [LT (16 °C)] compared with high temperature [HT (20 °C)] during different phases of the cultivation period for four chrysanthemum cultivars in Expt. 3.

Phase	Duration (d)	Delay in final harvest LT compared with HT (d)			
		Annecy	Delianne	Reagan	Supernova
I Long-day (LD) period	14	0	0	0	0
II Start short day (SD) to visible bud (VB)	16–21	3	3	4	2
III VB to harvest	36–42	3	3	4	2

Table 4. The effect of a temperature increase from low temperature [LT (16 °C)] to high temperature [HT (20 °C)] during three phases of the cultivation period in four cut chrysanthemum cultivars on total dry weight (TDW), stem length, number of internodes (NoI), average internode length (IL), number of flowers (NoF), and individual flower area (FA) measured at final harvest stage in Expt. 3.

	Temperature effect (LT–HT)		
	Phase I ^z	Phase II ^z	Phase III ^z
TDW (g/plant)	+1.3 ^y	+0.58 LT phase I ^x NS HT phase I	+0.60 Annecy NS Delianne –0.65 Reagan NS Supernova
Stem length (cm)	+4.2 ^y	+4.3 Annecy NS Delianne +4.5 Reagan NS Supernova	NS
NoI (no./plant)	+1.6 Annecy +1.9 Delianne +0.9 Reagan +2.1 Supernova	NS	NS
IL (cm/internode)	NS Annecy NS Delianne +0.11 Reagan NS Supernova	+0.09 Annecy NS Delianne +0.17 Reagan NS Supernova	NS
NoF (no./plant)	+4.8 Annecy +2.9 Delianne +3.5 Reagan +1.9 Supernova	+1.0 LT phase I ^x NS HT phase I	+1.7 ^y
FA (cm ² /flower)	NS	NS	NS Annecy –4.0 Delianne NS Reagan –6.2 Supernova

^zPhase I, long day (LD); phase II, start of short day (SD) to visible bud (VB); phase III, VB to flowering.

^ySame temperature effect for all cultivars.

^xSame temperature effect for all cultivars, but interaction between temperature in phase I and temperature in phase II.

^{ns}Difference between LT and HT is nonsignificant.

during phase II. The effect of temperature in phase III was dependent on the cultivar ($P = 0.008$), varying from a significant negative effect of higher temperature for ‘Reagan’ up to an increase in TDW with temperature in phase III for ‘Annecy’.

GROWTH CHARACTERISTICS. Although there were clear differences present between the cultivars in Expt. 2, when it comes to the growth characteristics during the LD period, all cultivars responded in a similar way to temperature (Table 5). Relative growth rate increased quadratically with temperature until an optimum around 24 °C. NAR also showed an optimum response to temperature but was only significantly lower at 15 °C. Leaf area ratio and SLA showed a linear increase with temperature, whereas LWR showed a quadratic response with a minimum around 22 °C. ‘Supernova’ had a lower RGR than the other three similar cultivars. Furthermore ‘Supernova’ had a low NAR. To the contrary, ‘Reagan’ had the highest NAR, but this was combined with a low LAR, because of a low LWR.

Absolute growth rate during SD showed an interaction between cultivar and temperature ($P = 0.003$; Fig. 1C). In both ‘Annecy’ and ‘Supernova’, temperature had a quadratic effect on AGR, with a similar optimum around 19 °C to 20 °C, but the later cultivar had a higher AGR. ‘Delianne’ and ‘Reagan’ did not show significant differences in AGR between the different temperature treatments. At all temperatures the AGR of ‘Reagan’ was clearly higher than that for the other cultivars.

STEM LENGTH, IAR, LEAF NUMBER, AND AVERAGE INTERNODE LENGTH. In all four cultivars, stem length increased linearly with temperature, but this increase was larger for ‘Reagan’ ($P < 0.001$) compared with the other three (Fig. 2A). An overall regression model could explain 96% of the variation observed in stem length. For all cultivars, temperature during phase I had a positive effect on stem length, whereas temperature during phase II only affected stem length in ‘Annecy’ and ‘Reagan’. In the last part of the SD period (phase III), temperature had no effect on stem length (Table 4).

Internode appearance rate (IAR) (Fig. 2B) and final leaf number (Fig. 2C) increased in all cultivars with temperature, but this effect was less strong in ‘Annecy’ compared with the other cultivars. The overall regression model could explain 81% of the variation present in IAR and 93% of the variation present in leaf number. ‘Delianne’ had the lowest leaf number at all temperature treatments. For all cultivars, leaf number only increased with temperature during phase I (Table 4), with ‘Reagan’ significantly less sensitive ($P = 0.026$).

Similar to leaf number, average internode length was increased linearly with temperature in all cultivars (Fig. 2D), and an overall regression model could explain 92% of the variation present. The increase in internode length with temperature was only marginal in ‘Supernova’ whereas it was greatest in ‘Reagan’. Higher temperature during phase I increased internode length of ‘Reagan’, whereas the average internode length of the other three cultivars was unaffected by temperature during phase I (Table 4). Higher temperature during phase II increased average internode length of ‘Annecy’ and ‘Reagan’, whereas the other two cultivars were unaffected by temperature during phase II. Temperature during phase III had no effect on internode length for any of the cultivars.

FLOWER CHARACTERISTICS. The number of flowers per plant (Fig. 3A) showed a significant interaction between cultivar and temperature ($P = 0.015$). For instance, in ‘Annecy’ no significant effect of temperature on flower number was found, whereas in ‘Delianne’ increased temperature had a negative effect on number of flowers. Contrary, ‘Reagan’ and ‘Supernova’ showed an increase in flower number with temperature from 15 °C to 24 °C. In all cultivars, higher temperature during phases I and III resulted in an increase in flower number, especially during phase I for the cultivars Annecy and Reagan (Table 4). The effect of temperature during phase II was dependent on the temperature during the previous phase ($P = 0.002$). When plants were cultivated at HT during phase I, the temperature during phase II did not have a significant influence on flower number, whereas plants cultivated with LT during phase I showed a small increase in flower number with temperature during phase II.

Individual flower size showed a significant interaction ($P = 0.047$) between cultivar and temperature (Fig. 3B). Temperature had no significant influence on the flower size of ‘Reagan’, whereas for the other three cultivars flower size showed a quadratic response, with a minimum that was cultivar dependent. Furthermore, ‘Delianne’ was more sensitive to temperature than ‘Annecy’ and ‘Supernova’. Temperature during phases I and II did not influence flower size in any of the cultivars, whereas flower size was reduced at HT during phase III for ‘Delianne’ and ‘Supernova’ (Table 4).

Table 5. Effect of temperature and cultivar on relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) of cut chrysanthemum during the long-day (LD) period in Expt. 2.

	RGR (g·g ⁻¹ ·d ⁻¹)	NAR (g·m ⁻² ·d ⁻¹)	LAR (cm ² ·g ⁻¹)	SLA (cm ² ·g ⁻¹)	LWR (g·g ⁻¹)
Temperature (°C)					
15	0.093 a	3.70 a	223	315	0.712 c
18	0.110 b	4.37 b	224	334	0.675 b
21	0.119 c	4.63 b	231	359	0.646 a
24	0.122 c	4.62 b	245	373	0.659 ab
F _{pr} ^z Linear	<0.001	0.006	0.047	0.005	0.004
Quadratic	0.008	0.036	0.267	0.677	0.017
Cultivar					
Annecy	0.110 b	3.91 a	248 c	343 b	0.726 b
Delianne	0.114 b	4.48 b	226 ab	319 a	0.709 b
Reagan	0.114 b	4.93 c	216 a	347 b	0.623 a
Supernova	0.105 a	3.98 a	234 b	371 c	0.633 a
F _{pr} ^z	0.005	0.001	0.009	0.002	< 0.001

^zF probability; significant levels less than 0.05 presented in bold type. Different letters indicate significant differences between treatments based on Student’s *t* test ($P = 0.05$).

Fig. 2. Stem length (A), internode appearance rate (IAR) (B), final leaf number (NoI) (C), and average internode length (IL) (D) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an $R^2_{adj} = 0.96$ (A), $R^2_{adj} = 0.81$ (B), $R^2_{adj} = 0.93$ (C), and $R^2_{adj} = 0.92$ (D). Vertical bars denote SE of regression = 2.22 (A), SE of regression = 0.0309 (B), SE of regression = 0.695 (C), and SE of regression = 0.0779 (D).

Discussion

This paper clearly demonstrates that cut chrysanthemum genotypes differ in their response to temperature for growth and quality aspects (e.g., Table 2). As in chrysanthemum, flower shape and color are important selection criteria. Flowers with special shapes and colors may result in higher market prices, which might compensate for lower growth rates. Therefore, the selection pressure in cut chrysanthemum has not only been in the direction of higher growth rates, resulting in a large variation for growth related traits.

REACTION TIME, TDW, AND GROWTH CHARACTERISTICS. Reaction time showed a quadratic response to temperature, and the minimum was cultivar dependent, varying from around 19 °C to 21 °C (Fig. 1A). An even wider range of variation had been established previously (De Jong, 1978). Especially early stages of flower initiation and development are known to be temperature sensitive (Karlsson et al., 1989a; Van Ruiten and De Jong, 1984). Also in this study temperature during the bud initiation phase had a relatively strong influence on reaction time (Table 3).

The response of TDW to temperature depended on the cultivar (Fig. 1B). This might be an explanation for varying responses in previous studies (reviewed by Van der Ploeg and Heuvelink, 2006). However, different temperature ranges and interactions with several other growing conditions (e.g., light level) might also play a role. Contrary to our results, Lepage et al. (1984) found that fresh weight of six cultivars all showed the same temperature (10 °C to 18 °C) response. The difference might originate from the way in which the cultivars were selected in our study; the four chrysanthemum cultivars in this study were not selected solely based on the temperature response for reaction time, but growth responses were also taken into account.

Total dry weight in chrysanthemum showed an exponential increase over time followed by a linear growth phase (Lee et al., 2002). The effects of temperature were clearly different in each phase and depended on genotype (Tables 4 and 5, Fig. 1C). Especially higher temperature during the LD period increased TDW for all cultivars (Table 4), as a result of an increase in RGR. The optimum temperature for RGR (Table 5) in our study was slightly lower than optimum temperatures reported for young tomato (*Lycopersicon esculentum* Mill.) (Hussey, 1965) and pansy (*Viola x wittrockiana* Gams.) (Adams et al., 1997) plants, both showing a maximum around 25 °C. In this study rooted cuttings (initial LAI between 0.31 and 0.57) were used, and internal shading resulted in a lower temperature optimum for RGR (Adams et al., 1997). To understand differences observed in RGR, RGR was separated into an assimilatory (NAR) and a morphological (LAR) component. Leaf area ratio showed a linear increase with temperature, as a result of an increase in SLA. This is in agreement with Acock et al. (1979), although in their research no effect on dry weight gain was found, probably because of the high initial LAI (2.7) so that most light was already intercepted. In our study, lower RGR at 15 °C was also incited by reduced NAR. However, between 18 °C and 24 °C, temperature had no effect on NAR. This is in line with Körner (2003), who reported that chrysanthemum crop photosynthesis also shows a rather flat optimum response to temperature. Temperature during the beginning of the SD period only affected TDW if the temperature during LD was low (Table 4). Reduced dry weight production during the LD and decreased SLA (thicker leaves) resulted in a lower LAI at LT. When LAI is low, not all the light is intercepted by the canopy, and therefore morphological changes, like decreased SLA, will affect light interception. As canopy growth proceeds, gradually more internal shading will occur and morphological changes will have less effect on plant growth.

The cultivar with the lowest RGR in this study, 'Supernova', also had a low NAR (Table 5). However, this NAR did not differ from the NAR of 'Annecy', but in the latter cultivar a low NAR was compensated by a high LAR. On the other hand, the cultivar with the highest NAR, 'Reagan', had the lowest LAR. Therefore in this study no clear relationship could be found between RGR and NAR or between RGR and LAR to explain differences between cultivars. This is in conflict with De Jong and Jansen (1992), who found RGR of 15 cultivars to be correlated with LAR, but this could be related to the lower number of cultivars used for our growth analysis.

Compared with the effect of temperature on RGR during the LD, the effect of temperature on AGR during the SD is relatively small or even absent (Fig. 1C). Furthermore, the optimum for 'Annecy' and 'Supernova' was located between

Fig. 3. Number of flowers (NoF) per plant (A) and individual flower area (FA) of the first basipetal flower (B) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an $R^2_{adj} = 0.85$ (A) and $R^2_{adj} = 0.93$ (B). Vertical bars denote SE of regression = 1.45 (A) and SE of regression = 2.54 (B).

19 °C and 20 °C, and therefore, far below the optimum for RGR. In the linear growth phase the crop is closed, and therefore most of the light is intercepted. Kohl and Thigpen (1979) also reported that above an LAI of 3, temperature did not affect dry weight gain.

Differences in TDW at flowering (Fig. 1B) can be explained by differences in reaction time, growth rate, or a combination of both. For instance in ‘Delianne’ and ‘Reagan’, the quadratic response of TDW to temperature was solely incited by a similar response in reaction time because AGR was unaffected by temperature (Fig. 2C), whereas in ‘Annecey’ the effect on reaction time was counteracted by an opposite effect on AGR. The negative influence of higher temperature during phase III on TDW in ‘Reagan’ (Table 4) must be a result of a shorter cultivation period. ‘Reagan’ had the longest extension of cultivation period at LT compared with HT (Tables 2 and 3). Furthermore, ‘Reagan’ had a higher AGR than the other cultivars and therefore differences will show up sooner. On the contrary, the positive effect of temperature during phase III on ‘Annecey’ is a result of a reduced AGR, which could not be compensated for by a longer growth period.

STEM LENGTH, IAR, FINAL LEAF NUMBER, AND AVERAGE INTERNODE LENGTH. Stem length increased with temperature as a result of increasing leaf (internode) number and average internode length (Fig. 2, Table 4). In previous research, an

optimum around 25 °C for IAR has been established (Carvalho et al., 2002; Larsen and Hiden, 1995). The temperature range in this study was below the optimum, which might explain why a linear response was found. Chrysanthemum starts to initiate flowers soon after the start of the SD period (Van Ruiten and De Jong, 1984) and, after the plant has induced the apical flower bud, it loses the ability to initiate new internodes. Not surprisingly, only temperature during the LD period influenced final leaf number (Table 4). After the flower buds have been initiated, stem elongation is solely the result of internode elongation. Although internode length is generally highly dependent on the difference between day and night temperature (DIF) (Carvalho et al., 2002; Karlsson et al., 1989b) and not 24-h average temperature, in this study average internode length increased with temperature even though DIF was 0 (Table 4, Fig. 3D). However, because in these experiments several internodes were already present when the experiment started and the final number of internodes differed between cultivars and temperature treatments, it is not possible to compare these results directly with measurements on individual internodes. Internode elongation in ‘Reagan’ follows a sigmoidal time course that, depending on temperature, takes between 17 and 24 d to complete (Carvalho et al., 2002). Therefore, most internodes have fully elongated by the time phase III starts, and temperature during this last phase had no influence on internode elongation and, subsequently, stem length.

FLOWER CHARACTERISTICS. The effect of temperature on flower characteristics depended on the cultivar (Fig. 3). This genetic variation in temperature response has been well established in previous research for both flower number (Lepage et al., 1984) and individual flower size (Vince, 1960; Willits and Bailey, 2000). Individual flower size of three cultivars showed a minimum ranging between 18 °C and 20 °C, depending on the cultivar (Fig. 3B). The increased flower size at sub- and supraoptimal temperatures might be related with increasing reaction times. However, this cannot explain why flower size in ‘Reagan’ did not increase at suboptimal temperatures. Therefore, different processes could be involved in contrasting cultivars.

A significant positive effect of temperature during phases I and III on number of flowers was previously reported for ‘Reagan’ (Carvalho et al., 2005) and was here confirmed for other chrysanthemum cultivars (Table 4). Additionally, a small increase in flower number with temperature during phase II was reported, but only if temperature during phase I was low. The positive effect of temperature on number of flowers during phase I and phase II is likely to be related with the increase in TDW, because a higher assimilate availability will result in a higher number of flowers (Carvalho and Heuvelink, 2003). The larger increase in flower number for ‘Annecey’ and ‘Reagan’ is probably associated with the smaller flower size in these two cultivars. Even though the formation of the apical flower bud takes place during phase II, lateral and second-order flowers continue to be formed during phase III, as a consequence of the basipetal progression of flower formation in chrysanthemum. Higher temperature during this phase mainly increases the number of flowers as a result of an increase in the percentage of flower buds (Carvalho et al., 2005). Furthermore, for ‘Delianne’ and ‘Supernova’, higher temperature during phase III increased the size of the first lateral flower, whereas flower size of the other two cultivars did not respond to temperature in any of the phases (Table 4). Because ‘Delianne’ and ‘Supernova’ were

also the cultivars with the largest flowers, differences could probably be detected more easily.

CONCLUDING REMARKS. Temperature influence on chrysanthemum varied greatly between cultivars, and temperature sensitivity depended on the phase of the cultivation period. This provides several opportunities for breeding more energy-efficient cultivars. The genetic variation for temperature response during the LD period is limited, but breeders could exploit the variation in growth parameters between cultivars to construct new lines with a higher RGR. This will have the result that the crop closes faster, at which stage temperature has less influence on crop growth. A higher RGR could be achieved by combining a high partitioning toward the leaves (high LWR), thin leaves (high SLA), and a high NAR. Genotypic differences were present for all these traits. During the SD, temperature influenced mainly the rate of development. Because the influence of temperature on development is highly cultivar specific, there are plenty of opportunities to select less temperature-sensitive genotypes. Furthermore, it is important that these genotypes do not show a reduced AGR at low temperature.

In addition, changes could be applied during the cultivation period. For instance, a more dynamic heating strategy could be applied. It is then important to keep the temperature during the LD period high so that enough biomass is formed. For growth, temperature during the SD is less important, but quality is influenced by temperature during the SD (Carvalho et al., 2005). Currently, this will run into practical problems in continuous chrysanthemum production, because in the same greenhouse compartment, plants at different stages of development are cultivated (Carvalho et al., 2005). However, as at this moment practical trials are being performed for a mobile cultivation system, and in the future might be feasible to adapt temperature better to stage of cultivation.

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