Thermostability of Cell Membranes as a Measure of Heat Tolerance and Relationship to Flowering Delay in Chrysanthemum

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Abstract. Identification of heat-tolerant chrysanthemum [*Dendranthema ×grandifolia* (Ramat.) Kitamura] genotypes for commercial production in hot areas of the world is desirable. The extent to which electrolyte leakage from chrysanthemum leaf discs, measured using a test for cell membrane thermostability (CMT), could be related to the delay in flowering induced by heat in the field-grown plants was determined. The relationship between the relative injury (RI) occurring in leaf tissue discs of chrysanthemum cultivars and treatment temperature was sigmoidal. A single temperature treatment at 50 °C resulted in injury values near the midpoint of the sigmoidal response curve and showed the greatest sensitivity in detecting genotypic differences in heat tolerance. The cultivars with a low RI value are those with the greater CMT and shorter heat-induced delay to flowering.

High temperatures during the growing season can have considerable detrimental effects on plant growth and yield (Hall, 1992). Techniques for rapid and easy identification of heat-tolerant genotypes during breeding programs for sub-tropical and tropical areas are desirable. Cell membrane thermostability (CMT), measured as electrolyte leakage from leaf discs over a range of temperatures, is a sensitive and rapid method to evaluate heat tolerance in plants (Sullivan, 1972; Wu and Wallner, 1993). Several studies have shown the effectiveness of CMT testing in detecting genetic variability for heat tolerance among several agronomic, fruit, and vegetable crops (Chen et al., 1982; Ingram and Buchanan, 1984; Lester, 1985; Martineau et al., 1979; Saadalla et al., 1990; Sullivan and Ross, 1979). Leaf CMT was highly correlated with fruit set and yield under high temperature conditions (Fokar et al., 1998; Martineau et al., 1979; Sullivan and Ross, 1979). However, little information is available on using CMT to indicate heat tolerance of floricultural crops.

In chrysanthemum (*Dendranthema ×grandifolia*), the phenomenon of a heat delay in time to flowering (anthesis) is induced by temperatures of 26 to 32 °C (Cathey, 1954; Cockshull, 1979; Karlsson et al., 1989; Whealy et al., 1987). For chrysanthemums grown during the summer, in greenhouses, or under tropical field conditions, high temperature limits production. Breeding heat-tolerant or heat-delay insensitive genotypes is vital (Anderson and Ascher, 2001). Shibata and Kawata (1987) measured differences in the degree of heat delay among Japanese chrysanthemum cultivars directly by growing the plants to flowering during summer and found that some genotypes may be useful for introducing heat tolerance. Screening for heat tolerance using field evaluation techniques is slow and frequently heat stress conditions during flowering are inconsistent (De Jong, 1989). Because thermotolerance at the seedling and flowering stages is generally well-correlated in other species, for example in wheat (*Triticum aestivum* L.) (Fokar et al., 1998; Saadalla et al., 1990) and cowpea [*Vigna unguiculata* (L.) Walp.] (Ismail and Hall, 1999), preliminary field screening with chrysanthemum could also be successful if carried out with young vegetative plants.

The present study examined heat tolerance in leaves taken from a wide range of young chrysanthemum cultivars, using leaf electrolyte leakage as a measure of CMT and then testing how well this related to the heat-induced delay in flowering of plants grown in the field under natural high temperatures.

Materials and Methods

Stock plants. Rooted chrysanthemum cuttings of the cultivars (Table 1) were planted in 21-cm-diameter plastic pots each containing sphagnum peat (Fafard No. 1, Conrad Fafard Inc., Agawam, Mass.), perlite and vermiculite mixed in equal volumes. After planting, the cuttings were grown in a greenhouse at a mean daily temperature of 27 °C under 11.5 to 12 h natural daylength augmented, to maintain vegetative growth, by a night break from 2200 to 0200 hr using incandescent lamps giving 2.2 μmol·m^{-2}·s^{-1} (PAR, 400 to 700 nm) at plant level. The plants were fertilized weekly with water-soluble 20.0N–4.4P–24.9K (The Scotts Co., Marysville, Ohio) at 300 ppm N. Tips of the shoots were pinched off above the sixth leaf after plants were established to encourage lateral growth.

Cell membrane thermostability. On 5 Nov. 2001, cuttings (6 to 8 cm long) of seven chrysanthemum cultivars (Fig. 1) were obtained from stock plants and were evaluated for CMT following procedures described by Martineau et al. (1979) with minor modifications. CMT was measured at 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 °C.

A sample for assay consisted of a paired set (25 °C control and another treatment) of five 9-mm-diameter leaf disc samples (53.7 ± 0.9 mg fresh weight) cut from a group of five recently fully expanded leaves with a cork borer. Prior to assay, the paired set of leaf discs was placed into two separate 75-mL test tubes and rinsed thoroughly with three rapid changes of distilled water.

Leaf discs were then placed in 25 × 150-mm test tubes containing 1 mL distilled water to prevent secondary water stress. Three tubes per treatment were placed in a heated, circulating water bath for 30 min at each temperature, while the control tubes were maintained at 25 °C for the same period. Distilled water (15 mL) was added to each tube following elevated temperature exposure. Samples were placed on an orbital shaker for 24 h at 10 ± 3 °C, and then solution conductivity was measured with

Table 1. Relative injury (RI) as determined by the cell membrane thermostability test at 25 and 50 °C for 30 min for 21 cultivars of chrysanthemum.

Cultivar	Relative injury (%)		
	25 °C[z]	50 °C[z]	Calibrated[y]
Dance Windmill	14.9 bcd[x]	42.2 g	32.1 g
Yellow Windmill	13.1 cd	43.2 fg	34.6 fg
Chiu-Yang	17.7 a	45.6 fg	34.6 fg
Fen-Yen	13.2 cd	52.3 efg	45.3 efg
Huang Tung-Chien	12.9 cd	57.0 def	50.6 def
Hsin Chung-Huang	12.0 d	62.1 cde	56.8 cde
White Ting-Tzu	12.2 cd	62.1 cde	56.8 cde
Hei Hsiao-Chu	16.9 ab	64.3 bcde	56.9 cde
Huang Ching-Chin	15.2 bc	65.7 bcde	59.6 bcde
Hua Chao Feng	13.1 cd	66.4 bcde	61.2 bcde
Chun Chih-Wan	13.1 cd	66.5 bcde	61.4 bcde
Pai Tung-Yang	13.4 cd	66.9 bcde	61.7 bcde
Pai Ting-Er	13.8 cd	67.1 bcde	61.8 bcde
Hung Kuei-Fei	12.0 d	69.3 abcd	65.2 abcd
Yellow Ting-Tzu	13.0 cd	69.5 abcd	64.9 abcd
Remix	12.9 cd	71.7 abcd	67.5 abcd
Hung-Yen	15.0 bc	73.3 abc	68.7 abc
Shih Wan-Hung	14.3 bcd	75.9 abc	71.9 abc
Chu-Feng	12.5 cd	77.2 abc	73.8 abc
Daymark	14.9 bcd	78.7 ab	75.0 ab
Yen Chih-Hung	14.0 cd	83.6 a	81.0 a

[z]Calculated as (initial conductivity/final conductivity) × 100.
[y]Calibrated RI = {1 − [1 − (T1/T2)]/[1 − (C1/C2)]} × 100.
[x]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

a conductivity/TDS meter (model 44600; Hach Co., Loveland, Colo.). The tubes then were capped with foil, autoclaved (121 °C, 1.2 kg·cm⁻²) for 15 min, cooled to 25 °C, and incubated an additional 24 h before final conductivity measurements were taken. Relative injury (RI) values at 25 and 50 °C were calculated as (initial conductivity/final conductivity) × 100. The calibrated RI was calculated as follows: RI (%) = {1− [1 − (T1/T2)]/[1 − (C1/C2)]} × 100, where T and C refer to conductance values for treatment and control vials, respectively, and subscripts 1 and 2 refer to initial and final conductance readings, respectively.

Twenty-one chrysanthemum cultivars were used to measure the initial conductivity of electrolytes leaking from leaf discs
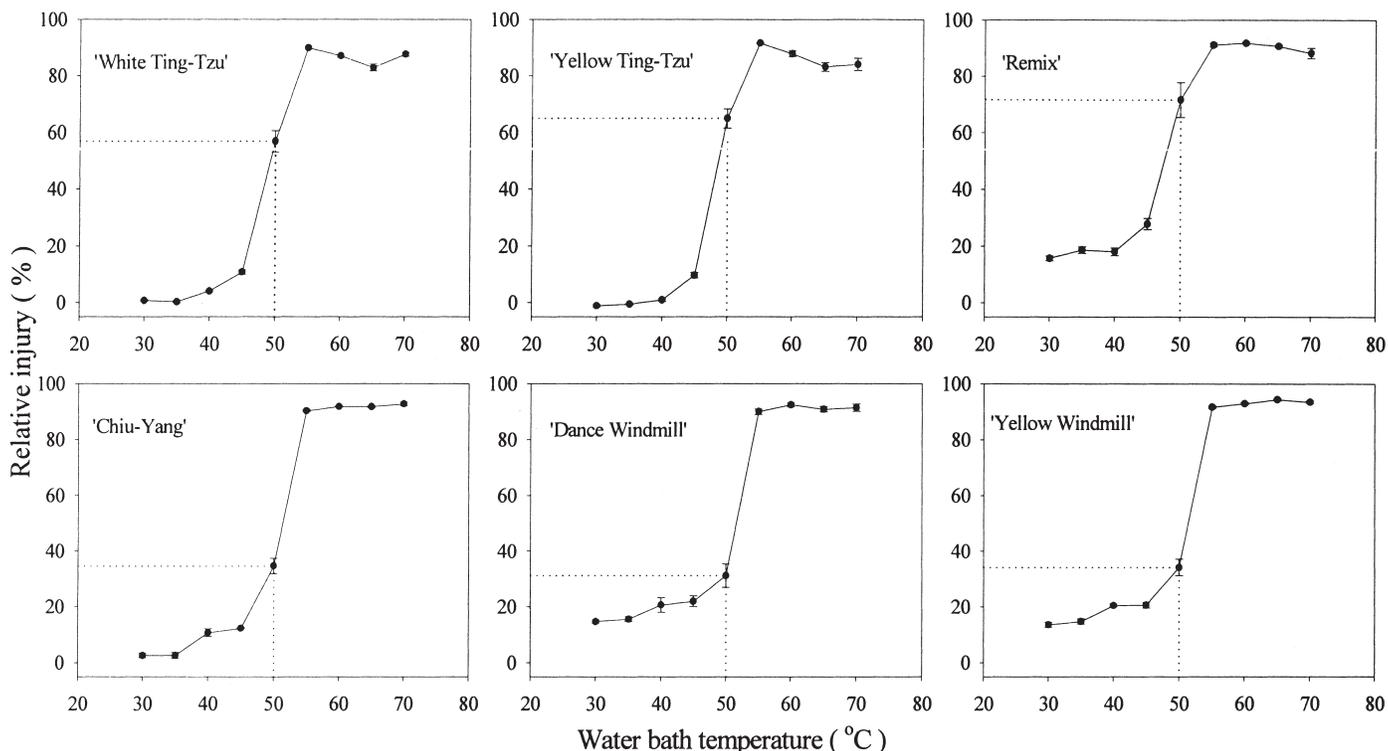

Fig. 1. Effect of water bath temperature on relative injury of chrysanthemum leaf discs. Bars represent standard error of the mean and are not visible if smaller than the symbol.

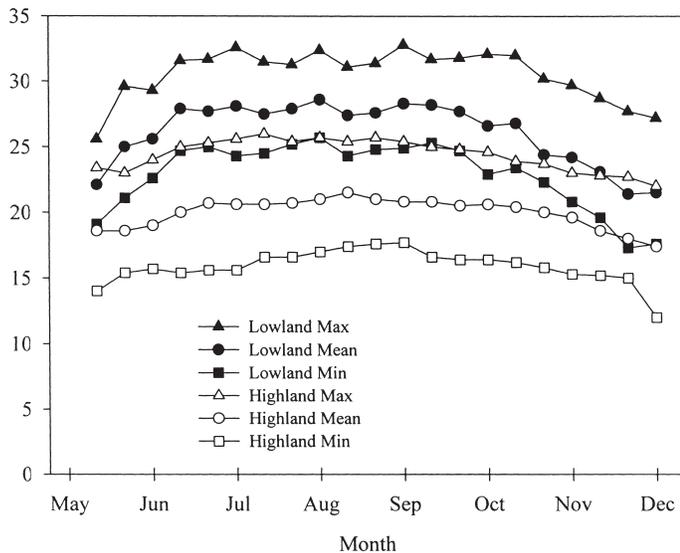J. Amer. Soc. Hort. Sci. 128(5):656–660. 2003.

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Fig. 2. The maximum, mean and minimum daily temperatures during the experimental period under lowland and highland conditions.

at 25 °C (control) and 50 °C for 30 min. Test tubes containing samples were autoclaved at 121 °C for 15 min and final solution conductivity was measured again at 25 °C.

FIELD EVALUATION. Rooted cuttings of seven chrysanthemum cultivars were obtained from the stock plants and were planted each month from May to August at Taichung District Agriculture Improvement Station (altitude 50 m, lowland) and at Chun-Yang (altitude 1100 m, highland) under natural field conditions. The thermal environment at the lowland location is typical for commercial chrysanthemum production in Taiwan, where heat stress is frequently considered a limiting factor for summer production.

On each planting date, four plants for each cultivar were set in plots consisting of four rows spaced at 10×12 cm within and between rows, respectively. Daylengths during the experimental period at each location were similar, ranging from 12 to 14.5 h. Air temperature and radiation were measured at the top of the plant canopy and logged every 30 min using screened thermocouples and pyranometer sensors (LI-200SZ, LI-COR, Lincoln, Nebr.) attached to a datalogger (LI-200SZ, LI-COR). The daily cumulative radiation received by the plants ranged between 2 and 4 MJ·m^{-2}·d^{-1}. Days from planting to flowering (first row of disc florets fully open in the terminal inflorescence) was recorded for each plant. Each plant was terminated at flowering or 150 d after planting if the terminal apex was still vegetative. Costat programming (CoHort Software, Berkeley, Calif.) was used for statistical analysis.

RELATION BETWEEN CMT AND HEAT DELAY. The degree of heat delay was calculated following the method of Shibata and Kawata (1987) as the difference between days to flowering in the seven cultivars planted at the lowland site in June and July, and July and August, respectively. Regression analysis was used to determine the relationship between the cultivars' calibrated RI value and their associated degree of heat delay.

Results

CELL MEMBRANE THERMOSTABILITY. The relationship between the RI value of leaf tissue discs of six chrysanthemum cultivars and treatment temperature was sigmoidal (Fig. 1). In the 50 °C treatment, the RI values were near the center of the inflection points of the response curves and showed the greatest genotypic differences in CMT. For example, the 50 °C treatment resulted in 65% and 68% RI in 'Yellow Ting-Tzu' and 'Remix', but only 32% and 35% in 'Chiu-Yang', 'Dance Windmill', and 'Yellow Windmill' respectively. This suggests that differences in heat tolerance of leaf tissues exist between chrysanthemum cultivars which are detectable by CMT.

The RI values of 21 cultivars at 25 °C differed, but showed a very narrow range (Table 1). Genotypic rankings for CMT were similar by both 50 °C or calibrated 50 °C calculations, where RI values varied markedly from 32% to 84%. Clearly, a single heat treatment at 50 °C can be used to screen large numbers of chrysanthemum cultivars.

FIELD EVALUATION. Two locations were chosen to measure the flowering time of chrysanthemums: one in the lowland and one in the highland. Temperatures differed markedly between the two sites as shown in Fig. 2. Temperature increased from May, was consistent between June and October, and then decreased in December when the experiment ended. From June to October, the maximum, mean and minimum temperatures were 32, 28, and 24 °C, respectively, at the lowland, and 25, 19, and 16 °C, respectively, at the highland.

Time from planting to flowering differed among cultivars and planting dates at the highland location (Table 2). The cultivars Huang-Yen, Daymark and Yen Chih-Hung consistently took longer to flowering (87 to 95 d), compared with other cultivars (63 to 78 d) planted in May or June. Plantings in July or August (mean temperature of 20 °C), flowered faster than those in May.

Plants grown at the lowland location were slower to flower than those grown on the highland, even when planted on the same date (Table 3). However, cultivars Fen-Yen and Huang Tung-Chien flowered after the same time period regardless of planting date between May and August when temperatures were high, suggesting that these are heat-delay insensitive chrysanthemums. The cultivars Huang-Yen, Daymark and Yen Chih-Hung on the other hand appeared to be heat-delay sensitive. First, these cultivars

Table 2. Days between planting and flowering of chrysanthemums under highland field conditions.

Cultivar	Planting month			
	May	June	July	August
Fen-Yen	74.2 cA[z]	64.3 bAB	52.8 cB	50.4 cB
Huang Tung-Chien	60.8 dA	63.7 bA	59.5 bA	50.3 cA
Huang Ching-Chin	78.2 bcA	64.3 bB	61.3 bB	59.4 cB
Pai Ting-Er	74.4 cA	68.4 bA	64.2 bB	66.2 bB
Huang-Yen	87.6 abA	94.2 aA	78.2 aB	78.3 aB
Daymark	94.4 aA	94.7 aA	78.1 aB	59.3 bcC
Yen Chih-Hung	94.5 aA	94.5 aA	78.6 aB	65.3 bC

[z]Mean separation within columns (lowercase) and rows (uppercase) by Duncan's multiple range test at $P \leq 0.05$.

Table 3. Days between planting and flowering of chrysanthemums under lowland field conditions.

| | Planting month | | | |
Cultivar	May	June	July	August
Fen-Yen	80.3 aA[z]	77.6 dA	68.4 cA	72.8 bcA
Huang Tung-Chien	80.2 aA	77.1 dA	68.3 cA	72.3 bcA
Huang Ching-Chin	80.1 aA	85.9 cA	68.7 cB	65.4 cB
Pai Ting-Er	84.3 aB	105.7 bA	82.6 bB	79.8 bB
Huang-Yen	---[y]	147.2 aA	124.1 aB	93.2 aC
Daymark	---	147.2 aA	117.4 aB	79.3 bC
Yen Chih-Hung	---	147.6 aA	117.6 aB	93.6 aC

[z]Mean separation within columns (lowercase) and rows (uppercase) by Duncan's multiple range test at $P \leq 0.05$.
[y]Denotes that plants did not flower within 150 d.

remained vegetative for 150 d after planting in May, when the mean temperature was 28 °C. Second, plants planted in June or July experienced high temperature for a longer time and were delayed in flowering more than those planted in August. Third, these heat sensitive cultivars did not flower until November or December, requiring ≈50% more days to flower than the heat insensitive cultivars planted in June or July.

RELATION BETWEEN CMT AND HEAT DELAY. The heat delay sensitive cultivars had higher RI values (Table 1) and a greater degree of heat delay (Table 3). Regressing the delay in days to flowering versus the associated, calibrated RI values of seven cultivars grown in the lowland showed good linear relationships ($P \leq 0.01$) for both planting in June and July ($R^2 = 0.93$), and July and August ($R^2 = 0.74$), respectively (Fig. 3).
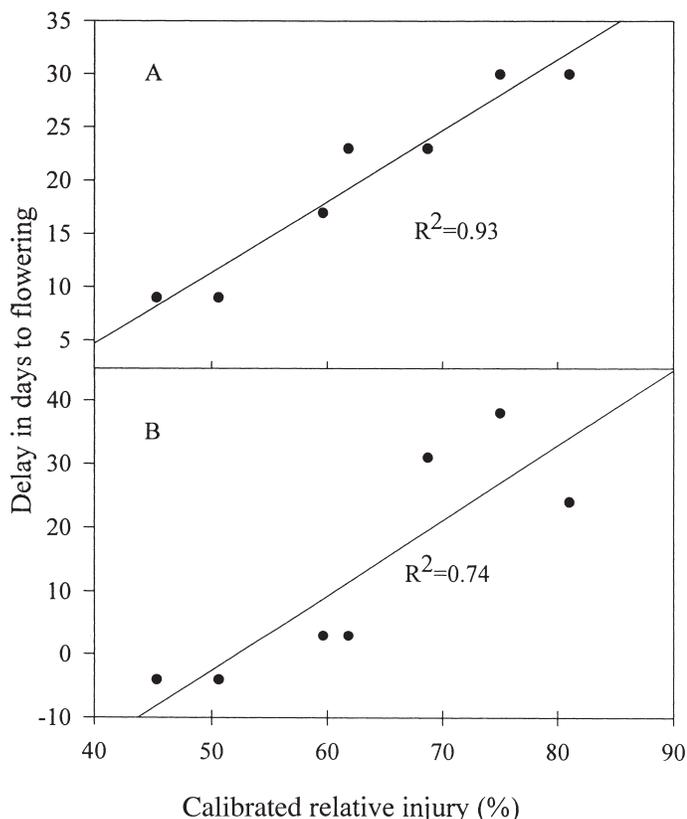

Fig. 3. Relationship between flowering delay and calibrated relative injury of chrysanthemum cultivars grown under lowland conditions. The degree of heat delay was calculated as the difference between days to flowering in plants planted in June and July (**A**), and July and August (**B**), respectively.

Discussion

The electrolyte leakage test is one of the most convenient methods of screening crops for heat tolerance (Lester, 1985; Martineau et al., 1979; Sullivan, 1972). The present study with chrysanthemum showed electrolyte leakage from leaf discs to have a sigmoidal response to increasing temperature (Fig. 1). Similar response curves have been reported for agronomic crops (Chen et al., 1982; Ismail and Hall, 1999; Martineau et al., 1979; Sullivan, 1972), fruit crops (Ahrens and Ingram, 1988; Ingram and Buchanan, 1984), and vegetable crops (Inaba and Crandall, 1988; Lester, 1985).

High temperature tolerance of plants varies with genotype (Ingram and Buchanan, 1984; Lester, 1985) but to derive injury response curves as shown in Fig. 1 for many individual plants would be tedious. From a practical perspective, it is easier to set up a single temperature bath than to set up many at different temperatures. A single temperature treatment at 50 °C resulted in injury values near the midpoint of the sigmoidal response curve and showed the greatest sensitivity in detecting genotypic differences in heat tolerance (Table 1). A treatment temperature around 50 °C has been successfully used to screen for heat tolerance in pepper (*Capsicum annuum* L.) (Anderson et al., 1990), groundnut (*Arachis hypogaea* L.) (Chauhan and Senboku, 1996), soybeans [*Glycine max* (L.) Merr.] (Martineau et al., 1979), and winter wheat (Saadalla et al., 1990). The present study showed that a temperature of 50 °C can be used to screen chrysanthemum cultivars with confidence. Furthermore, consistent genotypic differences in electrolyte leakage were observed in samples taken from plants grown in the field indicating that the technique is appropriate for screening plants from conventional field breeding programs.

Seven cultivars of chrysanthemum appeared to be day-neutral or quantitative short day plants as they flowered under summer long day conditions on the highland (Table 2). However, three of these cultivars (Huang-Yen, Daymark and Yen Chih-Hung) grown on the lowland site either remained vegetative or were delayed in flowering (Fig. 2, Table 3). Delays in time to anthesis of chrysanthemums by temperatures of 26 to 32 °C have been reported previously (Cockshull, 1979; Karlsson et al., 1989; Whealy et al., 1987). Two cultivars, Fen-Yen and Huang Tung-Chien, flowered within 80 d after planting in hot and long day conditions (Fig. 2; Tables 2 and 3), indicating their potential use for the introduction of heat tolerance for flowering to new cultivars.

Positive linear correlations existed between leaf electrolyte leakage and the degree of heat-induced flowering delay in chrysanthemum (Fig. 3). This is consistent with thermotolerance being correlated between the seedling and flowering stages among spring wheat cultivars (Fokar et al., 1998), winter wheat genotypes

(Saadalla et al., 1990), and cowpea (Ismail and Hall, 1999). This technique is simple, quick, and less expensive than a whole plant screen. Potentially, breeders could use the technique with early vegetative-stage leaf tissue from plants grown in field.

If cellular processes could be identified that were involved both in CMT and in heat-induced delay in flowering of chrysanthemum then not only would the physiology of both be clearer but more ways to manipulate flowering might be suggested. Photosynthetic capacity is a likely candidate. Flowering is known to be delayed by environmental conditions that reduce assimilate supply (Dinar and Rudich, 1985; Kinet, 1993). More specifically, exposure of cells to heat disrupts the membrane lipid bilayer which can impact photosynthetic activity (Raison et al., 1980). It would be of interest to examine possible relationships between heat-induced changes in membrane structure, rate of flowering and carbon assimilation.

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