Chlorophyll Fluorescence and Flowering Behaviour of Annual-Fruiting Raspberry Cultivars under Elevated Temperature Regimes

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Summary

The effects of a seven day period with increased temperature from a standard level of 20 °C to 27, 32 or 37 °C during flower initiation on apparent quantum efficiency (F_v/F_m), chlorophyll content and flowering behaviour in raspberry 'Autumn Bliss', 'Autumn Treasure', 'Erika', 'Fall Gold' and 'Polka' were investigated. The F_v/F_m decreased steadily from 0.82 to 0.70 on the seventh day at 37 °C in 'Autumn Bliss' and 'Fall Gold'. A significant diurnal variation in F_v/F_m, characterized by a midday depression and partial recovery in the evening was observed in 'Polka'. Plants of 'Autumn Bliss' exposed for seven days to 37 °C had 58% less chlorophyll *a* as compared to those grown at 20 °C. The chlorophyll *a/b* ratio decreased to 20% at 37 °C in 'Autumn Bliss', and 'Fall Gold'. The number of days to anthesis of the terminal flower was not significantly affected by the temperature treatments. The number of unopened axillary buds decreased at 37 °C in 'Autumn Bliss', 'Autumn Treasure', 'Fall Gold' and 'Polka'. The percentage of flowering lateral shoots per plant decreased by 16% at 37 °C in 'Autumn Bliss' whereas it increased by 7% at 37 °C in 'Autumn Treasure' and 'Erika'. The percentage of flower buds per plant decreased by 20% at 37 °C in 'Autumn Treasure' and 'Erika'. Thus the negative responses of heat stress across the temperature regimes was reflected in F_v/F_m in all cultivars, while there was a remarkable difference in chlorophyll content and flowering behaviour among cultivars. These responses suggest that there is a

potential difference of annual-fruiting raspberry cultivars in their inherent ability to adapt to heat stress, which was reflected in photosynthetic pigments and subsequent flowering behaviour.

Key words. chlorophyll content – photosynthesis - *Rubus idaeus* - heat stress - terminal flower

Introduction

Raspberry *(Rubus idaeus* L.*)* is an important soft fruit crop across cold and temperate regions of the world (HEIDE and SONSTEBY 2011; SONSTEBY and HEIDE 2012) and interest in raspberry production under open-field, high-tunnel and greenhouse conditions has been increasing (OLIVEIRA et al. 2002; DALE et al. 2003; DALE et al. 2005). Manipulation of the growth cycle in annual-fruiting raspberry cultivars allows a year-round production of fruit in the greenhouse (DALE et al. 2005). However, the effect of temperature on flower formation in annual-fruiting cultivars is being discussed (HEIDE and SONSTEBY 2011). The optimum temperature for annual-fruiting raspberry cultivation ranges from 16 to 24 °C, but some cultivars like 'Polka' grow well even at 30 °C (HEIDE and SONSTEBY 2011). In annual-fruiting raspberries, the environmental regulation of flower induction is not fully understood (CAREW et al. 2001; SONSTEBY and HEIDE 2010). But flowering is advanced by intermediate photoperiods (11-15 h) and temperatures between 20 and 25 °C (CAREW et al. 2001; SONSTEBY and HEIDE 2010; NERI et al. 2012). Most raspberry cultivars are poorly adapted to warm and humid conditions that may occur during summer (BALLINGTON and FERNANDEZ 2008).

Temperatures above optimum negatively affects plant growth in all developmental stages from shoot formation in the spring to flowering and fruit ripening. However, the upper temperature threshold for optimal growth varies significantly at different phenological and

49 growth stages. Excessively high temperature (10-15 °C above optimum) adversely affects
50 photosynthesis, respiration, evapotranspiration, membrane integrity and modulates hormone
51 and metabolite production (WAHID et al. 2007). Photosynthesis is one of the most heat-
52 sensitive processes in plants (BERRY and BJORKMAN 1980; WASID et al. 2007) and many
53 authors have shown that elevated temperature reduces net assimilation due to impairment of
54 CO_2 fixation, photophosphorylation and the electron transport chain (SALVUCCI and CRAFTS-
55 BRANDNER 2004a; SALVUCCI and CRAFTS-BRANDNER 2004b). Furthermore, high temperature
56 decreases photosynthetic efficiency, chlorophyll accumulation and regulates heat shock
57 proteins (EFEOGLU and TERZIOGLU 2009). MOLINA-BRAVO et al. (2011) reported a lower ratio
58 of variable to maximum chlorophyll fluorescence (F_v/F_m) in heat sensitive raspberry cultivars
59 with the lowest values in the afternoon. Moreover, heat stress may decrease the total
60 concentration of chlorophyll pigments, change the ratio of chlorophyll *a* to *b* (Chl *a/b*), and
61 chlorophyll to carotenoid content (*a+b/x+c*) in stressed leaves depending on the temperature
62 tolerance of the species (CAMEJO et al. 2005; EFEOGLU and TERZIOGLU 2009).
63 An increasing interest in producing raspberries in warmer climates and out-of-season in
64 protected cultivation has stimulated research aimed at a better understanding of the effects of
65 temperature and photoperiod on growth and fruiting (CAREW et al. 2000; CAREW et al. 2001;
66 DALE et al. 2003; SONSTEBY and HEIDE 2010). In this study, the effects of high temperature
67 during early flower initiation on flowering behaviour of five annual-fruiting raspberry
68 cultivars were investigated to pin point possible control mechanisms underlying the
69 differences between cultivars. The aim of the study was also to determine if chlorophyll
70 fluorescence may be used as a screening criterion for high-temperature sensitivity in annual-
71 fruiting raspberry cultivars.
72

73 ## Materials and Methods

Plant material and experimental conditions

One-year old cold-stored plants of five annual-fruiting raspberry cultivars; 'Autumn Bliss', 'Autumn Treasure', 'Fall Gold', 'Erika' and 'Polka' were obtained from the nursery Vester Skovgaard, Denmark, where they were lifted from the field in mid-November 2010 and kept in a dark cold-store at 2 °C before shipping to the Department of Food Science, Aarhus University on 20 January 2011. The plants were stored on site in a dark cold room (2 ±1°C) for additional 9, 10 or 11 weeks, which resulted in cold-storage for a total of 15, 16 or 17 weeks before forcing under greenhouse conditions. The canes were pruned to soil level and potted in 3.5 L pots containing 10-30 mm blonde peat substrate (Pindstrup No 4, Pindstrup Mosebrug A/S, Ryomgard, Denmark), with an electrical conductivity (EC) of 2-4 (mS cm^{-1}) and pH 6. Plants were forced at 20-25 °C and a photoperiod of 14 h until flower initiation. Microscopic observation of axillary buds from non-experimental plants was carried out five weeks after root sprouting to determine the time of flower initiation. Flower primordia were visible under the microscope after seven weeks (WILLIAMS 1959). A single primary shoot was maintained per pot during the experimental period. Plants were fertigated once a day to pot capacity using a nutrient solution with an EC of 2.16 mS cm^{-1} containing 40 mg L^{-1} NH_4-N, 165 mg L^{-1} NO_3-N, 44 mg L^{-1} P and 257 mg L^{-1} K.

Heat stress treatments

When plants reached the stage of flower initiation (~ seven weeks after root sprouting), they were transferred to three climate chambers (MB-teknik, Broendby, Denmark) at 27, 32 or 37 °C, with a day length of 14 h similar to the greenhouse. There were three plants per cultivar, treatment and cold-storage time (15, 16 and 17 weeks). The temperature treatment was given for a seven day (~168 h) period. A fourth set of plants remained in the greenhouse at temperature ranging from 20 to 25 °C and 14 h light conditions as reference. In the climate

chambers, the irradiance was constant at 350 µmol m^{-2} s^{-1} PAR and RH was 60% ±5% for all replications. All plants were fertigated with a complete nutrient solution to pot capacity at 08.00 am and 05.00 pm.

Measurement of chlorophyll fluorescence

During the seven days of heat treatment, chlorophyll fluorescence was measured on the third leaf (≈75% expanded) from the top of the shoot between 12.00 am to 02.00 pm each day to minimize the diurnal effects of temperature and light on photosynthesis. A pulse-amplitude modulated fluorometer (MiniPam; Heinz Walz GmbH, Eiffeltrich, Germany) was used on the upper surface after 30-min dark adaptation using a standard leaf clip. Initial fluorescence (F_o), when plastoquinine electron acceptor pool (Q_A) is fully oxidized and maximum fluorescence (F_m), when Q_A is transiently fully reduced, were recorded for photosystem II and variable fluorescence ($F_v = F_m - F_o$) and maximum quantum efficiency (F_v/F_m) were calculated. The F_v/F_m readings were taken on the third leaf (~75% expanded) from the top of the shoot in 'Polka' between 08.30 and 09.00 am, 12.00 am and 02.00 pm, and 05.30 and 06.30 pm to describe diurnal variations in apparent quantum efficiency.

Chlorophyll pigment

At the end of the heat stress treatment, half of each leaf used to measure F_v/F_m was collected and weighed and immediately frozen in liquid nitrogen and stored at -80 °C. The samples were freeze-dried for 48 h and homogenised into a fine powder. Approx. five mg of the homogenised freeze-dried tissue was weighed in a 15 mL test-tube and 100 µL distilled water was added. After 10 min of hydration, 8 mL of 96% ethanol was added before shaking in a vortex at approx. 250 rpm for a min. The samples were wrapped in aluminium foil and incubated at room temperature in an exhaust hood overnight. Samples were vortexed for one

124 min and the absorbance was measured on the supernatant at 470.0, 648.6, 664.2 and 750 nm
125 using spectrophotometer (UV-1700, Shimadzu, Japan). The chlorophyll *a*, chlorophyll *b*,
126 chlorophyll *a/b* and the ratio of chlorophyll (*a*+*b*) to carotenoid (*x*+*c*) were calculated as
127 described by LICHTENTHALER (1987).
128
129 ***Plant growth and flowering behaviour***
130 After the seven day treatment at elevated temperature regimes in climate chambers, plants
131 were transferred to an open high tunnel (Atoplan Longlife film, Vorden, The Netherlands),
132 and the third node from top of the shoot was marked to indicate new shoot and leaf
133 development after stress treatment. The plants were placed in rows on a ground cover with
134 black Mypex with an inter-row spacing of 1 m and 0.30 m between plants. Plants were drip-
135 irrigated with a complete nutrient solution. For each plant, the day of anthesis of the terminal
136 flower was recorded and plant growth and flowering behaviour were evaluated 30 days after
137 anthesis of the terminal flower. Days to anthesis of the terminal flower was counted from the
138 day of root sprouting when forcing them in greenhouse. The following were recorded: leaf
139 area (LI-3100 Leaf Area Meter, LI-COR, Lincoln, USA) of leaves developed above the
140 marked node, total number of lateral shoots, number of flowering lateral shoots and number
141 of unopened axillary buds. The percentage of unopened flower buds was calculated as:
142 Unopened flower buds (%) = (Unopened flower buds)*100/ (Unopened flower buds + flowers
143 and fruit)
144
145 ***Experimental design and statistical analysis***
146 The experiment was a split plot design with three cold-storage duration as main-plot and
147 temperature as sub-plot. Repeated (F_v/F_m) measurements were conducted every day during the
148 seven day temperature treatment. Statistical analysis was carried out using the SAS procedure

149 PROC MIXED (SAS Inst. Inc., Cary, NC). Each cultivar was analysed separately. Data were
150 tested for normal distribution and homogeneity of variance before analysis. The percentage
151 and proportional data were arcsine transformed prior to statistical analysis, original mean
152 values are shown in figures and tables. Mean differences within cultivar, temperature and cold
153 storage duration were separated using Tukey Kramer's test at $P \leq 0.05$.
154

155 ## Results

156

157 ### *Chlorophyll fluorescence*

158 Three-way interaction between cold-storage duration, temperature and days of temperature
159 stress was found for all cultivars (Table 1 and Fig. 1). The F_v/F_m decreased with increased
160 stress period at 27, 32 and 37 °C and the highest reduction was from 0.82 to 0.70 at 37 ºC at
161 day seven in 'Autumn Bliss' and 'Fall Gold'. While in 'Autumn Treasure' and 'Erika', the
162 F_v/F_m was reduced to 0.72 at day seven at 37 °C. A similar pattern for F_v/F_m during the period
163 of increased temperature was found for plants cold-stored for 15 and 16 weeks but for plants
164 cold stored for 17 weeks, F_v/F_m dropped more quickly between days one and five. There was
165 an abrupt drop in F_v/F_m at 32 and 37 °C in all cultivars until day five of stress treatment.
166

167 ### *Diurnal variations in F_v/F_m in 'Polka'*

168 Similar to the other four cultivars, midday F_v/F_m in 'Polka' decreased from 0.82 to 0.71 at the
169 end of the seven day stress period (Fig. 2). However, the F_v/F_m ratio remained constantly
170 higher in the morning and evening as compared with midday ($P<0.001$). When statistical
171 analysis was carried out using cold-storage duration (C), temperature (T), days of temperature
172 stress (S), and time of day (D) as class variables, the four-way (C*T*S*D) and three-way

173 (C*T*D) interactions were not significant while C*T*S and T*S*D were significant for F_v/F_m
174 ($P<0.05$).
175
176 ***Chlorophyll pigments***
177 The effect of temperature on chlorophyll pigments varied with cultivar. The Chl *a* and Chl *a/b*
178 significantly decreased with increasing temperature in 'Autumn Bliss', 'Fall Gold' and
179 'Polka', while there was no effect in 'Autumn Treasure' and 'Erika' (Fig. 3A). The decrease
180 in Chl *a* at 37 °C ranged from 20 to 58% in 'Autumn Bliss' and 'Fall Gold', respectively,
181 when compared with greenhouse conditions and resulted in yellowing of the upper leaves.
182 Similarly, Chl *a/b* decreased from 5 to 20% in 'Autumn Bliss', 'Fall Gold' and 'Polka' at
183 37 °C (Fig. 3B). The chlorophyll to carotenoid $(a+b)/(x+c)$ ratio significantly decreased in
184 'Erika', with increased temperature (Fig. 3C). Therefore 'Autumn Treasure' and 'Erika' could
185 not be regarded as heat sensitive according to these parameters.
186
187 ***Growth and flowering***
188 The leaf area of the main shoot developed between a seven day period at 37 °C and 30 days
189 after anthesis of the terminal flower was significantly lower ($P<0.05$) in 'Autumn Treasure',
190 'Erika', 'Fall Gold', and 'Polka' and the reduction ranged from 68 to 82% compared with
191 reference plants grown in greenhouse. The number of unopened axillary buds at the main
192 shoot decreased significantly after seven days at 37 °C in 'Autumn Bliss', 'Autumn Treasure',
193 'Fall Gold' and 'Polka' whereas the number of lateral shoots per plant was increased in
194 'Autumn Bliss' (175%), 'Erika' (66%) and 'Fall Gold' (31%) compared with greenhouse
195 conditions (Table 2). The percentage of flowering lateral shoots per plant decreased by 16%
196 in 'Autumn Bliss' after exposure to 37 °C but other cultivars were not affected by the
197 temperature treatments. The height of the main shoot following a seven day stress period at

37 °C was significantly reduced in ‘Autumn Treasure’, ‘Erika’ and ‘Polka’. The number of days to anthesis of the terminal flower was not influenced by increased temperature in any of the five cultivars (Fig. 4) but the percentage of unopened flower buds was decreased up to 22% in ‘Autumn Treasure’ and ‘Erika’ by a seven day period at 37 °C compared to greenhouse conditions. However, the number of ripe fruits at 30 days after anthesis of the terminal flower was not affected by increased temperatures during flower initiation (data not shown).

The effect of cold-storage duration on growth and flowering behaviour is presented in table 3. The leaf area of the main shoot developed after heat stress period increased significantly on ‘Autumn Bliss’ and ‘Erika’. The number of unopened axillary buds, number of lateral shoots per plant, number of flower and buds per lateral and main shoot height were influenced by longer cold-storage duration in ‘Autumn Bliss’ and ‘Fall Gold’. The number of flowers and unopened buds per lateral was 43 in ‘Autumn Bliss’ and 36 in ‘Fall Gold’ at longer period of cold-storage period (17 weeks). However, other cultivars were not significantly affected by cold-storage period.

Discussion

Chlorophyll fluorescence

The effect of extreme temperatures on plant growth, development and reproductive behaviour is complex due to the combined effect of environment and genetic factors. The damage caused by high temperatures includes a wide range of structural and functional changes in plants (GEORGIEVA et al. 2000). At temperatures above the optimum, the apparent quantum yield declines due to inhibition of PSII activity (BERRY et al. 1980). The heat stress has a

direct effect on the PSII photo-oxidizing site and decreases the emission of the variable chlorophyll fluorescence (GEORGIEVA et al. 2000). The dark-adapted value of F_v/F_m is therefore a sensitive indicator of maximal photosynthetic performance with optimal values around 0.83 for most plant species (BJORKMAN and DEMMING 1987). In our observations, F_v/F_m decreased steadily from 0.82 to 0.70 at 37 °C after 168 h of exposure. This decrease in F_v/F_m was only a weak and recoverable effect and therefore not likely to have any major impact on the D1 protein repair system (LEIPNER 2007). In our experiment, F_v/F_m for 'Polka' was always higher in the morning and evening than at midday regardless of temperature treatment and period of cold-storage. The midday F_v/F_m decreased with increased stress period and temperature ($P<0.001$) . The depression at midday and the partial recovery in the evening indicated that photoinhibition was reversible in 'Polka'. Moreover, it was observed that F_v/F_m did not fully recover in the morning after consecutive stress periods thus the repair mechanism was not sufficient**.** The diurnal changes; the highest Fv/Fm in the morning, minimum at noon and gradually recovered in late afternoon was observed in soybean plants (KAO and FORSETH 1992). In contrast to our results, the highest effect of heat stress was observed in the afternoon of heat susceptible raspberry cultivars (MOLINA-BRAVO et al. 2011). The significantly stronger depression of F_v/F_m in plants cold-stored for 17 weeks, as compared to 15 and 16 weeks, may imply the operation of a quantitative chilling effect, but this could not be validated by the present study. The F_v/F_m measurement indicates that all five cultivars have an almost similar response with regard to heat tolerance.

Chlorophyll pigments

Elevated temperature regimes affect the total concentration of chlorophyll pigments in leaves depending on the thermotolerance capacity of the species (CAMEJO et al. 2005; GUO et al. 2006; EFEOGLU and TERZIOGLU 2009). The Chl *a/b* ratio is an indicator of the functional

247 pigment equipment and light adaptation/acclimation capacity of the photosynthetic apparatus.
248 Chl *b* is found exclusively in the pigment antenna system, whereas Chl *a* is present in the
249 reaction centres of PSI and PSII as well as in the pigment antenna (GUO et al. 2006). We
250 observed that the Chl *a* and Chl *a/b* decreased significantly at high temperature in 'Autumn
251 Bliss' and 'Fall Gold' compared to greenhouse conditions. The low Chl *a/b* suggests a
252 decrease in the ratio of reaction centres compared to light harvesting proteins while the lower
253 Chl *a* content suggests a decrease in light harvesting capacity (ADAMS and BARKER 1998).
254 The chlorophyll concentration decreased in 'Sutsuma' mandarin, when the temperature was
255 increased to 38 °C for a 15-days stress period (GUO et al. 2006). The measured level of Chl *a*
256 and Chl *a/b* indicate that 'Autumn Bliss' and 'Fall Gold' are less heat tolerant while 'Autumn
257 'Treasure' and 'Erika' were not heat sensitive according to these parameters.

258

259 ***Growth and flowering***

260 Due to their temperate origin, the plasticity of current raspberry cultivars to adapt to high
261 temperature is limited (BALLINGTON and FERNANDEZ 2008). Increased temperature up to
262 24 °C advanced anthesis and increased number of leaves in 'Autumn Bliss' (CAREW et al.
263 2003; HEIDE and SONSTEBY 2011). In our study, the opening date of the terminal flower was
264 not significantly affected by heat stress during flower initiation, presumably because stress
265 was imposed for a short period. However, at high temperature (37 °C), anthesis of the
266 terminal flower in 'Autumn Bliss', 'Fall Gold' and 'Polka' tended to be advanced, while in
267 'Autumn Treasure' and 'Erika', it tended to be delayed. SONSTEBY and HEIDE (2010)
268 observed that flowering and fruit maturation was advanced by elevated temperature from 20
269 to 26 °C in 'Autumn Bliss' but delayed in 'Autumn Treasure' above 20 °C. There were a
270 higher number of lateral shoots in 'Autumn Bliss' in the shorter cold-storage period. The
271 result is surprising because the chilling periods used were very much longer (>15 weeks) than

those suggested by other workers to satisfy the chilling requirement (TAKEDA 1993; CAREW et al. 2001). Chilling is not an absolute requirement of annual-fruiting raspberries but cold treatment advances the day-to-flower opening in many annual cultivars (TAKEDA 1993; HEIDE and SONSTEBY 2011). Others reported that flowering was advanced when chilling duration increased from 0 to 10 weeks in 'Autumn Bliss' (CAREW et al. 2001). As chilling duration increased, the rate of vegetative growth increased and days-to-first flower opening decreased in 'Autumn Bliss. Similarly, cold treatment affected flower bud development. For example, non-chilled 'Heritage' plants developed 15 flowering lateral shoots, while plants receiving >750 chilling units had 25 flowering lateral shoots (TAKEDA 1993). Therefore low temperature exposure also known as vernalization prior to shoot growth is needed for flower bud initiation (TAKEDA 1993; HEIDE and SONSTEBY 2011). Our results are in agreement with CAREW et al. (2001), who reported that cold-storage influences vegetative and flowering behaviour of raspberry cultivars. The differences could also be due to the loss of carbohydrates during cold-storage and differences in climate conditions in the greenhouse, although the temperature was maintained similar for each replication, while light and RH obviously were not fully controlled in greenhouse as compared to climate chambers.

Conclusion

Short exposure of annual-fruiting raspberry cultivars to high temperature decreases midday F_v/F_m and chlorophyll content. A decline in the efficacy of photosystem II under elevated temperature regimes at midday and partial recovery at evening in 'Polka' may indicate coordinated changes in the photosynthetic apparatus and processing that might help plants to survive in heat stress. The results suggest that heat tolerance of annual-fruiting raspberry grown in warm regions could be improved by selecting cultivars with high photochemical activity as manifested by high apparent quantum efficiency (F_v/F_m). Furthermore, as chilling

297 increases, in our case cold-store duration, the rate of vegetative growth increases in 'Autumn
298 Bliss' and 'Fall Gold'. Moreover, heat stress enhances earlier flowering in 'Autumn Bliss'
299 and delayed in 'Autumn Treasure', indicating distinct cultivar differences. In commercial
300 production, this information may be useful for manipulating and optimizing fruit production
301 in glasshouses and outside in warmer regions. Therefore evaluation of raspberry germplasm
302 for cultivation in warmer areas should be performed. However, we suggest that longer
303 exposure than the seven day period and above 37 °C should also be examined to understand
304 the effects of heat stress in detail.

305

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309

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Figure Captions

Fig. 1. The effect of heat stress treatment (days) and preceding cold-storage duration (15, 16 or 17 weeks) on F_v/F_m of five annual-fruiting raspberry cultivars during a seven day period. On day 0, F_v/F_m was measured in the greenhouse before transfer to climate chamber (n = 3). Vertical bars indicate standard error of mean (n = 3).

Fig. 2. The effect of heat stress treatment (days) and preceding cold-storage duration (weeks) on diurnal variation in F_v/F_m in 'Polka' during a seven day stress period. F_v/F_m was not measured for day 0 and 7 in the morning and evening. Vertical bars indicate standard error of mean (n = 3 for midday and n = 9 for morning and evening measurements).

Fig. 3. The effect of heat stress treatment (days) on changes in leaf (A) chlorophyll *a*, (B) chlorophyll *a/b* and (C) Chlorophyll to carotenoid $(a+b)/(x+c)$ in percent of greenhouse conditions (20 °C) in five annual-fruiting raspberry cultivars. Negative and positive values indicate a decrease or increase compared to greenhouse conditions. Vertical bars indicate standard error of mean (n = 9). Mean separation was done using Tukey Kramer's test at $P \leq 0.05$ and different letters within cultivar indicate significant difference.

Fig. 4. The effect of a seven day heat stress treatment on anthesis (days) of the terminal flower (A) and changes in unopened flower buds in percent of greenhouse conditions (B) in five annual-fruiting raspberry cultivars. Negative and positive values indicate a decrease or increase compared to greenhouse conditions. Vertical bars indicate standard error of mean (n = 9). Mean separation was done using Tukey Kramer's test at $P \leq 0.05$ and different letters within cultivar indicate significant difference.

Table 1. Main effects and interactions of cold-storage duration, temperature and days of temperature stress period on quantum efficiency (F_v/F_m) in five annual-fruiting raspberry cultivars using PROC MIXED with stress period as repeated variable.

Treatments	Df	'Autumn Bliss'	'Autumn Treasure'	'Erika'	'Fall Gold'	'Polka'
Cold store duration (C)	2	<0.0001	<0.0001	<0.0001	<0.0001	0.003
Temperature (T)	3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C*T	6	0.031	0.0002	<0.0001	0.003	ns
Stress period (S)	7	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C*S	14	0.005	<0.0001	<0.0001	<0.0001	ns
T*S	21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C*T*S	42	<0.0001	<0.0001	0.007	<0.0001	0.014

Df: degree of freedom; ns: not-significant at P>0.05

Table 2. Effect of a seven day period of elevated temperature during flower initiation on growth and flowering behavior in five annual-fruiting raspberry cultivars.

Temperature	Leaf area (cm^{-2}) †	Unopened axillary buds $plant^{-1}$ ¥	No of lateral shoots $plant^{-1}$ ¥	Flowering lateral shoots $plant^{-1}$ (%)¥	Number of flower and bud $lateral^{-1}$ ¥	Main shoot height (cm)
			'Autumn Bliss'			
Greenhouse (20 °C)	1948 ^{a}	8^{b}	7^{c}	95.0^{ab}	35 ^{a}	145 ^{a}
27 °C	1717 ^{a}	10^{a}	6^{c}	96.8^{a}	37 ^{a}	145 ^{a}
32 °C	1469 ^{a}	7^{bc}	9^{b}	92.7^{ab}	31 ^{a}	136 ^{a}
37 °C	1185 ^{a}	5^{c}	12^{a}	85.7^{b}	29 ^{a}	131 ^{a}
			'Autumn Treasure'			
Greenhouse (20 °C)	3569^{a}	5^{ab}	10 ^{a}	96.0 ^{a}	42 ^{a}	189 ^{a}
27 °C	2741^{ab}	7^{ab}	11 ^{a}	97.1 ^{a}	49 ^{a}	178 ^{a}
32 °C	3566^{a}	9^{a}	10 ^{a}	99.0 ^{a}	54 ^{a}	178 ^{a}
37 °C	1135^{b}	3^{b}	15 ^{a}	100.0 ^{a}	42 ^{a}	138 ^{a}
			'Erika'			
Greenhouse (20 °C)	9455^{a}	8 ^{a}	6^{b}	98.8 ^{a}	84 ^{a}	219 ^{a}
27 °C	8946^{ab}	8 ^{a}	9^{ab}	100.0 ^{a}	60 ^{a}	215 ^{a}
32 °C	6996^{b}	5 ^{a}	9^{ab}	99.0 ^{a}	68 ^{a}	218 ^{a}
37 °C	2498^{c}	5 ^{a}	10^{a}	99.1 ^{a}	56 ^{a}	173 ^{b}
			'Fall Gold'			
Greenhouse (20 °C)	2658^{a}	9^{a}	8^{ab}	86.7 ^{a}	30 ^{a}	150 ^{a}
27 °C	1215^{b}	8^{ab}	7^{b}	78.4 ^{a}	27 ^{a}	130^{ab}
32 °C	1568^{ab}	9^{a}	8^{ab}	88.7 ^{a}	28 ^{a}	139 ^{ab}
37 °C	862^{b}	5^{b}	11^{a}	91.4 ^{a}	28 ^{a}	120 ^{b}
			'Polka'			
Greenhouse (20 °C)	5256^{a}	7^{ab}	7 ^{a}	99.0 ^{a}	52 ^{a}	163 ^{a}
27 °C	3065^{ab}	9^{a}	8 ^{a}	98.8 ^{a}	53 ^{a}	135^{ab}
32 °C	3323^{ab}	9^{a}	6 ^{a}	92.3 ^{a}	55 ^{a}	148 ^{ab}
37 °C	974^{b}	5^{b}	9 ^{a}	98.3 ^{a}	51 ^{a}	117 ^{b}

Different letters within the same column and cultivar indicate significant difference at $P < 0.05$ by Tukey-Kramer test; †: Leaf area of the leaves developed after the temperature stress period; ¥: Data were log transferred prior to statistical analysis but original mean values are shown.

Table 3. Effect of cold-storage period on the growth and flowering behavior in five annual-fruiting raspberry cultivars.

Cold-storage period	Leaf area (cm^{-2}) †	Unopened axillary buds $plant^{-1}$ ¥	No of lateral shoots $plant^{-1}$ ¥	Flowering lateral shoots $plant^{-1}$ (%)¥	Number of flower and bud $lateral^{-1}$ ¥	Main shoot height (cm)
			'Autumn Bliss'			
15 weeks	1171[b]	5[b]	10[a]	87.1[b]	25[b]	133[b]
16 weeks	1274[ab]	9[a]	7[b]	91.4[ab]	32[ab]	126[b]
17 weeks	2294[a]	8[a]	8[b]	99.1[a]	43[a]	158[a]
			'Autumn Treasure'			
15 weeks	2160[a]	8[a]	12[a]	96.4[a]	48[a]	171[a]
16 weeks	2935[a]	5[b]	12[a]	99.3[a]	48[a]	170[a]
17 weeks	3158[a]	5[b]	11[a]	98.4[a]	46[a]	171[a]
			'Erika'			
15 weeks	4878[c]	7[a]	10[a]	100.0[a]	64[a]	196[b]
16 weeks	6735[b]	6[a]	8[a]	98.0[a]	72[a]	208[ab]
17 weeks	9309[a]	6[a]	8[a]	100.0[a]	65[a]	214[a]
			'Fall Gold'			
15 weeks	1034[a]	6[b]	10[a]	80.0[a]	28[ab]	128[b]
16 weeks	1692[a]	9[a]	8[b]	82.0[a]	22[b]	126[b]
17 weeks	2000[a]	10[a]	8[b]	96.0[a]	36[a]	150[a]
			'Polka'			
15 weeks	2211[a]	8[a]	8[a]	97.0[a]	49[a]	131[a]
16 weeks	3860[a]	8[a]	8[a]	99.3[a]	58[a]	146[a]
17 weeks	3392[a]	7[a]	7[a]	95.2[a]	53[a]	147[a]

Different letters within the same column and cultivar indicate significant difference at $P < 0.05$ by Tukey-Kramer test; †: Leaf area of the leaves developed after the temperature stress period; ¥: Data were log transferred prior to statistical analysis but original mean values are shown.

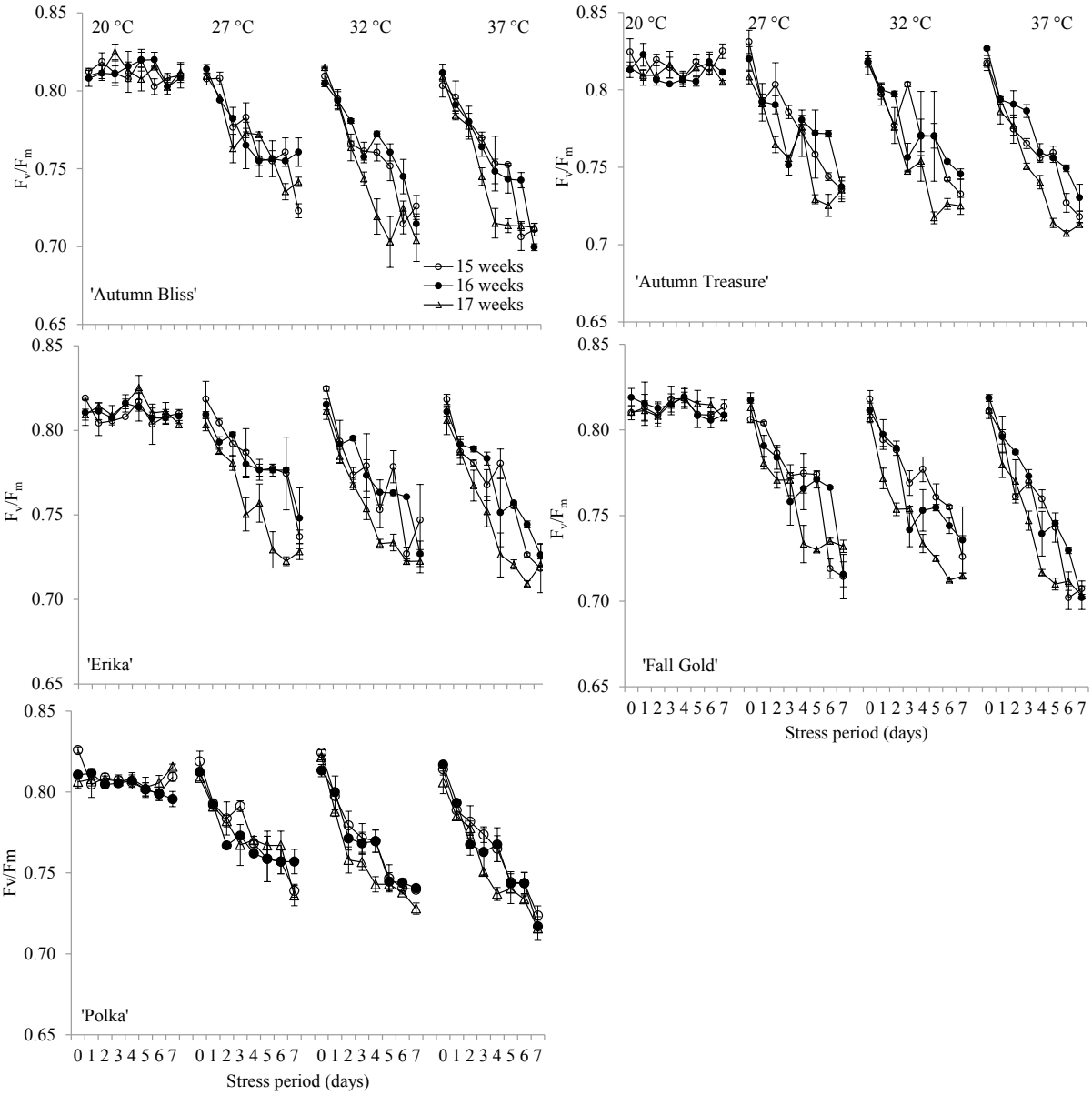
20 °C
27 °C
32 °C
37 °C
15 weeks
16 weeks
17 weeks
'Autumn Bliss'
'Autumn Treasure'
'Erika'
'Fall Gold'
'Polka'
Fv/Fm
Stress period (days)

Figure 1.

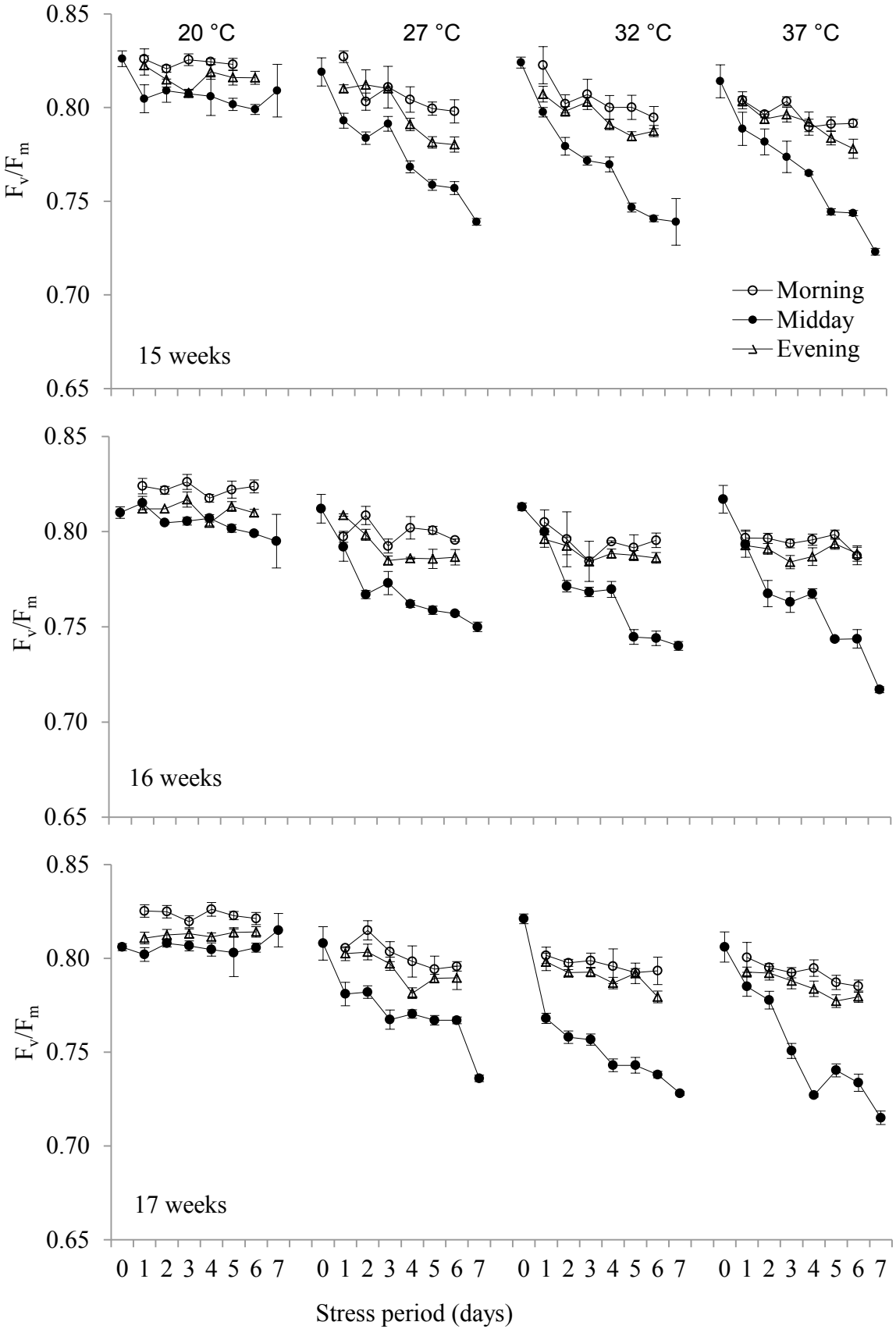
20 °C
27 °C
32 °C
37 °C
Morning
Midday
Evening
15 weeks
16 weeks
17 weeks
F_v/F_m
0.85
0.80
0.75
0.70
0.65
0 1 2 3 4 5 6 7
Stress period (days)

Figure 2.

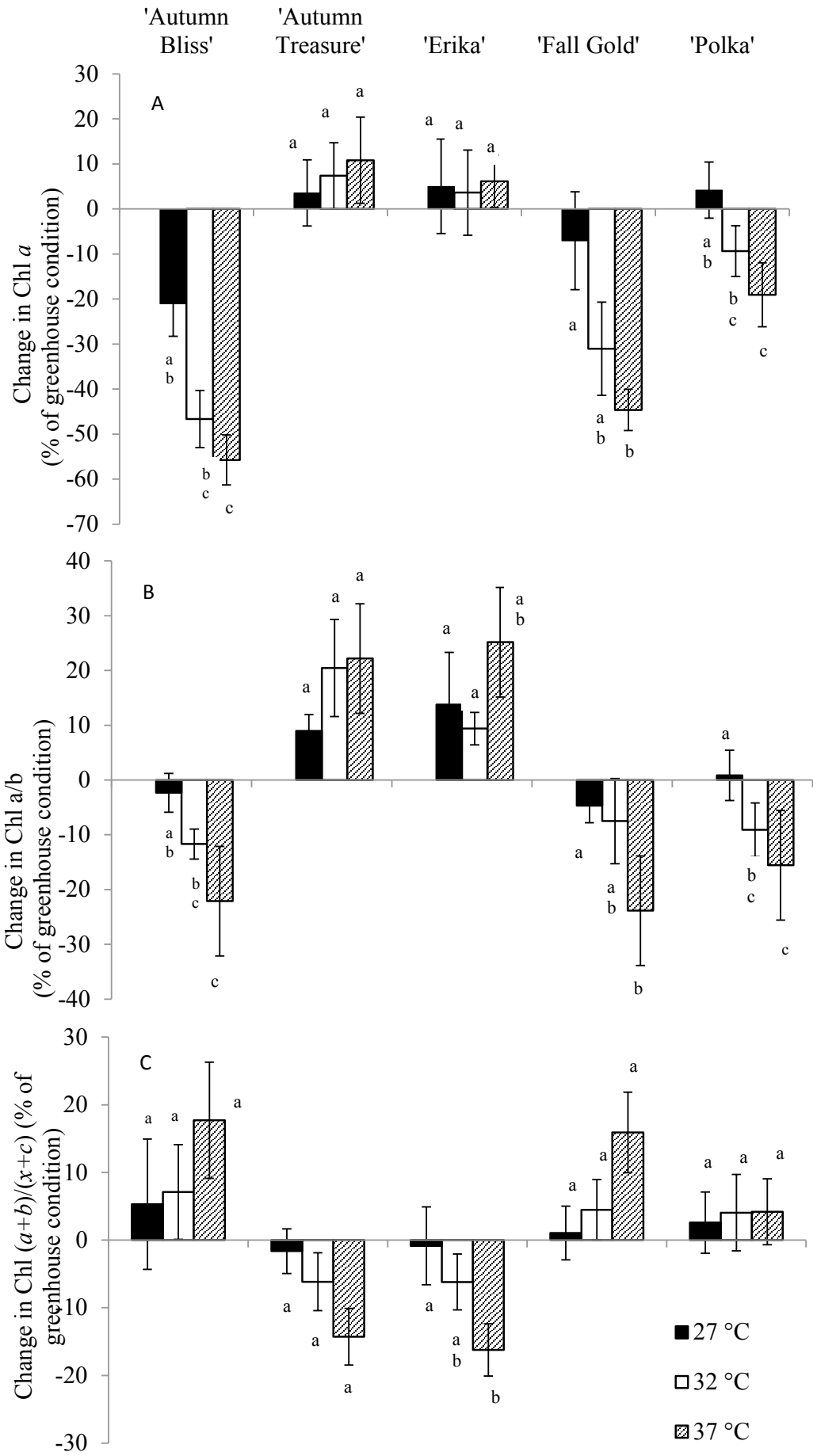
'Autumn Bliss'
'Autumn Treasure'
'Erika'
'Fall Gold'
'Polka'
A
B
C
Change in Chl a (% of greenhouse condition)
Change in Chl a/b (% of greenhouse condition)
Change in Chl (a+b)/(x+c) (% of greenhouse condition)
27 °C
32 °C
37 °C

Figure 3.

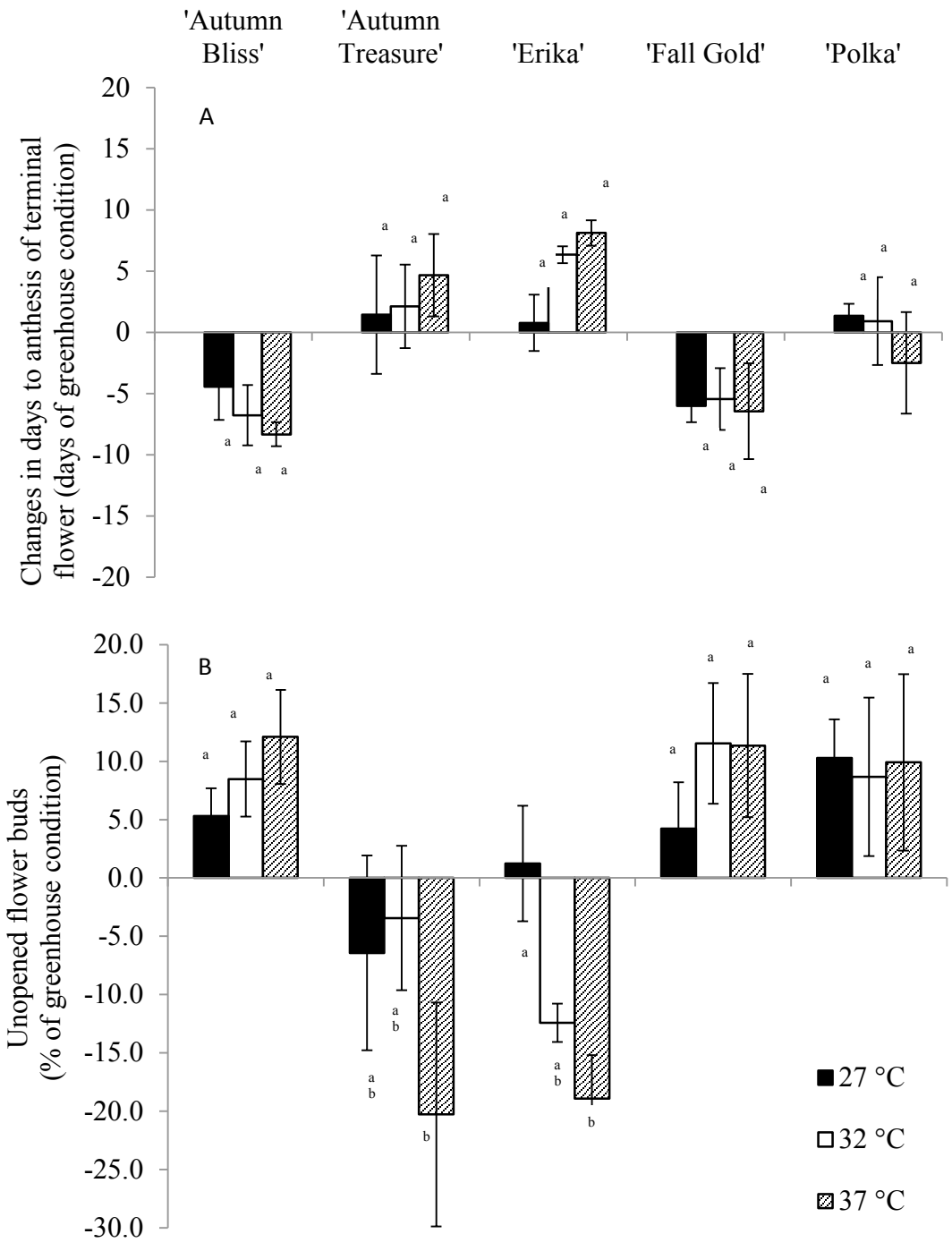
'Autumn Bliss'
'Autumn Treasure'
'Erika'
'Fall Gold'
'Polka'
A
Changes in days to anthesis of terminal flower (days of greenhouse condition)
20
15
10
5
0
-5
-10
-15
-20
B
Unopened flower buds (% of greenhouse condition)
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
27 °C
32 °C
37 °C

Figure 4.