EFFECTS OF LIGHT AND TEMPERATURE ON PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES IN HYBRID GERANIUM AND MARIGOLD

By

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ABSTRACT

EFFECTS OF LIGHT AND TEMPERATURE ON PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES IN HYBRID GERANIUM AND MARIGOLD

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Time to visible flower bud (<0.5 cm diameter) in hybrid geraniums was negatively correlated to light intensity as measured by quantum flux density (QFD) in the 400-700 nm range at a given temperature. Time required from visible bud to flower anthesis was negatively correlated with temperature while light had little effect. Leaf thickness, number of palisade layers, and specific leaf weight each were negatively correlated with temperature. Specific leaf weight was positively correlated with QFD. Net photosynthetic rate (PN) ranged from 5-38 mg CO_2 $dm^{-2}hr^{-1}$ for temperatures of 10-37^{o}C and optimum PN was obtained at 20-32^{o}C. The Q_{10} for respiration for mature hybrid geranium leaves was approximately 2.2. Flowering was accelerated with high temperature (32-35^{o}C) and high light (350-800 μE $m^{-2}s^{-1}$) treatments applied for 9 days to 3-5 week old plants, however peduncle length and flower number were decreased.

Flowering time, dry weight, total leaf area, and vegetative height of marigolds based on QFD (50-600 μE $m^{-2}s^{-1}$), and day and night temperature (10-32^{o}C) were determined through response surface techniques. Predicted total flowering time ranged from 20-70 days and was temperature dependent, however time to visible bud was light dependent at high QFD (400-600 μE $m^{-2}s^{-1}$) and low night temperatures (10-15^{o}C) but was temperature dependent at all levels of QFD at 26^{o}C night temperature. Time from visible bud to flower ranged from 12-30 days and was temperature dependent at 10°C night temperature but light dependent at low levels of QFD (50-300 μE $m^{-2}s^{-1}$). Dry weight ranged from 0.1-2.1 g and maximum dry weight occurred at high QFD (400-600 μE $m^{-2}s^{-1}$), high day temperature (22-30°C) and low night temperature (10-15°C). Vegetative height was greatest (14 cm) at high day temperature (30°C) and low night temperature (10-15°C) while maximum leaf surface area (400 cm^2) occurred only at 10°C night temperature and high day temperature (25-30°C).

Total foliar anthocyanin was negatively correlated to cumulative temperature and QFD. Conditions for maximum chlorophyll (7.0 mg dm^{-2}) were high QFD (500-600 μE $m^{-2}s^{-1}$) and low day temperature (10-15°C) and 10-15°C night temperature but at 26°C night temperature high QFD and high day temperature (28-30°C) were necessary for maximum chlorophyll production. There was no correlation either between anthocyanin and chlorophyll content or between foliar phosphorus or potassium and anthocyanin in marigold leaves.

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Guidance Committee:

The journal-article format was adopted for this dissertation in accordance with departmental and university requirements. Sections I-III were prepared and styled for publication in the _Journal of the American Society of Horticultural Science_. Section IV was prepared and styled for publication in _HortScience_.

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INTRODUCTION

The greenhouse environment is more amenable to environmental control than any other facet of horticulture. Night and day temperature may be routinely set by growers and incoming light may be decreased through shading practices or increased by supplemental light when necessary. Sophisticated equipment exists in greenhouses today to allow for precise environmental control, however optimum light-temperature regimes for various plant processes may be different. The manipulation of light and temperature to control flowering and growth is a necessary part of a growing strategy if the greenhouse operator is to be efficient in growing of the plant as well as in the market-place.

Light and temperature cannot be considered alone in dealing with plant responses. Their interaction results in changes in reproductive and morphological responses which will certainly affect the flowering and vegetative habit and in turn the overall appearance of the plant.

Temperature and light play a major role in physiological responses such as flowering, growth and pigment formation in bedding plants and optimum light-temperature regimes may be predicted which will give optimum plant responses. Hybrid geraniums have recently become a stable part of the bedding plant industry and marigolds continue to occupy a significant portion of the bedding plant market. These plants were studied due to their popularity as well as the relative lack of physiological information available for these crops.

The objectives of this research were (a) to investigate the effects of light and temperature on flowering, leaf structure, and physiological process in hybrid geraniums, (b) to attempt to accelerate flowering time in hybrid geraniums with heat and light treatments during the seedling stage and (c) to determine light and day-night temperature combinations to optimize reproductive and morphological processes in marigolds.

Developing a better understanding of the effects of light-temperature interactions on floral crops will aid in attaining a more efficient and knowledgeable floricultural industry.

SECTION I

THE EFFECT OF TEMPERATURE AND QUANTUM FLUX DENSITY ON THE MORPHOLOGY, PHYSIOLOGY, AND FLOWERING OF HYBRID GERANIUMS

THE EFFECT OF TEMPERATURE AND QUANTUM FLUX DENSITY ON THE MORPHOLOGY, PHYSIOLOGY, AND FLOWERING OF HYBRID GERANIUMS

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Additional Index Words: leaf thickness, leaf mesophyll, photosynthetic rate, Q_{10}, specific leaf weight

Abstract: Hybrid geraniums (*Pelargonium* x *hortorum* Bailey) 'Sooner Red' were grown at temperatures ranging from 10°C - 32°C and at various quantum flux densities. The time to visible bud stage (<0.5 cm diameter) was negatively correlated to quantum flux density at a given temperature. The time required from visible bud to flower stage was negatively correlated with temperature while light had no effect. Leaf thickness, number of palisade layers and specific leaf weight were negatively correlated with temperature and specific leaf weight was positively correlated with quantum flux density at a given temperature. Net photosynthetic rate (PN) ranged from 5-38 mg CO_2 $dm^{-2}hr^{-1}$ for temperatures of 10-37°C and optimum PN was obtained at 20-32°C. The Q_{10} of respiration for hybrid geranium leaves was approximately 2.2.

Flowering time in hybrid geraniums has been shown to be directly related to quantum flux density (QFD) (6,7,9,23) in the 400-700 nm range and is measured as the time from seeding to first flower anthesis (1,21,32). Heins (17) demonstrated visible bud to flower delay at low temperatures and the effect of low temperature on delaying hybrid geraniums flowering has been reported (21). However separation of effects of light and temperature at various stages of flowering has not been reported. Friend (13) and Schull (28) indicated that environment had a marked effect on leaf thickness and surface area, however little information on temperature effects on leaf morphology in ornamental crops is available. Temperature effects have been studied in relation to leaf respiration (3,12,30,34) and photosynthetic behavior (4,29,31) in many crops but little has been done with hybrid geraniums. This study evaluates temperature and QFD effects on flowering stages, leaf morphology, specific leaf weight, respiration and photosynthetic rate of hybrid geranium.

Materials and Methods

To determine if stage of flowering was independent of temperature and QFD, two easily recognizable stages of flowering were chosen: 1) visible bud stage; bud <0.5 cm in diameter and 2) first flower anthesis. Geraniums were grown under 18 hr/day of 0, 10, 30, 50, 75, 100 or 375 $\mu E\ m^{-2}s^{-1}$, constant temperature of 21 ± $2^{o}C$, and RH of 40-60% in controlled environment chambers. Light sources were cool white fluorescent tubes. Plants were watered when necessary with a water soluble fertilizer at 200 ppm N of 20N - 8.7 P - 16.7 K. The time to reach visible bud from seeding and the time from visible bud to flower were determined at each light level. Specific leaf weight was determined by measuring the area of mature leaves from plants grown under each light level with a Lambda leaf area meter (LI-3000), immediately immersing the leaves in liquid N_2, freeze drying them, and measuring dry weight. The effect of temperature on flowering stage was determined by transferring geraniums of similar physiological age from greenhouse conditions (21^{o} ± $5^{o}C$ daylight) at the visible bud stage into growth chambers at 10, 15, 21, 26 or 32 ± $2^{o}C$ temperatures. QFD was either

150 or 375 µE $m^{-2}s^{-1}$ for 18 hr/day. The number of days to flower, number of flowers per inflorescence, and the diameter of flowers were recorded. Specific leaf weight was determined for each temperature regime.

Leaf thickness and anatomy at different temperatures was determined from leaf discs of newly exposed and mature leaves of 10 week old plants grown at 10, 15, 21, 26 or 32° ± 2°C. Leaves were exposed to 150 ± 20µE $m^{-2}s^{-1}$ QFD. The discs were immediately fixed in FAA (50% ethyl alcohol, 10% formaldehyde, 5% glacial acetic acid, and 35% distilled water), dehydrated with graduated ethanol series to tertiary butyl alcohol, and infiltrated with paraplast (18,19). Ten µm sections were cut on a microtome mounted on a glass slide with Weaver's fixer and stained with Safranin-Fast Green. Total leaf x-section thickness, number of palisade layers and palisade and mesophyll thickness were determined using a Wild microscope (125X) fitted with an eyepiece micrometer.

Mature geraniums grown under fall and winter light at a constant 21° ± 5°C temperature were used to determine leaf photosynthetic rate (Pn), (mg CO_2 $dm^{-2}hr^{-1}$) at temperatures ranging from 10-37°C. Pn was determined using an open gas analysis system as described by Sams and Flore (26). Environmental conditions within leaf chambers were ±1°C, and 346-358 ppm CO_2 and relative humidity of 40-70%. Flux density was varied by using neutral density filters and CO_2 concentration was measured with a Beckman 865 differential CO_2 analyzer. Leaf area was determined with a Lambda leaf area meter immediately upon removal of the leaf from the chamber.

Respiration rates of 10, 20 and 27°C were determined using excised leaves from plants grown under greenhouse conditions. Leaf petioles were placed in distilled water in 10 ml tubes and placed in 250 ml jars. A constant flow of air through the jars was maintained. CO_2 concentration was measured by gas chromatography every 3 hours until CO_2 concentrations stabilized (9-12 hrs). Respiration rates (mg CO_2 $dm^{-2}hr^{-1}$) were calculated and Q_{10} determined.

Results and Discussion

Flower Development: The time to visible bud stage (VB) appears to be dependent on QFD at the temperatures tested. There were no significant differences in the time required between VB to flower if enough light was present to allow bud development (Table 1). However, the VB to flower stage is highly dependent on temperature (Fig. 1) regardless of QFD's employed in this study. Temperature decreases of 32-15° increased time of VB to flower in a nearly linear fashion, however there was greater increase in time from VB to flower between 15 and 10 compared with other adjacent temperature treatments, resulting in a quadratic response. Numerous theories have been proposed dealing with flower induction (11,35) and a great deal more is known about the action of an inductive control than about its production or chemical nature. Flowering theory has dealt at length with photoperiodic effects on LD or SD plants and although geranium is thought to be strictly day neutral (10,24), no work has been done to determine if different flowering stages exhibit independent photoperiodic tendencies. The effect of photosynthesis on flower induction has not been well dealt with and has been dismissed by some researchers (14,27), however Purohit and Tregunna (25) showed that continuous CO_2 at high light level induced flowering of Silene under SD conditions, presumably as a result of increased photosynthesis. The light levels which accelerated visible bud to flower time (10-375 $\mu E\ m^{-2}s^{-1}$) also caused the steepest slope of the photosynthetic curves (Fig. 6). The implication is that increased photosynthetic rate may be a controlling mechanism to start the inductive process, perhaps by rapidly expanding the photosynthate pool. Once initiation is completed, flower development is more dependent on temperature than on light. The coldest temperature (10^oC) resulted in flower diameter equal to that of 26^o, however the greatest diameter occurred at 15^o and decreased as temperatures were raised to 32^o regardless of QFD (Fig. 2). The number of flowers per inflorescence decreased significantly at 32^o compared with other temperatures regardless of QFD (Table 2).

Leaf Thickness and Specific Leaf Weight: Leaf thickness, palisade thickness, number of palisade layers and ratio of palisade to total thickness decreased with increasing temperatures while the ratio of mesophyll to total thickness increased (Fig. 3, 4a-d). Leaf thickness may be an important factor in protection from photo-destruction of photosynthetic pigments (5) under saturating light conditions. Some cool growing temperatures during high light periods may be beneficial to maintain chlorophyll stability.

Plants grown at 32^oC died before they had produced enough new growth for sampling purposes. Any new leaves were totally chlorotic indicating a thermal breakdown of chlorophyll. Specific leaf weight (SLW) was correlated with light intensity (Fig. 5) at a given temperature as has been previously reported (2,4). However, as temperature decreases, SLW increases (Fig. 6) and care must be exercised to compensate for temperature effects when using SLW as a predictive tool of light responses. Attempts were made to minimize shading of mature leaves by excising new growth, however the rather low R^2 (Fig. 5) may be partly due to differences in light.

Photosynthetic Efficiency and Q_{10}: Temperatures of 15-32^o had little effect on photosynthetic curves (Fig. 7a-c), as has been found with other crops (13,15,33) however, temperature extremes (10 and 37) resulted in lower saturation points while compensation points decreased with decreasing temperatures (Table 3). We suggest from the data that plants growing in temperature ranges from 20-32°C more fully utilize light energy compared with those grown outside this range. The curves of PN appear to be well fitted to both the natural log as well as the second order polynomial of quantum flux density (Table 4). Transfer of leaves from low to high light or vice versa can have a significant effect on apparent photosynthesis, compensation point and saturation point (8,16,20). The leaves of the plants with which we worked were predisposed to PN rates determined by fall and winter light conditions. The PN values we obtained may be different from those which would have resulted had plants been grown at the QFD's tested.

The Q_{10} of geraniums is approximately 2.2 over the range of temperatures tested (Table 5), thus hybrid geraniums fall within the Q_{10} range associated with most plants at temperatures of 10-30° (12,22). Prolonged exposure to temperatures of 32° however, caused death of the plant within 3-4 weeks. This is due to the lack of significant change in PN rates with temperature while respiration rates were approximately doubling resulting in markedly reduced daily net gain of photosynthate. Thermal breakdown of chlorophyll at high temperatures also contributed to the plants demise.

Some practical implications of this study are as follows:

1) Flowering has been shown to have at least two nearly independent stages and growers should concentrate their effort on maximizing light before buds are visible. Temperature control then becomes most important in regulating crop time.
2) Lowering growing temperatures below 15° increases time from VB to flower stage and decreases flower diameter although flower number is not

adversely affected.

3) Higher growing temperatures result in thinner leaves, higher respiration rates and lower SLW. Extremes of temperature results in lower net photosynthetic rates thus decreasing dry matter production and consequently growth.

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Table 1. The effect of quantum flux density on the flowering of hybrid geranium 'Sooner Red'.[z]

Light ($\mu E\ m^{-2}s^{-1}$)	Seed to bud (days)	Bud to flower (days)
0	died	-
10	no growth[x]	-
30	growth, no bud	-
50	87 a[y]	20 a
75	76 b	20 a
100	67 c	19 a
375	58 d	18 a

---

[z] 21-23°C

[y] Mean separation within columns by HSD (.05).

[x] As measured by gain in fresh weight.

Table 2. The effect of temperature on the number of flowers per inflorescence at two different quantum flux densities.

Temperature	Quantum flux density ($\mu E\ m^{-2}s^{-1}$) 125-150	350-400
10	40.0 a[z]	41.4 a
15	47.0 a	49.4 a
20	44.0 a	47.4 a
25	45.6 a	43.0 a
32	26.6 b	21.2 b
Significance		
QFD ns		
Temperature **		

[z] Mean separation in columns by HSD (.05).

Table 3. The effect of temperature on light compensation and light saturation of hybrid geranium 'Sooner Red'.

	Temperature (C)					
	10	15	20	25	32	37
Compensation[x] point	25	38	46	68	75	71
Saturation[y] point*	700	700	900	1000	1100	700

*$\mu E\ m^{-2} s^{-1}$

[x] Calculated using linear portion of photosynthetic curves.

[y] Estimation based on upper region of photosynthetic curves.

Table 4. Percent of variation (R^2) of PN at various temperatures accounted for by two functions of quantum flux density.

Temperature	Function $b_0 + b_1 X$	 $b_0 + b_1X + b_{11}X^2$
10	87	94
15	91	92
20	86	88
25	97	97
32	98	99
37	90	94

X = Quantum flux density

Table 5. The effect of leaf temperature on dark respiration of hybrid geranium 'Sooner Red'.

Temperature (°C)	Respiration rate (mg CO_2 $dm^{-2}hr^{-1}$)	Q_{10}
10	2.71	
20	6.22	2.3
27	9.04	2.1*

* extrapolated

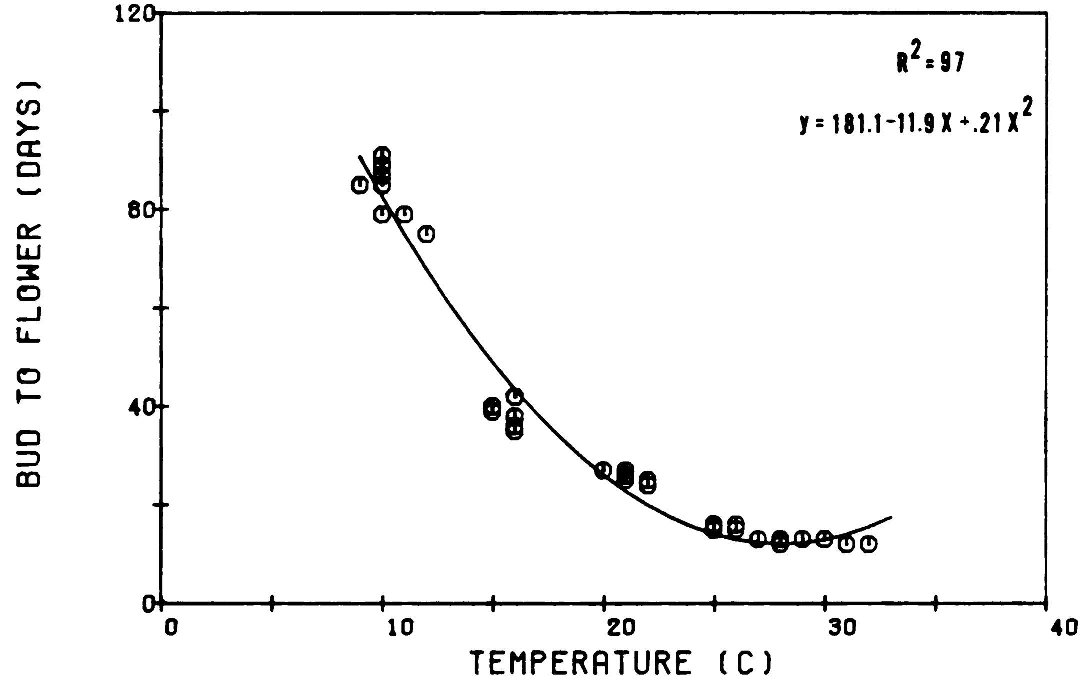


Figure 1. The effect of temperature on the number of days from visible bud to flower anthesis in hybrid geranium 'Sooner Red'.

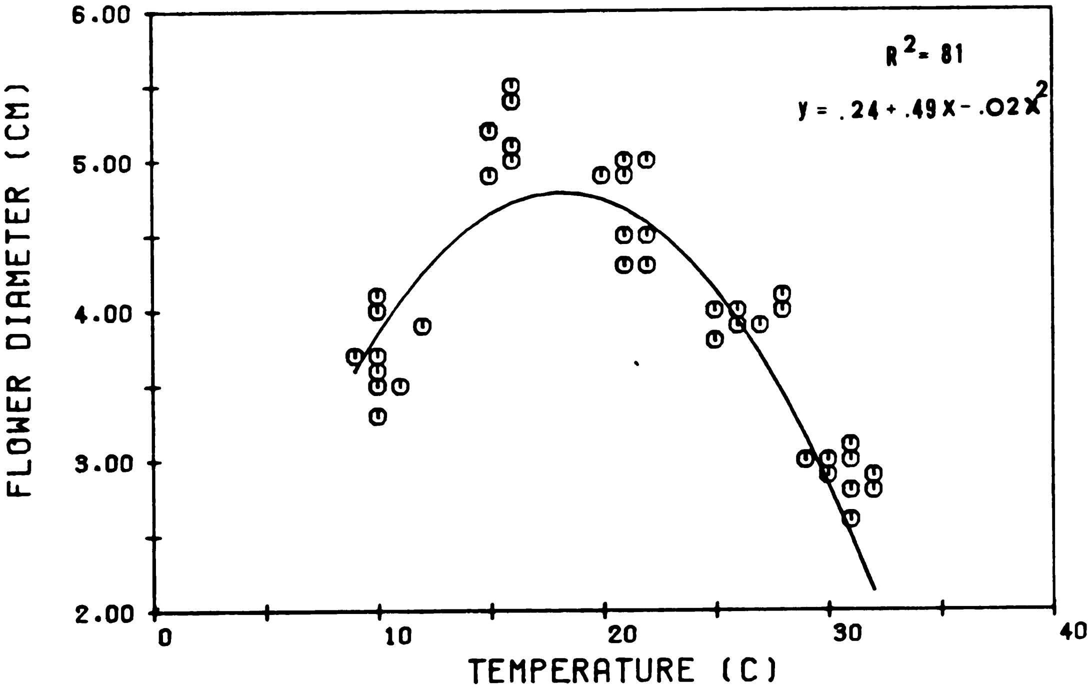


Figure 2. The effect of temperature on the flower diameter of hybrid geranium 'Sooner Red'.

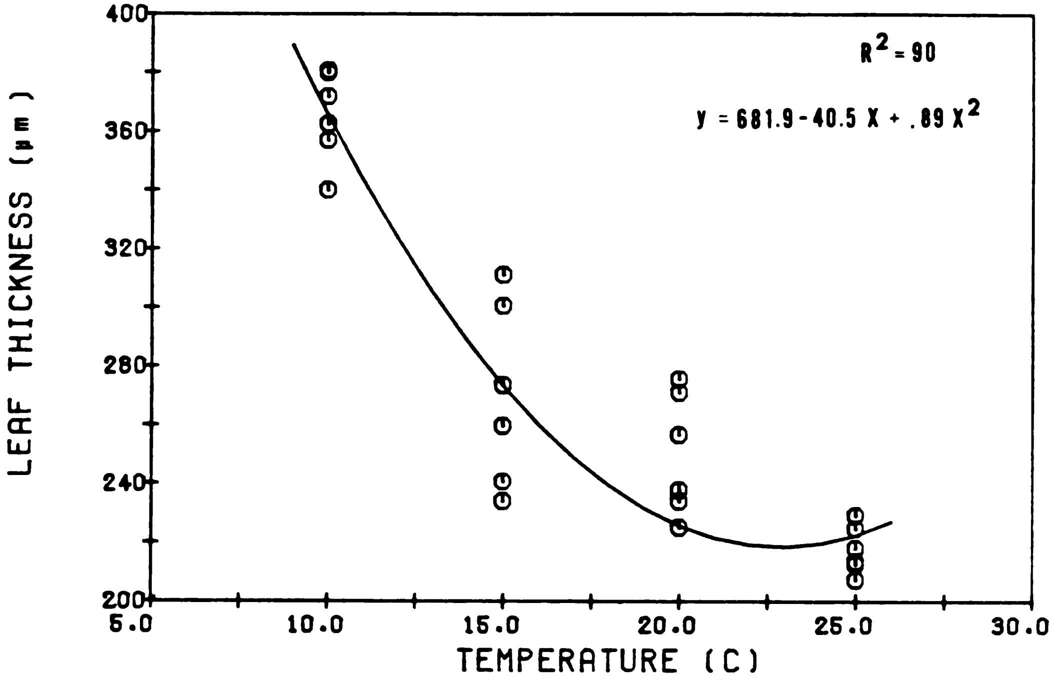


Figure 3. The effect of temperature on leaf thickness of hybrid geranium 'Sooner Red'.

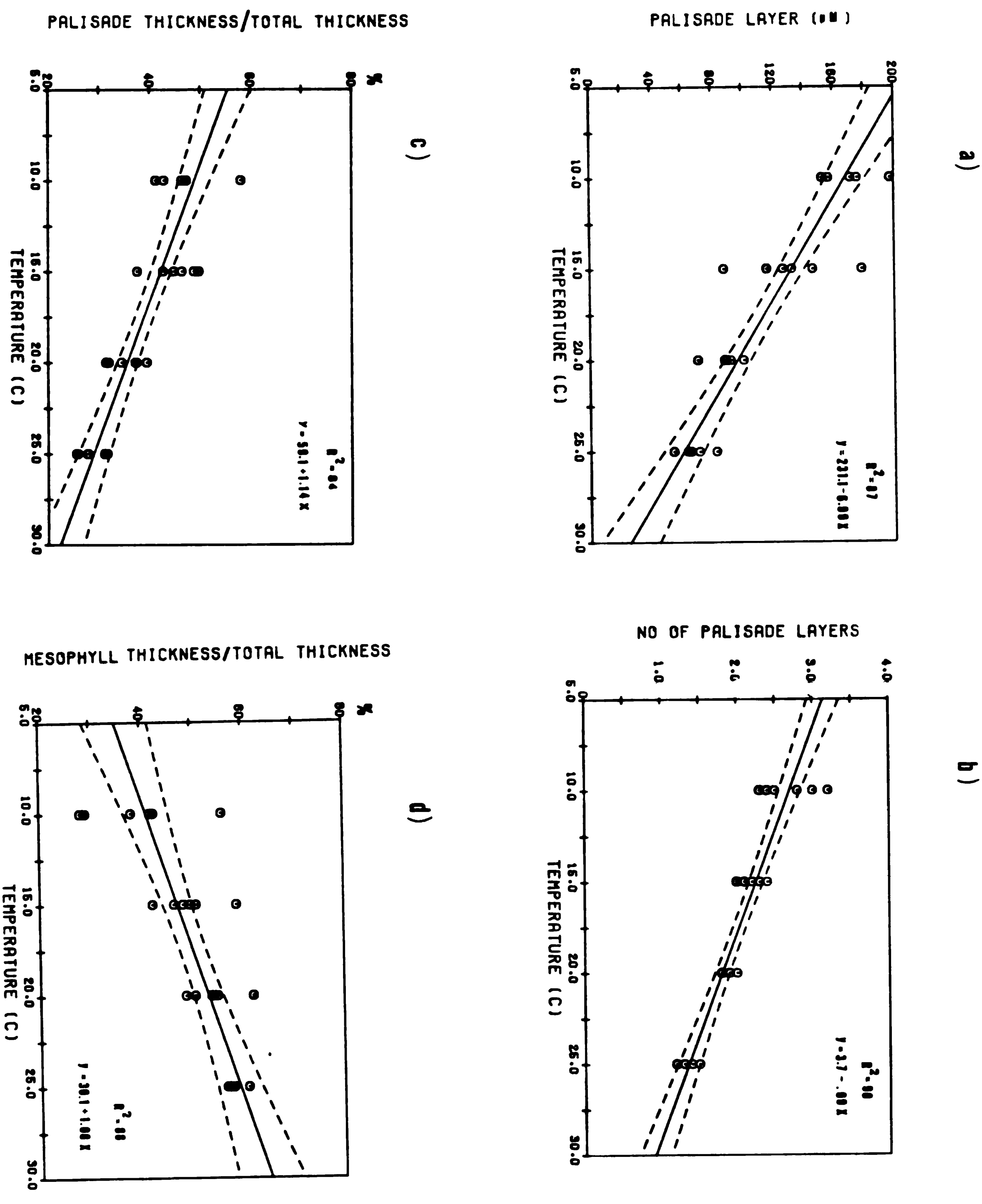
Figure 4. The effect of temperature on leaf structure of hybrid geranium 'Sooner Red'. Broken lines represent 95% confidence limits of true regression line.

a) Effect of temperature on palisade layer

b) Effect of temperature on the number of palisade layers.

c) Effect of temperature on the ratio of palisade thickness to total leaf thickness

d) Effect of temperature on the ratio of spongy mesophyll thickness to total leaf thickness


PALISADE THICKNESS/TOTAL THICKNESS
c)
%
20
40
60
80
5.0
10.0
15.0
20.0
25.0
30.0
TEMPERATURE (C)
R^2=.84
Y = 58.1+1.14X
PALISADE LAYER (µM)
a)
0
40
80
120
160
200
5.0
10.0
15.0
20.0
25.0
30.0
TEMPERATURE (C)
R^2=.87
Y=231.1-6.08X
MESOPHYLL THICKNESS/TOTAL THICKNESS
d)
%
20
40
60
80
5.0
10.0
15.0
20.0
25.0
30.0
TEMPERATURE (C)
R^2=.88
Y = 30.1+1.08X
NO OF PALISADE LAYERS
b)
0
1.0
2.0
3.0
4.0
5.0
10.0
15.0
20.0
25.0
30.0
TEMPERATURE (C)
R^2=.88
Y=3.7-.08X

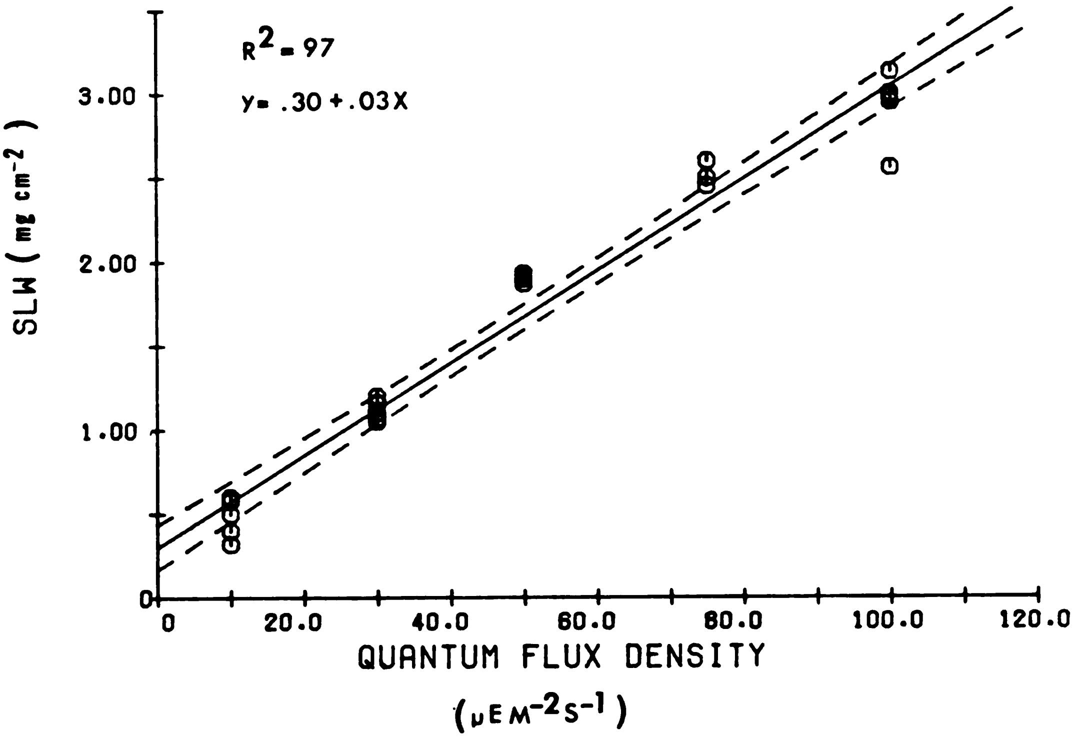


Figure 5. The effect of quantum flux density on the specific leaf weight of hybrid geranium 'Sooner Red'. Broken lines represent 95% confidence limits of true regression line.

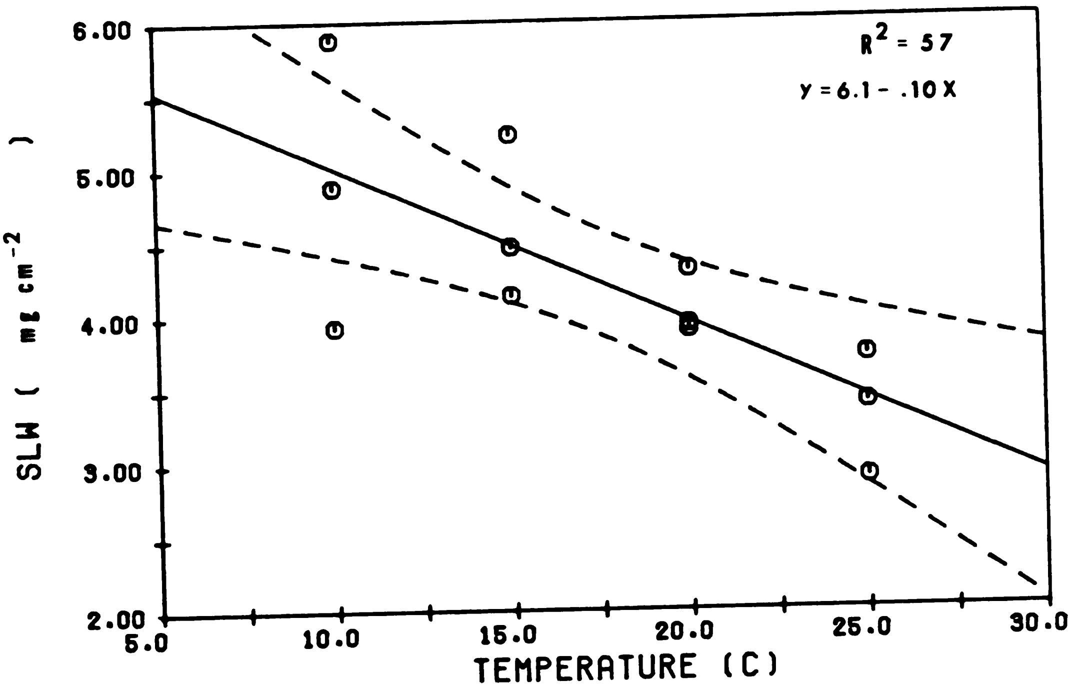


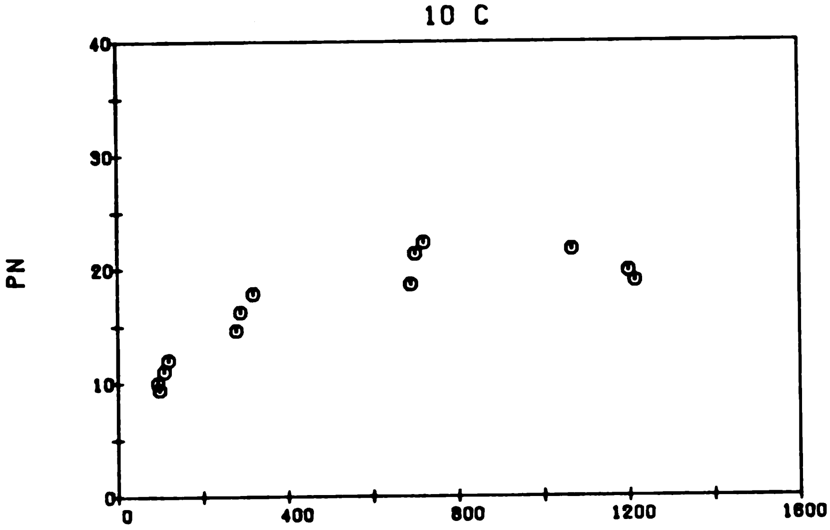
Figure 6. The effect of temperature on the specific leaf weight of hybrid geranium 'Sooner Red'. Broken lines represent 95% confidence limits of true regression line.

Figure 7. The effect of quantum flux density ($\mu E\ m^{-2}s^{-1}$) on net photosynthesis (PN) (mg CO_2 $dm^{-2}hr^{-1}$) of hybrid geranium 'Sooner Red' at various temperatures.

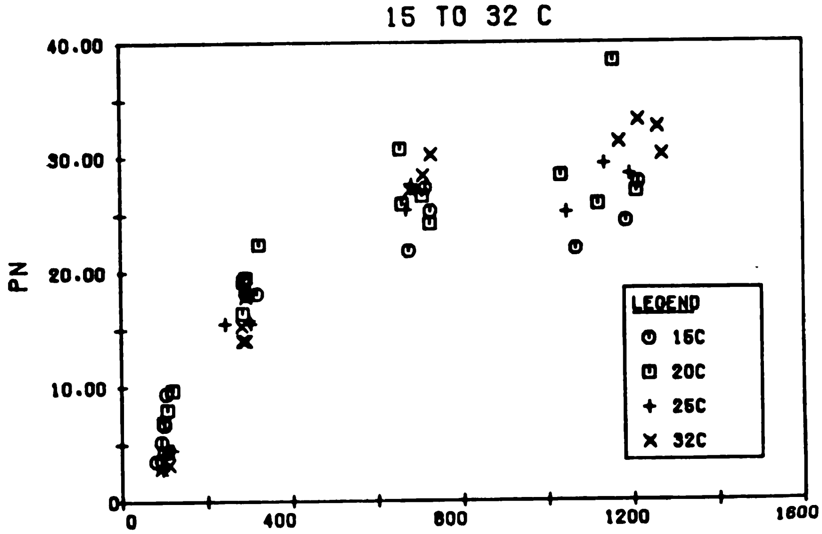
a) Effect of 10°C constant temperature on PN

b) Effect of 15-32°C constant temperature on PN

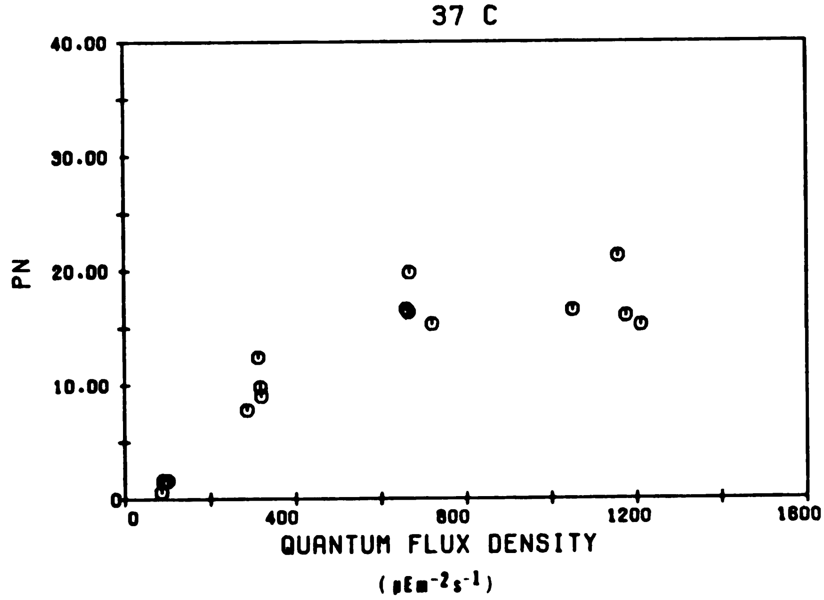
c) Effect of 37°C constant temperature on PN a)


10 C
PN
0
10
20
30
40
0
400
800
1200
1600

b)


15 TO 32 C
PN
0
10.00
20.00
30.00
40.00
0
400
800
1200
1600
LEGEND
⊙ 15C
⊡ 20C
+ 25C
× 32C

c)


37 C
PN
0
10.00
20.00
30.00
40.00
0
400
800
1200
1600
QUANTUM FLUX DENSITY
(μEm⁻²s⁻¹)

SECTION II

DETERMINATION OF FLOWERING TIME AND VEGETATIVE HABIT OF *TAGETES PATULA* THROUGH RESPONSE SURFACE TECHNIQUES

DETERMINATION OF FLOWERING TIME AND VEGETATIVE HABIT OF *TAGETES PATULA* THROUGH RESPONSE SURFACE TECHNIQUES

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Abstract: The prediction of flowering time, dry weight, total leaf area and vegetative height of *Tagetes patula L.* based on day temperature, night temperature and quantum flux density are demonstrated. High temperatures (30°C) decrease flower time regardless of QFD and greatest leaf surface area was caused by high QFD. As night temperatures increased, maximum leaf area occurred at lower day temperatures than those necessary for fastest flowering time. Response surface techniques for characterization of each response as well as the relative importance of each factor are discussed. The application of this technique to horticultural research is discussed.

Environmental effects on flowering and growth of greenhouse crops have been studied extensively. Temperature (7,13,19,22,23), light (6,9,12) and their interaction (8,14,16,21,24) on flowering and/or growth of many crops have been reported, however little has been done with bedding plants. Research on environmental control of greenhouse crops has dealt with one or two factors but has not shown how a change in one factor may be compensated by a change in a second factor to maintain a desired response. In trying to elucidate environmental conditions which produce a given response, experiments with factorial combination of treatments usually result in only one or two combinations which yield the desired response. A full complement of factorial combinations to test three factors at p levels results in p^3 experiments which may be too costly or too time consuming, especially if each experiment is lengthy. Response surface techniques minimize the number of experimental treatments required to adequately cover a given range of factors (11,15,17) and often incorporate a composite design (2,3,4). Response surface experiments have been used in other fields (10,17,18) but have not received much attention in the plant sciences. If levels of each factor in a 3-factor composite design are coded, a 15 point central design in three factors would appear as in Figure 1.

In our experiment, we wished to determine the effect of quantum flux density (QFD) in the 400-700 nm range, night temperature and day temperature on the flowering and growth of Tagetes patula, a dwarf french hybrid marigold. Specifically we determined all possible combinations of these three factors which would result in characterization of flowering time, growth (dry weight, total leaf surface area) and vegetative height. Recent work with hybrid geraniums (1,20) has shown that QFD and temperature affect different stages of flowering independently, therefore we determined if this was also true for marigolds. Tagetes species are long day, short day or day neutral plants (5), however Tagetes patula 'Petite Yellow' is day neutral with respect to flowering. It is a popular, widely grown bedding plant and served as the experimental plant.

Materials and Methods

Seeds of Tagetes patula L. 'Petite Yellow' were germinated under mist in their growing containers and placed in growth chambers[1] at appropriate treatment combinations (Table 1) approximately 10 days after sowing. Temperatures fluctuated ±2°C and QFD varied ±10 $\mu E\ m^{-2}s^{-1}$ and the photoperiod was 16 h light, 8 h dark. Plants were grown in an artificial peat lite medium and were fertilized with 20-20-20 water soluble fertilizer to provide 200 ppm N at each irrigation. Plants were leached as needed to prevent soluble salt accumulation. The 15 point composite design covered the surface of 10-32° in 5 levels for both day and night temperature and 50-600 $\mu E\ m^{-2}s^{-1}$ in four levels for QFD. The highest QFD level was not possible due to physical limitations of growth chambers used. In the treatment design (Fig. 1), the 8 vertices of the cube form a 3 variable, 2 level factorial. The ninth treatment is at the middle of the cube and was replicated 5 times to provide an independent measure of error. The remaining 6 treatments were placed at predetermined points ±2 units along the three axes. The time to reach visible bud stage (<0.5 cm in diameter) was recorded and data for dry weight, vegetative height and total leaf surface area were gathered at flower anthesis. Regression coefficients were determined and isoquants of similar responses were drawn for each parameter recorded. In order to adequately describe the surface and contain linear, quadratic and interaction terms, a second order model in the form of

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$
$$+ b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

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[1] Shere-Gillete, Marshall, MI

was selected where $\hat{y}$ is the measured response, x_1, x_2, x_3 are the actual variables for QFD, day temperature (DT) and night temperature (NT) respectively and the b's are the regression coefficients.

Results and Discussion

The means of the experimental values for all treatment combinations are given in Table 2. High day temperatures of 32°C resulted in bud development, however many plants died before reaching anthesis. In both stages of flowering, as well as total time to flower, linear and/or quadratic terms of QFD were highly significant (Table 3). Day and night temperature, and their interaction, were significant in the visible bud to flower stage. This corresponds with results found with geraniums (1,20) and lilies (25) namely that temperature is highly correlated to flower development once initiation has occurred. Day temperature significantly affects the time to reach visible bud, although night temperature has little significance (Table 3). Surface response plots (Fig. 2 a-c) for time to reach visible bud indicate that at cold night temperatures (10°) minimal time is obtained at very high light levels over a small range of day temperatures. However as night temperature increased, a lower optimum (i.e., less time) occurred over a wide range of light but over small day temperature ranges (Fig. 2). When night temperatures are high, day temperatures appear to be the limiting factor. High day temperatures and high light cause increased PN which may compensate for increased respiration losses under high night temperatures. When night temperatures drop, light appears to be limiting for bud formation. Low night temperatures likely caused decreased metabolism and high light levels may have been necessary for more rapid growth and flowering.

Temperatures above $30^{\circ}C$ resulted in death of the flower bud, therefore plots were truncated at that point. Visible bud to flower (Fig. 3 a-c) appear to be temperature dependent under cool night temperatures, however as night temperatures rise (Fig. 3c), the response becomes more light dependent with the response increasing with increasing QFD. This appears to be opposite to the time to visible bud stage and reflects some independence from one another in the stages in marigold flowering. It also indicates that QFD is very important in marigolds for flower development under high (>26) night temperatures but not under cool temperatures. Minimum total days to flower (Fig. 4 a-c) is a combination of high light and high day temperatures regardless of night temperature. However, the optimum response is lower (i.e., fewer days) with lower night temperatures compared with high temperatures. This confirms the work of many researchers (13,19) in that lower night temperatures enhanced flowering and growth of greenhouse crops compared with warm night temperatures. A summary of minimum flowering times under different light intensities and temperatures are given in Table 4.

Growth. Vegetative parameters chosen were dry weight, total leaf surface area and vegetative height at flowering as overall appearance and plant "quality" are most dependent on these factors. A second order model of the same form used for flowering responses was calculated and the regression coefficients and their significances are shown in Table 5. In all growth responses, linear and/or quadratic trends of QFD and day temperature were highly significant. Day-night temperature interaction was highly significant in vegetative height and leaf surface area while QFD x NT was the only interaction with dry weight. Response surface plots of dry weight (Fig. 5 a-c) indicate that maximum dry weight accumulation occurred with cool day temperatures and high light, regardless of night temperature. The lowest dry weight accumulation was under low light conditions. With marigolds, high light results in the highest dry weight even when night temperatures are high. The range of dry weight accumulation is narrower and the lowest dry weights do not occur at high night temperatures (Fig. 5c). These predictions show dry weight accumulation at flowering but not the rate of dry weight accumulation. Dry weight accumulation at flowering indicated that temperature is very important but the rate of dry weight increase appeared to be a function of QFD (Table 6). Maximum plant growth occurred during weeks 6 to 8 when growth under 150 μE $m^{-2}s^{-1}$, and week 4 when grown under 375 μE $m^{-2}s^{-1}$ except when day temperatures were extreme (10 or 32^{o}). At these temperatures, maximum increase in growth under 375 μE $m^{-2}s^{-1}$ was delayed until week 6. Maximum growth occurred at weeks 3 to 4 when plants were subjected to 600 μE $m^{-2}s^{-1}$. The large flush of growth at QFD's of 150 to 600 μE $m^{-2}s^{-1}$ did not occur at 50 μE $m^{-2}s^{-1}$ indicating expected slow growth under winter light conditions.

An optimum for vegetative height could not be assessed, however extremely tall plants would be unsuitable for shipping and excessively dwarfed plants would likely be unmarketable. High temperatures caused elongation of internodes of various species and result in tall plants. Response surface plots for vegetative height (Fig. 6 a-c) indicated that as day temperatures rise, height increases, regardless of night temperature. High QFD (500-600 μE $m^{-2}s^{-1}$) and low QFD (<100 μE $m^{-2}s^{-1}$) resulted in shorter plants regardless of temperature. The tendency towards shorter plants was most evident at high night temperatures ($>26^o$).

Large total leaf area would be beneficial for more carbohydrate production as well as the aesthetics of the plant. Low night temperature, coupled with low day temperature resulted in less surface area (Fig. 7 a-c), however the largest amount of leaf area occurred with cool night temperature ($10-15^o$) compared with higher night temperatures. At low night temperature, the smallest leaf area occurred over a wide range of light but as night temperature increased, the range of QFD became narrower for any one isoquant. The largest total leaf area at high night temperatures occurred at the highest QFD and mid range day temperatures. Higher day temperatures were necessary for high leaf area at lower night temperatures. Temperatures appear to play a major role in control of leaf surface area. Light affects carbohydrate production through PN and hormonal movement which controls cell division, elongation and growth. Temperature affects overall metabolism such as cell division and cell elongation and thus leaf expansion. Day temperatures necessary for maximum leaf area at given night temperatures and light levels were lower than those necessary for minimum flowering time (Table 4). The grower must make decisions based on the overall plant appearance as well as flowering time.

The ability to predict growth and flowering responses would be of great importance to the floriculture industry. More control of the environment is possible in the greenhouse than in any other commercial facet of horticulture. Studies of this type can be used to develop answers to questions related to timing, appearance and yield of a greenhouse crop under a wide range of light and temperatures. Other factors could be included in the model such as humidity, soil temperatures,

CO_2, etc., however light and temperature are easily measured, and controlled and adequately describe the growth and flowering of this crop. Computer programs could be built around experimental data and optima for any crop predicted. Significant values of independent variables would allow growers to make more intelligent decisions when varying the environment to obtain desired flowering and growth.

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Table 1. Actual and coded values for treatment combinations used in composite design.

	QFD ($\mu E\ m^{-2} s^{-1}$)	Temperature (c) Day	Temperature (c) Night	Coded Values QFD	Coded Values DT	Coded Values NT
1.	50	21	21	-2	0	0
2.	150	15	15	-1	-1	-1
3.	150	15	26	-1	-1	1
4.	150	26	15	-1	1	-1
5.	150	26	26	-1	1	1
6.	375	21	21	0	0	0
7.	375	21	10	0	0	-2
8.	375	21	32	0	0	2
9.	375	32	21	0	2	0
10.	375	10	21	0	-2	0
11.	600	15	26	1	-1	1
12.	600	26	26	1	1	1
13.	600	15	15	1	-1	-1
14.	600	26	15	1	1	-1

Table 2. Experimental values[1] obtained for treatment combinations of composite design.

	Treatment QFD	DT/NT	Days to visible bud	Days from VB to flower	Dry weight (g)	Vegetative height (cm)	Total leaf area (cm^2)
1.	50	21/21	36.8	29.0	0.14	2.35	74.8
2.	150	15/15	33.8	31.7	0.28	2.25	107.8
3.	150	26/15	21.2	31.9	0.69	5.65	186.7
4.	150	26/15	22.3	28.5	0.80	7.55	233.0
5.	150	26/26	22.5	23.4	0.66	4.90	97.6
6.	375	21/21	17.0	27.0	1.23	6.41	285.9
7.	375	21/21	17.1	29.2	1.00	5.99	300.5
8.	375	21/21	17.9	27.4	1.05	6.42	280.6
9.	375	21/21	16.8	26.5	0.99	5.95	260.2
10.	375	21/21	18.5	27.1	1.23	6.92	305.4
11.	375	21/10	19.7	27.3	1.00	5.22	263.5
12.	375	21/32	16.5	25.8	1.40	6.45	338.0
13.	375	32/21	16.8	-	-	13.85	-
14.	375	10/21	25.2	35.6	1.02	4.05	102.8
15.	600	15/26	16.8	23.1	1.02	4.00	187.1
16.	600	26/26	13.8	19.0	1.43	5.52	227.7
17.	600	15/15	27.5	23.1	1.62	3.71	174.4
18.	600	26/15	15.6	21.5	2.15	6.42	374.1

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[1] Means of 60 observations

Table 3. Regression coefficents and significance levels for flowering responses in Marigold 'Petite Yellow'.

Regression Coefficient		Days to visible bud (R^2=93)	Sign.	Visible bud to flower (R^2=90)	Sign.	Total days to flower (R^2=92)	Sign.
Constant	b_0	42.62		85.25		110.75	
QFD	b_1	$-0.19\text{x}10^{-2}$	.845	$-0.73\text{x}10^{-1}$	.000	$-0.89\text{x}10^{-1}$	.002
DT	b_2	-1.28	.012	-1.84	.041	-0.89	.374
NT	b_3	0.58	.127	-2.03	.005	-1.45	.150
$(QFD)^2$	b_{11}	$-0.39\text{x}10^{-4}$	.000	$0.92\text{x}10^{-4}$	.000	$0.72\text{x}10^{-4}$	.002
$(DT)^2$	b_{22}	$0.34\text{x}10^{-1}$	.003	$0.11\text{x}10^{-1}$	.569	$-0.37\text{x}10^{-1}$	.046
$(NT)^2$	b_{33}	$-0.92\text{x}10^{-3}$	.889	$0.37\text{x}10^{-2}$	.755	$-0.23\text{x}10^{-2}$	.895
QFD x DT	b_{12}	$0.53\text{x}10^{-3}$	.060	$-0.56\text{x}10^{-3}$	.251	$-0.13\text{x}10^{-4}$	.985
QFD x NT	b_{13}	$0.17\text{x}10^{-3}$	.522	$-0.87\text{x}10^{-4}$	.856	$0.11\text{x}10^{-3}$	.881
PT x NT	b_{23}	$0.34\text{x}10^{-3}$	.889	$0.90\text{x}10^{-1}$	.000	$0.51\text{x}10^{-1}$	.092

Table 4. Optima[1] for total flowering time and total leaf area at various levels of QFD and night temperatures.

QFD ($\mu E\ m^{-2}s^{-1}$)	Night temp.	Day temp. req'd. for min. flowering	Minimum flowering time (days)	Day temp. req'd. for max. leaf area	Maximum leaf area (cm^2)
50-125	10	30	41-47	30	170-250
	15	30	41-47	26	90-170
	20	30	42-47	21	80-140
	25	30	42-47	16	120-170
	30	30	41-47	12	200-250
130-300	10	30	31-39	30	270-390
	15	30	31-40	27	190-300
	20	30	32-40	23	160-250
	25	30	32-40	19	180-250
	30	30	32-40	13	250-300
305-400	10	30	27-30	30	410-450
	15	30	28-30	29	310-350
	20	30	28-31	25	200-280
	25	30	28-31	20	260-270
	30	30	28-31	15	300-310
405-600	10	30	24-27	30	460-500
	15	30	25-27	30	360-390
	20	30	25-27	27	290-300
	25	30	25-28	22	270-280
	30	30	25-27	17	280-310

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[1] Based on fortran program developed at MSU.

Table 5. Regression coefficients and significance levels for vegetative responses in Marigold 'Petite Yellow'.

Regression coefficient		Dry weight (R^2=90)	Sign.	Vegetative height (R^2=90)	Sign.	Total leaf area (R^2=85)	Sign.
Constant	b_0	-0.72		-10.649		-7.91	
QFD	b_1	$0.59\text{x}10^{-2}$	.000	$0.30\text{x}10^{-1}$	.000	$0.71\text{x}10^{-2}$	.030
DT	b_2	$-0.62\text{x}10^{-1}$	.346	$-0.57\text{x}10^{-1}$	.832	0.62	.000
NT	b_3	$0.54\text{x}10^{-1}$	.289	0.80	.007	0.18	.135
$(\text{QFD})^2$	b_{11}	$-0.20\text{x}10^{-5}$	.086	$-0.36\text{x}10^{-4}$	.000	$0.10\text{x}10^{-4}$	.000
$(\text{DT})^2$	b_{22}	$0.31\text{x}10^{-2}$	.040	$0.23\text{x}10^{-1}$	.000	$-0.81\text{x}10^{-2}$	.019
$(\text{NT})^2$	b_{33}	$0.13\text{x}10^{-2}$	.171	$-0.35\text{x}10^{-2}$	.468	$0.37\text{x}10^{-2}$	.082
QFD x DT	b_{12}	$0.46\text{x}10^{-4}$	.213	$-0.27\text{x}10^{-4}$	.892	$0.21\text{x}10^{-3}$	.020
QFD x NT	b_{13}	$-0.16\text{x}10^{-3}$	.000	$-0.13\text{x}10^{-3}$	.518	$-0.77\text{x}10^{-4}$	.362
DT x NT	b_{23}	$0.23\text{x}10^{-2}$	.131	$-0.28\text{x}10^{-1}$	.002	$-0.15\text{x}10^{-1}$	.000

Table 6. The effect of quantum flux density, and day and night temperature on the rate of dry weight accumulation of Marigold 'Petite Yellow'.

Treatment Number			Rate of dry weight gain (mg/wk)									
			Week									
#	QFD	DT/NT	1	2	3	4	5	6	7	8	9	10
1.	50	21/21	4	5	4	8	40	40	40	50	50	40
2.	150	15/15	4	8	18	40	100	150	40	20	10	-
3.	150	15/26	4	7	12	30	70	120	30	20	10	-
4.	150	26/15	4	26	70	98	142	153	70	30	10	-
5.	150	26/26	5	2	5	60	80	98	160	97	10	-
6.	375	21/21	5	70	180	260	260	170	50	20	-	-
7.	375	21/10	5	45	150	310	205	100	20	10	-	-
8.	375	21/32	5	105	160	410	200	96	40	20	-	-
9.	375	32/21	5	10	20	70	190	200	130	85	60	-
10.	375	10/21	5	10	20	70	150	370	150	76	50	10
11.	600	15/26	6	50	100	190	290	90	30	-	-	-
12.	600	26/26	8	92	140	290	250	80	20	-	-	-
13.	600	15/15	8	82	110	200	400	98	80	60	20	-
14.	600	26/15	8	160	305	430	130	20	-	-	-	-

- Indicates at least 50% of the plants have reached anthesis.

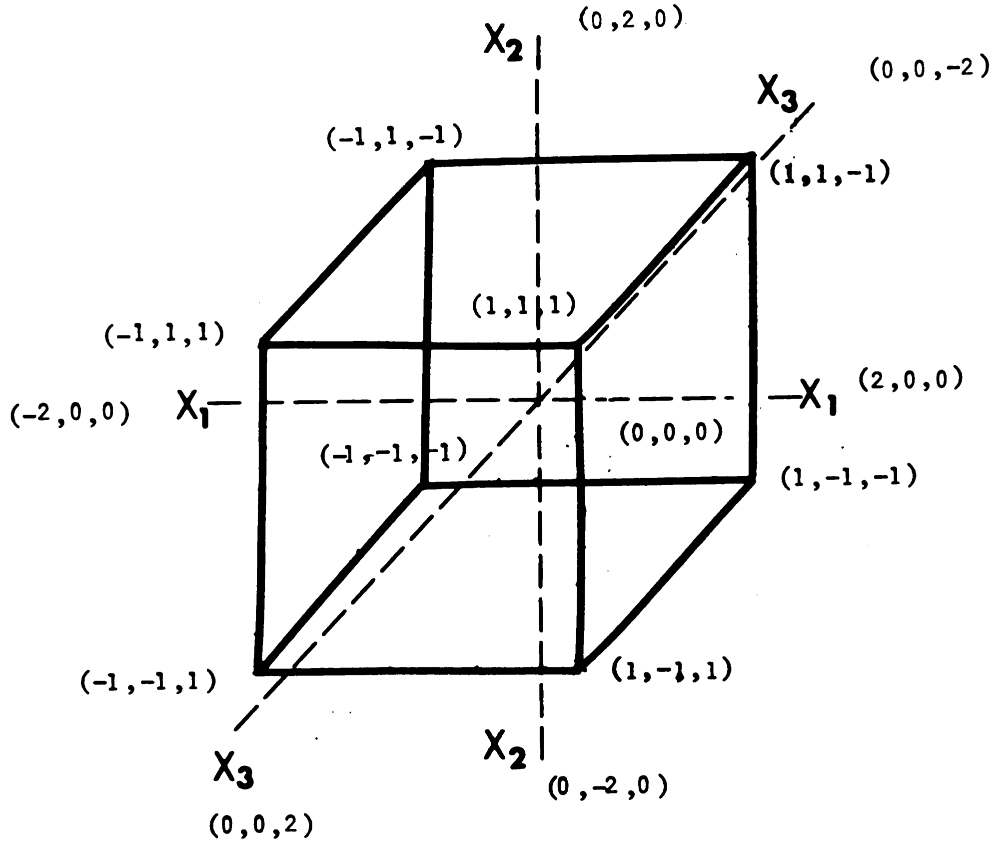


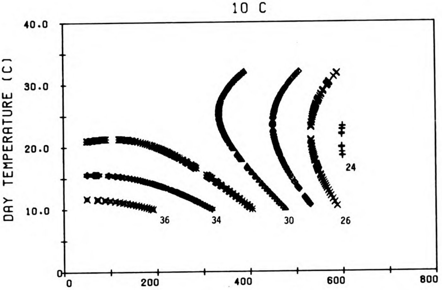
Figure 1. A 15 point central composite design for response surface techniques. X_1, X_2, and X_3 are QFD, day temperature, and night temperature respectively. Numbers refer to coded values.

Figure 2. The effect of quantum flux density and day and night temperature on the time to reach visible bud stage. Each isoquant differs by 2 days.

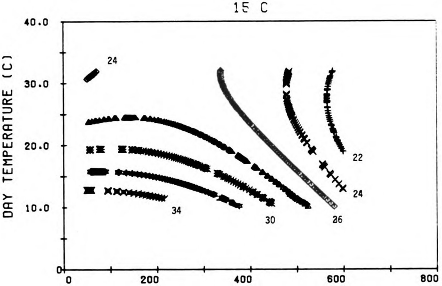
(a) Night temperature 10°C

(b) Night temperature 15°C

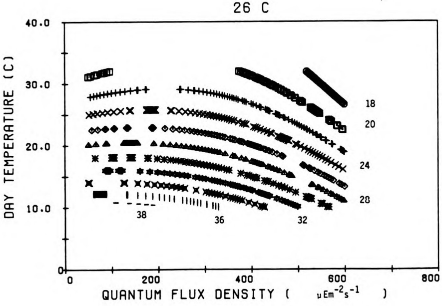
(c) Night temperature 26°C


10 C
40.0
30.0
20.0
10.0
0
DAY TEMPERATURE (C)
0
200
400
600
800
24
36
34
30
26

(a)


15 C
40.0
30.0
20.0
10.0
0
DAY TEMPERATURE (C)
0
200
400
600
800
24
22
24
34
30
26

(b)


26 C
40.0
30.0
20.0
10.0
0
DAY TEMPERATURE (C)
0
200
400
600
800
QUANTUM FLUX DENSITY ($\mu Em^{-2}s^{-1}$)
18
20
24
28
38
36
32

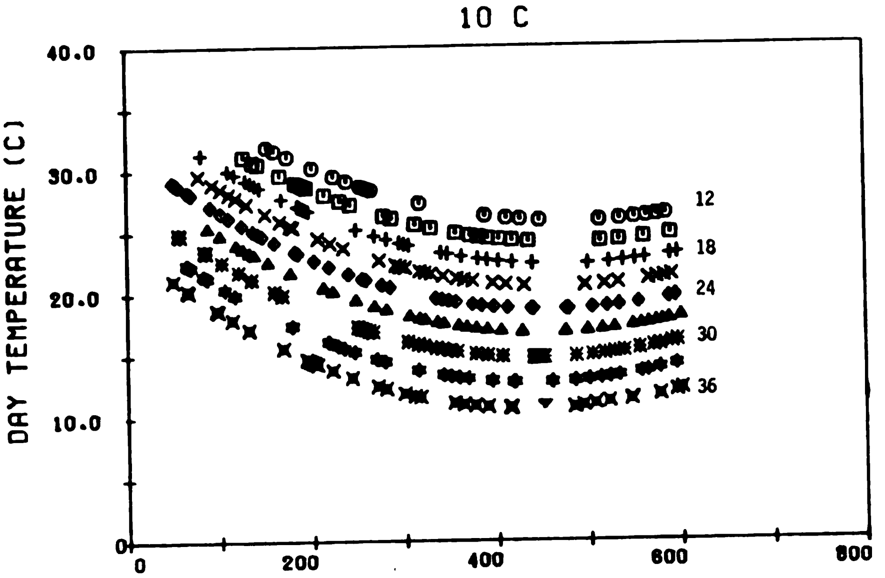
(c)

Figure 3. The effect of quantum flux density and day and night temperature on the time from visible bud to flower anthesis. Each isoquant differs by 3 days.

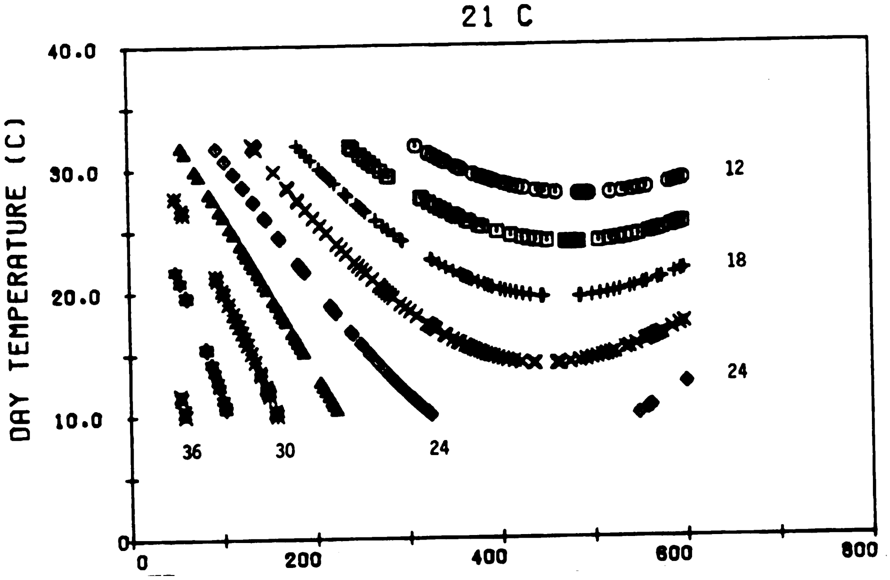
(a) Night temperature 10°C

(b) Night temperature 21°C

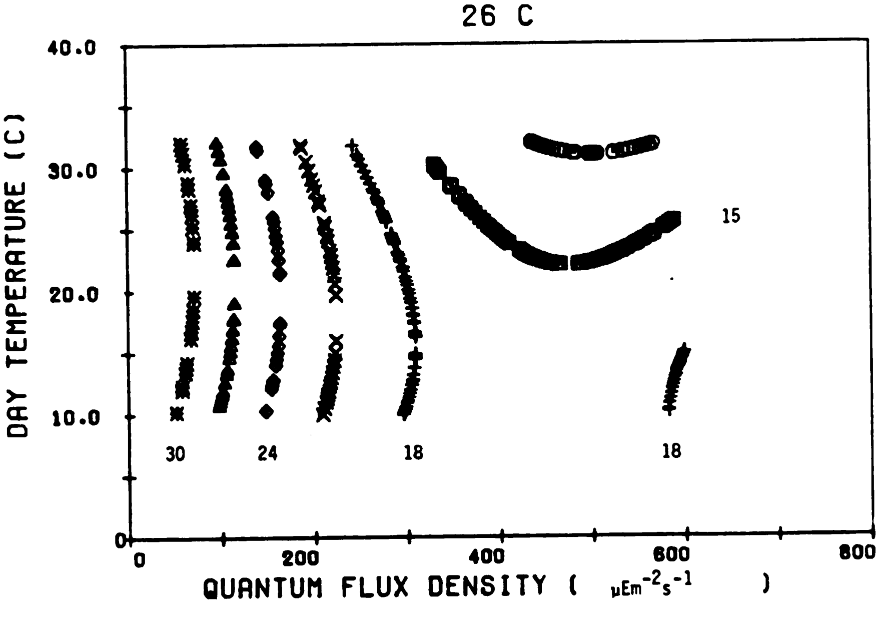
(c) Night temperature 26°C


10 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
12
18
24
30
36

(a)


21 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
12
18
24
36
30
24

(b)


26 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
QUANTUM FLUX DENSITY (μEm⁻²s⁻¹)
15
30
24
18
18

(c)

Figure 4. The effect of quantum flux density and day and night temperature on total days to flower. Each isoquant differs by 5 days.

(a) Night temperature 10°C

(b) Night temperature 15°C

(c) Night temperature 26°C

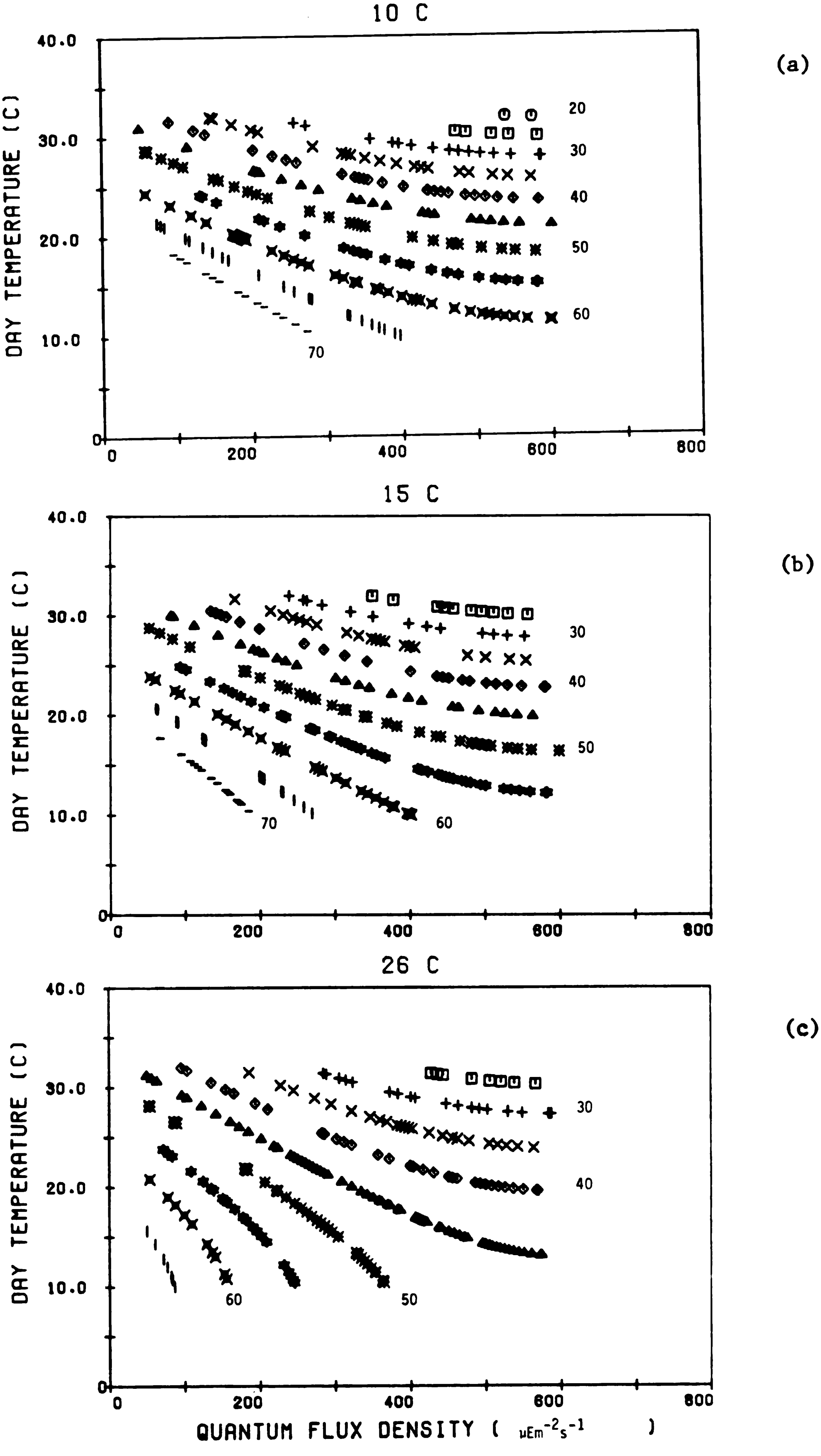

10 C
(a)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
20
30
40
50
60
70
15 C
(b)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
30
40
50
60
70
26 C
(c)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
30
40
50
60
QUANTUM FLUX DENSITY (μEm⁻²s⁻¹)

Figure 5. The effect of quantum flux density and day and night temperature on dry weight at time of flower anthesis. Each isoquant differs by 0.3 g.

(a) Night temperature 10°C

(b) Night temperature 15°C

(c) Night temperature 20°C

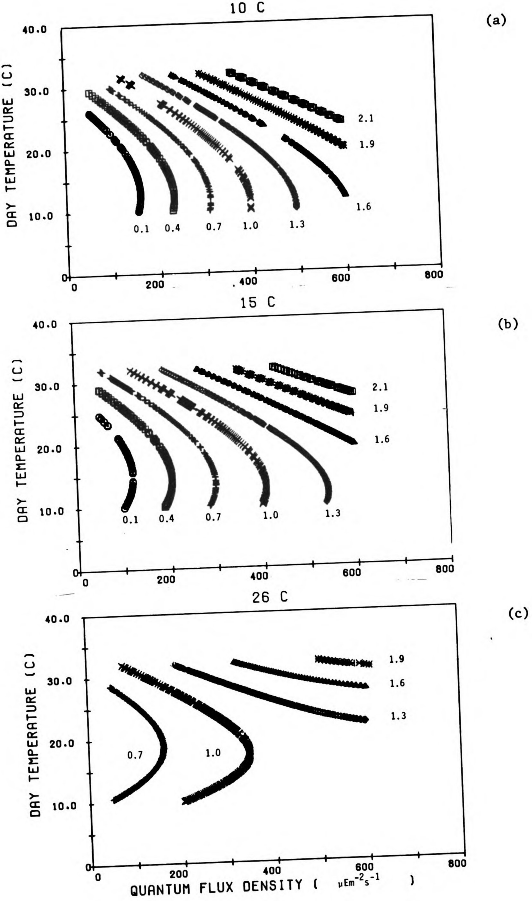

10 C
(a)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
2.1
1.9
1.6
0.1
0.4
0.7
1.0
1.3
15 C
(b)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
2.1
1.9
1.6
0.1
0.4
0.7
1.0
1.3
26 C
(c)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
1.9
1.6
1.3
0.7
1.0
QUANTUM FLUX DENSITY (µEm-2s-1)

Figure 6. The effect of quantum flux density and day and night temperature on the vegetative height at time of flower anthesis. Each isoquant differs by 2 cm.

(a) Night temperature 10°C

(b) Night temperature 15°C

(c) Night temperature 26°C

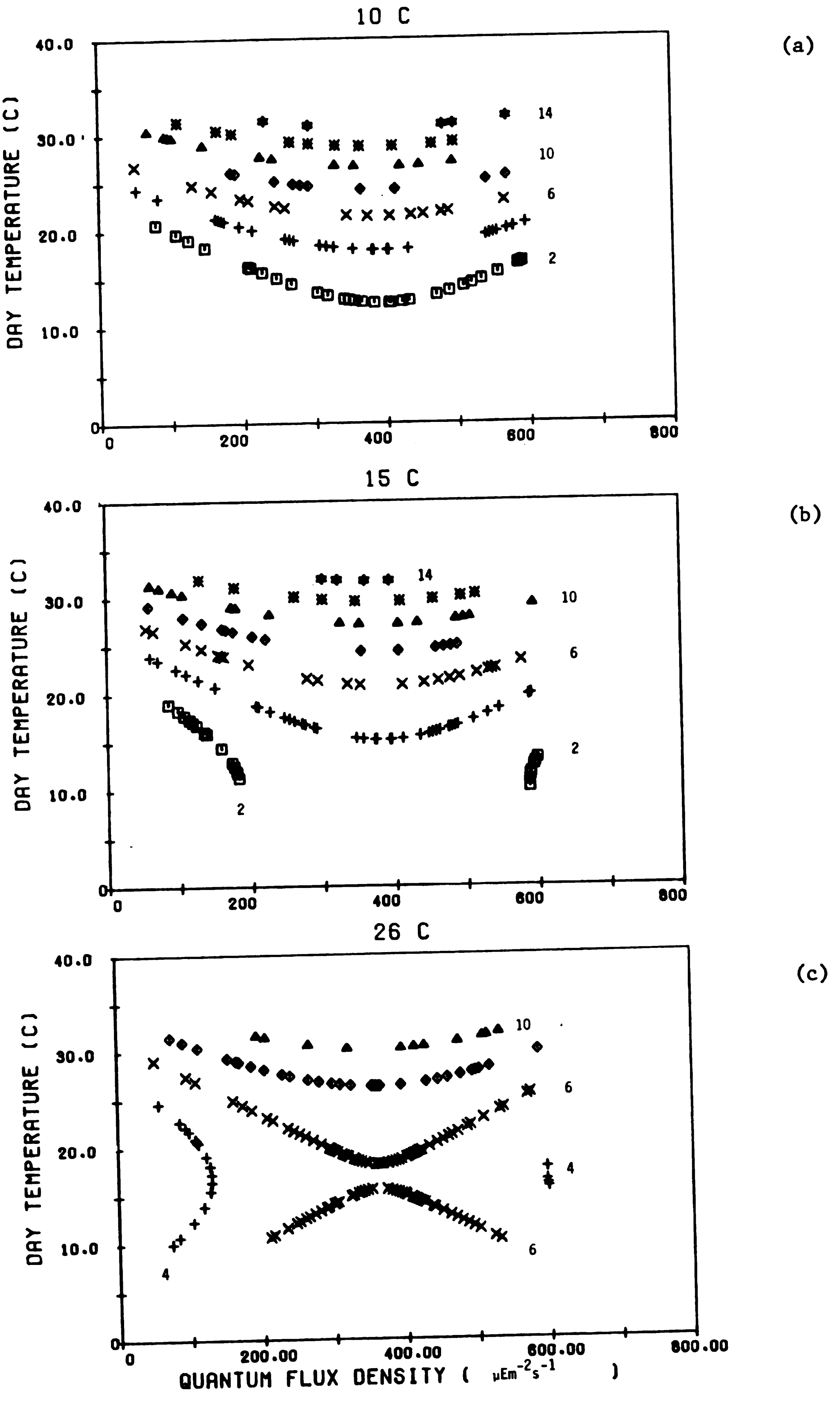
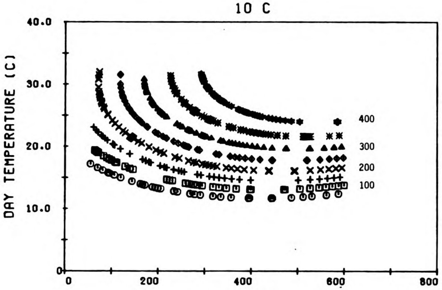

10 C
(a)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
14
10
6
2
15 C
(b)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
14
10
6
2
2
26 C
(c)
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200.00
400.00
600.00
800.00
10
6
4
6
4
QUANTUM FLUX DENSITY (μEm-2s-1)

Figure 7. The effect of quantum flux density and day and night temperature on total leaf surface area at time of flower anthesis. Each isoquant differs by 50 cm^2.

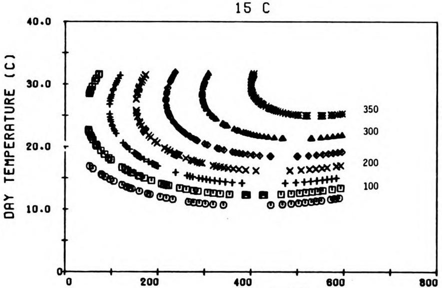
(a) Night temperature 10°C

(b) Night temperature 15°C

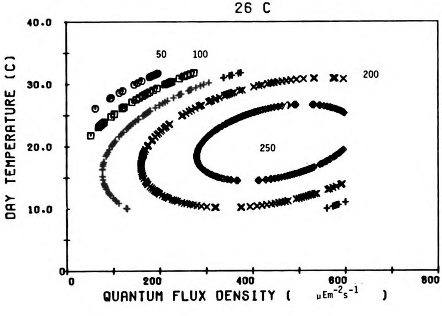
(c) Night temperature 26°C


10 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
400
300
200
100

(a)


15 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
350
300
200
100

(b)


26 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
0
200
400
600
800
50
100
200
250
QUANTUM FLUX DENSITY (μEm-2s-1)

(c)

SECTION III

THE EFFECT OF QUANTUM FLUX DENSITY, DAY AND NIGHT TEMPERATURE AND PHOSPHORUS AND POTASSIUM STATUS ON ANTHOCYANIN AND CHLOROPHYLL CONTENT IN MARIGOLD LEAVES

THE EFFECT OF QUANTUM FLUX DENSITY, DAY AND NIGHT TEMPERATURE AND PHOSPHORUS AND POTASSIUM STATUS ON ANTHOCYANIN AND CHLOROPHYLL CONTENT IN MARIGOLD LEAVES

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Additional Index Words: response surface

Abstract: A temperature of 10°C resulted in the greatest synthesis of anthocyanins in marigold foliage regardless of whether cold was applied during the day or night. Response surface techniques were used to determine combinations of light and temperature which result in equal levels of anthocyanin and chlorophyll. Predicted responses indicate that as night temperature increased from 10 to 26°C, a wide range of quantum flux densities and day temperatures resulted in low anthocyanin content. Night temperature of 10°C and high day temperature resulted in the same chlorophyll content as 26°C night temperature and low day temperature if quantum flux density was low. No significant correlation was found between anthocyanin level and foliar phosphorus or potassium.

The french marigold (Tagetes patula L.) is of major importance to the bedding plant industry and ranks second in volume sales in the United States and Canada (42). Although primarily known for its yellow-orange flowers, the foliage is important to the overall appearance of the plant. Anthocyanins in the leaves affect the hue of the plant and its marketability. The environment under which the plant is grown can have a marked affect on chlorophyll and total anthocyanin content of the foliage (4,25,27,32).

The synthesis of anthocyanins appear to be related to increased light intensities (13,16,32), low temperatures (8,28,34), and the high irradiance response (HIR) mechanism (11,12). Carbohydrate metabolism may play a role (10,35,40), however Mancinelli *et al*. (27) used photosynthetic inhibitors and dismissed photosynthesis as an active participant in anthocyanin synthesis. Anthocyanins in the leaves of plants such as copper beach and red cabbage do not inhibit photosynthesis indicating the continued presence and function of chlorophyll (37). Seedlings deprived of nitrogen, phosphorus, or potassium often show increased anthocyanin content in crops such as lettuce (45), and apple fruit (44). Tomatoes are very sensitive to phosphorus levels (22) and potassium deficiency has accelerated anthocyanin production in some crops (39,43). The relative effects of day versus night temperature on anthocyanin synthesis have been studied by Hanan (20) using red carnations. He found that decreased day temperature led to increased anthocyanin production while Frey-Wyssling and Blank (16) found that red cabbage colored best at 30°C day compared with 10°C. Cool night temperatures were optimum in apple fruits (41) while average temperatures 2 days before sampling were found by Creasy *et al*. (10) as the limiting factor in anthocyanin production in apples. Anthocyanins occur as glycosides of anthocyanins and are usually simple monosides of cyanidin in dicot leaves (21). Anthocyanins have been fully identified in 11 genera of plants of Compositae and cyanidin 3-glycoside (usually glucoside, galactoside, or arabinoside) is the most prevalent anthocyanin of the family (21). The interaction of chlorophyll and anthocyanin has received little attention in floriculture and its effect on crop appearance but Reger (33) found that an induced chlorosis in apples resulted in enhanced anthocyanin production.

This study investigates (i) the effect of temperature on total anthocyanin and chlorophyll content of the foliage, (ii) the interaction of quantum flux density and temperature on total anthocyanin, (T Acy) and total chlorophyll (T Chl) and (iii) the correlation between phosphorus and potassium with T Acy in leaves.

Materials and Methods

Two separate experiments were conducted to study the environmental effects of T Acy and chlorophyll.

Temperature: Plants of *Tagetes patula* L. cv. Petite Yellow were direct seeded in a 1:1 peat-vermiculite media and were placed in growth chambers 10 days after sowing. Cool white fluorescent lights provided 400 ±10 $\mu E\ m^{-2}s^{-1}$ at the following temperatures: 1: 21 day, 21 night; 2: 21 D, 21 N; 3: 21 D, 32 N; 4: 32 D, 21 N; 5: 10 D, 21 N. Temperatures fluctuated ±2°C and photoperiod was 16 h light and 8 h dark. Plants were fertilized with water soluble fertilizer at 200 ppm of 20 N, 8.7 P, 16.7 K and leached every 7 days to prevent soluble salt buildup. All treatments were maintained for 3 weeks before plants were excised.

Pigment analysis:

Chlorophyll: Four leaf discs were immediately macerated after excision in 2 ml cold 80% acetone. Two 2 ml washes followed and the total sample was centrifuged at 5000 rpm (Sorvall, model RC2B, head SS34). The supernatent was stored in the dark at 0°C while 2 ml of solvent were added to the pellet and recentrifuged. The total supernatent was brought to a final volume of 8 ml and read on a Gilford 220 spectrophotometer at

645 nm (chl b) and 663 nm (chl a). Extraction and analysis was done under dim white light. Calculations for chlorophyll a, chlorophyll b, and total chlorophyll were derived from equations of Arnon (3).

Anthocyanin: A method adapted from Fuleki and Francis (17) was used for T Acy analysis. The following abbreviated procedure was used with 95% ethanol:1.5 N HCL (85:15) as the solvent: Three gm of leaf tissue and 40 ml solvent were macerated in a blender, washed twice with 10 ml solvent and centrifuged twice at 4500 rpm for 25 minutes. The supernatent was poured through #1 Whatman filter and washed 3 times with equal volumes of petroleum ether in a separatory funnel to eliminate masking by chlorophyll. The ethanol phase was washed 3 times with equal volumes of hexane to extract carotenoids and other polar pigments. The ethanol phase was diluted to an appropriate volume and the wavelength of maximum absorbance was determined on a Beckman scanning spectrophotometer. One aliquot was adjusted to pH 1 (21) and another to pH 4.5 to eliminate breakdown products and precursors (18). The absorbance of T Acy was read at 532 nm and specific Acy equivalents were determined (29). T Acy were calculated using extinction coefficients for specific anthocyanins (17).

Light-Temperature Interaction: Fifteen environmental treatment combinations were arranged in a 3 factor central composite design (6) in 5 levels of day and night temperatures (10-32^{o}C) and 4 levels of quantum flux density (QFD) measured in the 400-700 nm range (Table 1). A second order model was fitted for each pigment and response surface plots were drawn (2). All treatments were carried out in growth chambers similar to the previous experiment. Pigment and nutrient analysis were determined 21 days after placement in the chambers. Phosphorus and potassium levels in the foliage were determined by photoelectric spectrometry similar to methods of Kenworthy (23).

Results and Discussion

Temperature: Temperature significantly affected T Acy, chlorophyll b, and total chlorophyll, but not chlorophyll a (Table 2). A highly significant negative correlation between cumulative temperature (^{o}/day) and T Acy indicated that total amount of heat (cold) regulates T Acy production regardless of whether the heat (cold) occurs during the day or night. We hypothesize that an increase in T Acy with cold temperature may be related to stress induced ethylene synthesis. Although we did not measure C_2H_4, its production under stress likely activates the phenylalanine ammonia lyase enzyme (7,15), an integral part of the Acy biosynthetic pathway (11,38). It is possible to consider increased foliar Acy in marigolds as an example of chilling induced ethylene generated Acy synthesis. There appeared to be no strong correlation between T Acy and T Chl in the foliage (r=.55) indicating that T Acy does not appear to increase by competition for substrate with chlorophyll production confirming other work (14,27).

Light-Temperature Interaction: Experimental data and regression coefficients were recorded for T Acy and T Chl for points on the composite design (Table 3). T Acy was temperature dependent and the lowest values for T Acy occurred at the highest day temperatures (Fig. 2). However the highest values of T Acy occurred at the upper QFD range regardless of night temperature. Acy synthesis is light mediated (21,16) and phytochrome appears to be the only pigment involved in its synthesis (5,26,31). Plants synthesize more Acy in the light compared with dark (21) and as light was increased to 600 $\mu E\ m^{-2}s^{-1}$, more T Acy was formed. It is unlikely that 600 $\mu E\ m^{-2}s^{-1}$ was sufficient to trigger the HIR involved with T Acy synthesis and therefore light appears to be less important for Acy production compared with temperature in this study. Trends appeared to be the same for all night temperatures although as night temperatures rose, a wider range of QFD and day temperature cause the lowest response.

Response plots of T Chl appear to be both light and temperature dependent (Fig. 3). Minimum levels of T Chl occurred only at 10 and 26°C nights. At 10°C nights, maximum T Chl occurred at high QFD and low day temperatures whereas at 26°C nights, warm day temperatures and high or low QFD resulted in the same effect. High T Chl occurred in wheat (30) at 15 or 28°C with high light, however high temperatures (32°C) in hybrid geranium resulted in solarization of chlorophyll (1). Other work with hybrid geraniums has shown that very high QFD (>1000 $\mu E\ m^{-2}s^{-1}$) resulted in less chlorophyll than at low light levels (36). We were not able to attain sufficiently high light levels to test those findings with marigolds.

We found no significant correlation between T Acy in leaf tissue and phosphorus, potassium, or a combination of these nutrients ($r=.26-.46$). The data indicate that nutrient uptake was sufficient however under conditions beneficial to T Acy synthesis, utilization or translocation of these nutrients may have been reduced. The synthesis of Acy reported as a result of nutrient deficiencies may be more realistically explained on the basis of stress ethylene. Nutrient deficiency and low temperature may simply be secondary causes of anthocyanin synthesis.

The practical ramifications of this work are significant as growers lower night temperatures to conserve fuel. Lower temperatures increase anthocyanin levels which change the hue of the plant. Energy conservation has resulted in an increase in polyethylene-type growing structures and there is less incident light reaching the plant compared with glass structures. This will cause lower chlorophyll levels regardless of temperature.

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Table 1. Treatment combinations of day and night temperatures and quantum flux density.[z]

Treatment	Quantum Flux Density ($\mu E\ m^{-2}s^{-1}$)	Day Temperature (C)	Night Temperature (C)
1	50	21	21
2	150	15	15
3	150	15	26
4	150	26	15
5	150	26	26
6	375	21	10
7	375	10	21
8	375	32	21
9	375	21	32
10	375	21	21
11	600	15	15
12	600	15	26
13	600	26	15
14	600	26	26

z Treatments chosen according to (2).

Table 2. The effect of temperature on foliar anthocyanin and chlorophyll content in 'Petite Yellow' marigold.

Temperature (C)		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Total[1] Anthocyanin
Day	Night	(mg dm^{-2})	(mg dm^{-2})	(mg dm^{-2})	(mg g^{-1})
10	21	3.08 a[z]	1.37 ab	4.45 ab	1.68 a
21	10	2.57 a	1.12 c	3.69 bc	0.65 b
21	21	3.14 a	1.12 c	4.26 a	0.24 c
21	32	3.62 a	1.29 b	4.91 a	0.20 c
32	21	3.75 a	1.46 a	5.21 a	0.01 d

[1] Cyanidin 3-glycoside equivalents (max.λ =532 nm)

[z] Mean separation in columns by HSD (.05)

Table 3. Total chlorophyll, total anthocyanin, phosphorus and potassium levels in 'Petite Yellow' marigold foliage.

Treatment	Total Chlorophyll ($mg\ dm^{-2}$)	Total Anthocyanin ($mg\ g^{-1}$)	Phosphorus (ppm)	Potassium (ppm)
1	2.59	0.09	10537	4390
2	3.15	0.43	13108	4013
3	3.15	0.30	12133	5545
4	4.03	0.23	10032	4725
5	5.26	0.17	10160	4411
6	3.97	0.19	8790	3859
7	3.47	0.59	7654	2739
8	4.87	1.43	11034	4299
9	4.91	0.05	10011	4234
10	4.19	0.28	6953	2659
11	6.57	0.76	14290	4380
12	5.46	0.78	10701	4190
13	5.78	0.66	11232	5645
14	5.98	0.68	11276	4969

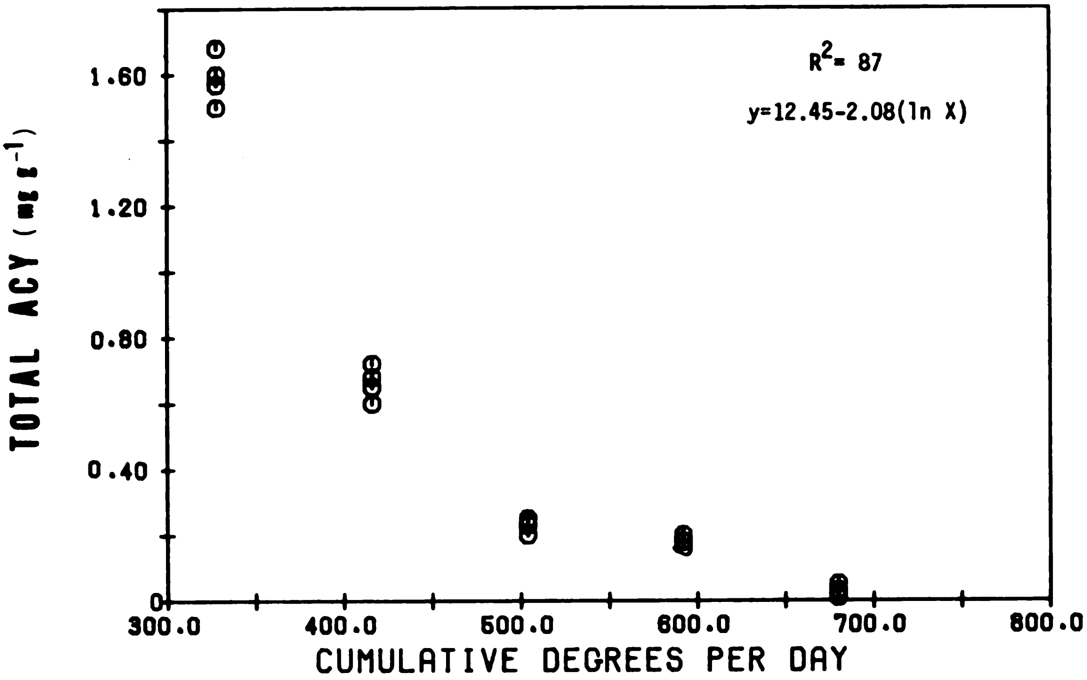


Figure 1. The effect of cumulative temperature on total anthocyanin (Total Acy) content in leaves of marigold 'Petite Yellow'.

Figure 2. Effect of day temperature and quantum flux density at various night temperatures on the total anthocyanin content in leaves of marigold 'Petite Yellow'.

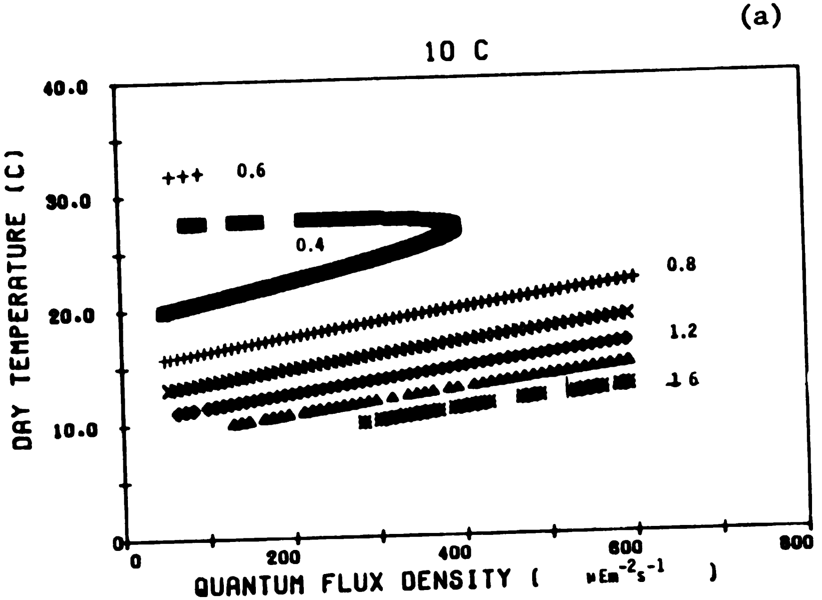
a) Night temperature 10°C

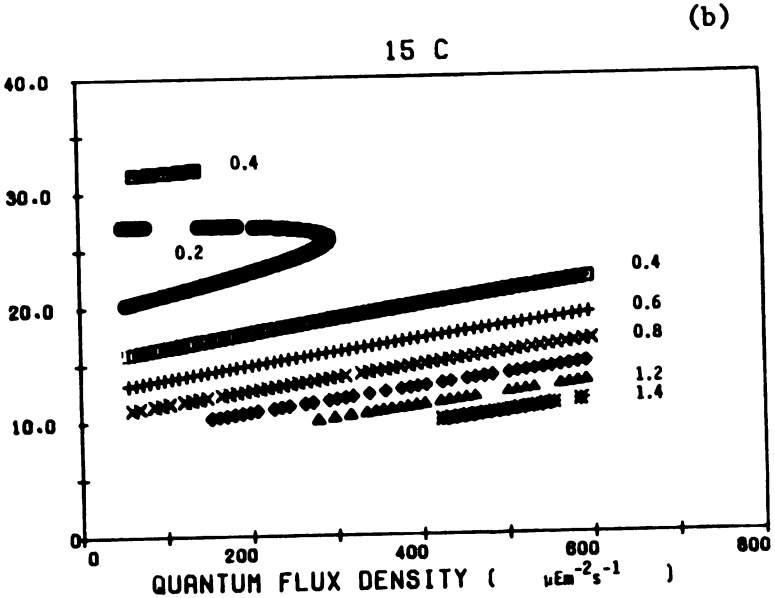
b) Night temperature 15°C

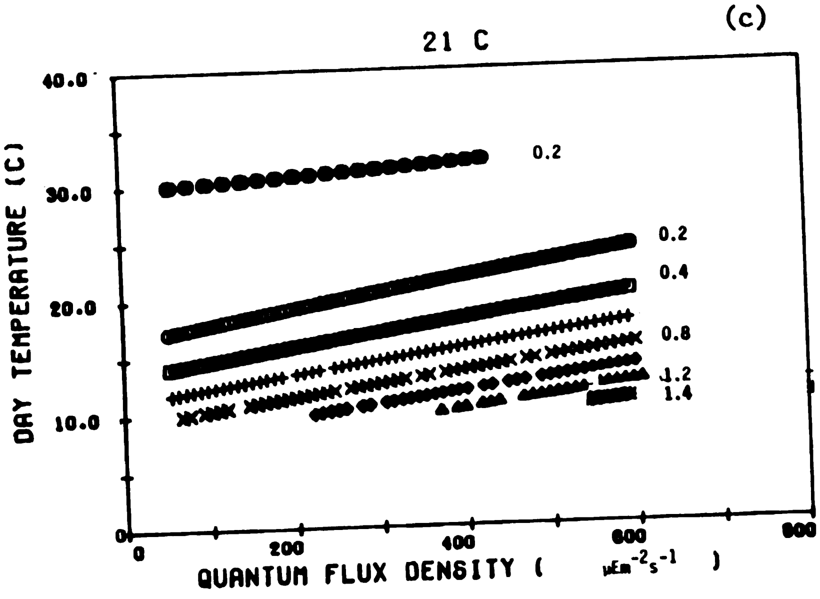
c) Night temperature 21°C

d) Night temperature 26°C

Numbers associated with each isoquant are total anthocyanin (mg g^{-1}). Each isoquant differs by 0.2 mg g^{-1}.


(a)
10 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
200
400
600
800
QUANTUM FLUX DENSITY ($\mu Em^{-2}s^{-1}$)
+++ 0.6
0.4
0.8
1.2
1.6


(b)
15 C
40.0
30.0
20.0
10.0
0
200
400
600
800
QUANTUM FLUX DENSITY ($\mu Em^{-2}s^{-1}$)
0.4
0.2
0.4
0.6
0.8
1.2
1.4


(c)
21 C
DAY TEMPERATURE (C)
40.0
30.0
20.0
10.0
0
200
400
600
800
QUANTUM FLUX DENSITY ($\mu Em^{-2}s^{-1}$)
0.2
0.2
0.4
0.8
1.2
1.4

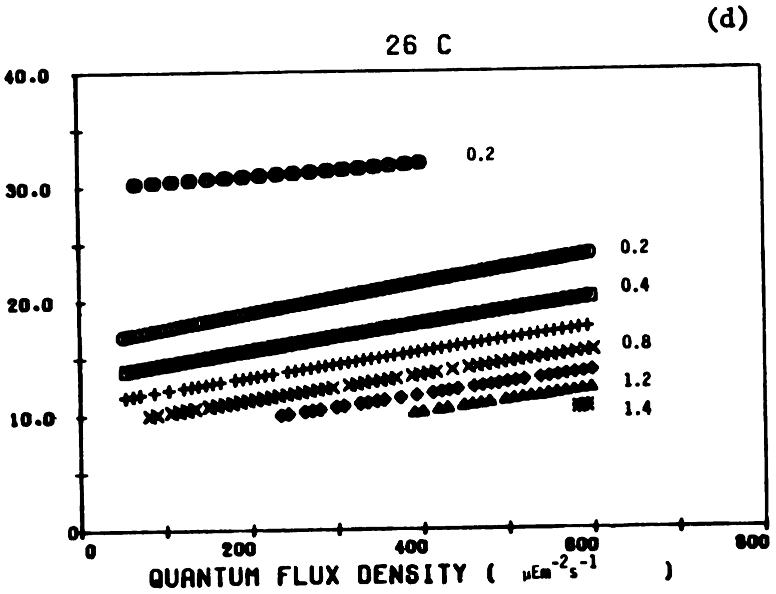

(d)
26 C
40.0
30.0
20.0
10.0
0
200
400
600
800
QUANTUM FLUX DENSITY ($\mu Em^{-2}s^{-1}$)
0.2
0.2
0.4
0.8
1.2
1.4

Figure 3. Effect of day temperature and quantum flux density at various night temperatures on the total chlorophyll content in leaves of marigold 'Petite Yellow'.

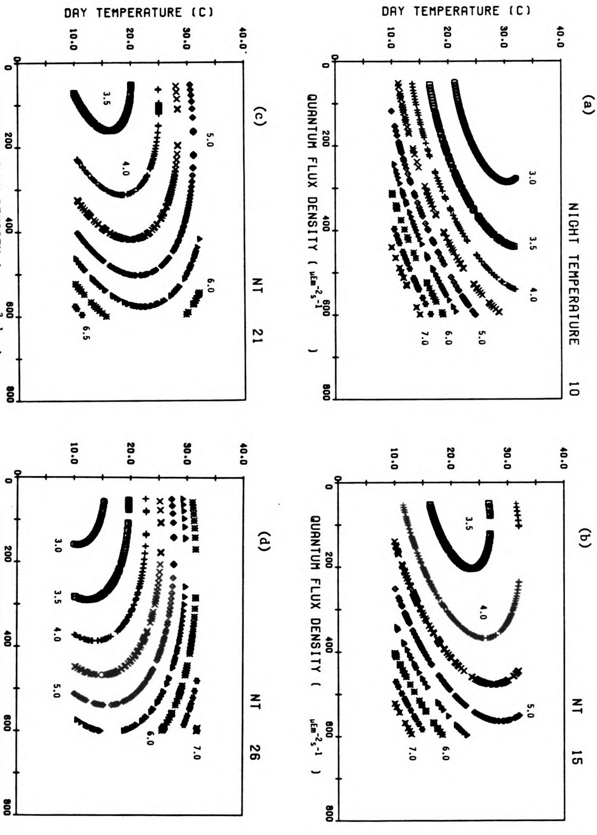
a) Night temperature 10°C

b) Night temperature 15°C

c) Night temperature 21°C

d) Night temperature 26°C

Numbers associated with each isoquant are total chlorophyll (mg dm^{-2}). Each isoquant differs by 0.5 mg dm^{-2}.


NIGHT TEMPERATURE
(a) 10
(b) NT 15
(c) NT 21
(d) NT 26
DAY TEMPERATURE (C)
QUANTUM FLUX DENSITY (μEm^{-2}s^{-1})
0
200
400
600
800
10.0
20.0
30.0
40.0
3.0
3.5
4.0
5.0
6.0
6.5
7.0

SECTION IV

FLOWERING POTENTIAL IN HYBRID GERANIUMS AS A RESULT OF EARLY HEAT AND LIGHT TREATMENT

FLOWERING POTENTIAL IN HYBRID GERANIUMS AS A RESULT OF EARLY HEAT AND LIGHT TREATMENT

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Abstract: Temperatures of 32-35°C combined with 350-800 $\mu E\ m^{-2}s^{-1}$ photosynthetically active quantum flux density reduced flowering time, height, and number of flowers per inflorescence of *Pelargonium* X *hortorum* Bailey compared with greenhouse grown plants under northern winter conditions. Three and 5 week plants were more responsive to treatments than 7 week plants. Treatments lasting 9 days reduced flower time compared with 3 or 6 day treatments however all treatments which decreased flower time were likely unmarketable.

Most cultivars of hybrid geranium require at least 100 days to flower under northern greenhouse conditions (1,7) and up to 150 days under winter conditions. Time to visible bud is dependent on light while temperature controls time from visible bud to anthesis (2). Supplemental light decreased flower time in many studies (4,6,11) and was most effective in the seedling stage when applied for at least 4 weeks (3,5). The role of temperature in flowering has received most attention in vernalization studies (9,10) in biennials and perennials but little has been done with annuals. Temperature affects the latter stages of flower development however there are no reports of the effect of temperature on flowering when applied during seedling development.

High temperature in early stages of geranium development caused thermal breakdown of chlorophyll (2) and eventual death but also resulted in early bud initiation.

This study investigated the effects of high temperature (26-37°C) and quantum flux density (70-1500 μE $m^{-2}s^{-1}$) combinations, duration of treatment, and plant age on flowering in hybrid geraniums.

Seeds of hybrid geranium 'Sooner Red' were germinated under intermittent mist in a soilless media. In a preliminary study (experiment 1), 10 day old plants were placed in growth chambers at 375 ± 10 μE $m^{-2}s^{-1}$ and 10, 21, or 32 ± 2°C constant temperature. Cool white fluorescent lights provided a 16 hr photoperiod for 4 weeks. Plants were placed in the greenhouse on February 15, 1979 with day-night temperatures of 21 ± 5, 18 ± 2°C and were allowed to flower. Fertilization with 20-16-12.2 (N-P-K) liquid fertilizer per irrigation provided 200 ppm N. Control plants were grown continuously in the greenhouse. The time to visible bud (<0.5 cm diameter) and time to first flower anthesis were recorded. Tukey's ω test was used for mean separation (HSD) (15).

In the second experiment, 3, 5, or 7 week old plants were placed in growth chambers for 10 days starting December 7, 1979 to provide the following constant temperature and light combinations (°C, μE $m^{-2}s^{-1}$): treatment 1: 32, 70; treatment 2: 32, 800; treatment 3: 36, 300; treatment 4: 36, 300; treatment 5: 36, 1500. High pressure sodium lights for high light levels were suspended above polyethylene chambers equipped with thermostatically controlled forced air heaters to maintain desired temperature. Temperature fluctuated ± 3°C and light ± 20 μE $m^{-2}s^{-1}$. After treatment, plants were placed in the greenhouse (21 ± 5 day, 18 ±

2^oC night) with control plants. The time to visible bud and first flower anthesis, flower diameter, number of flowers per inflorescence, and vegetative and total height were measured. Treatment means were compared with control by Tukey's ω test.

To determine proper treatment duration (experiment 3), 5 week old plants were placed at constant 26, 32, or 35 ± 2^oC and 375 ± 15 (high), 70 ± 10 (medium), or 8 ± 2 μE $m^{-2}s^{-1}$ (low) for 3, 6, 9, or 12 days in growth chambers similar to those used in experiment 1. Plants treated for 3 days were placed in the greenhouse April 6, 1980 and every 3 days until treatments were completed. Greenhouse day temperatures fluctuated with ambient temperatures and ranged from $20-30^oC$ and night temperatures were 18 ± 3^oC. Time to first flower, vegetative and total height were measured and treatment means compared with control plants by Tukey's ω test.

Experiment 1. Time to visible bud (VB) and days to flower were reduced at 32 and increased at 10^oC (Table 1). Under normal greenhouse temperatures ($18-26^oC$), bud initiation in hybrid geraniums is primarily dependent on light, however these data indicate that temperatures outside this range also influence initiation. High temperature treatment may have caused transition from vegetative to reproductive habit through nutrient mobilization (12), breakdown of flower inhibitors (16), or hormone redistribution (8,13,14). Early temperature treatments caused destruction of chlorophyll in all leaves but did not affect flower development time.

Experiment 2. Temperature and light combinations of 32^oC-800 μE $m^{-2}s^{-1}$ and 35^oC-300 μE $m^{-2}s^{-1}$ reduced flowering time compared with control plants regardless of age, however 3 and 5 week plants flowered earlier than 9 week plants (Table 2). High temperature (>35°C) and high light (>1000 μE $m^{-2}s^{-1}$) caused death of young plants while 7 week plants were less susceptible to damage. Plants treated with 32°C-70 μE $m^{-2}s^{-1}$ did not flower earlier than control plants regardless of age. In general, vegetative and total height, number of flowers per inflorescence, and peduncle length were reduced compared with control for treatments which accelerated flowering time. Flower diameter was reduced significantly only in 7 week plants at 35°C, 300 μE $m^{-2}s^{-1}$ compared with control. Although flowering time was reduced at 32-800 and 35°C, 300 μE $m^{-2}s^{-1}$, the decrease in the number of flowers per inflorescence seriously limits its marketability.

Experiment 3. Thirty-seven degrees and 12-15 days killed most plants regardless of light level. High light levels reduced flowering time more than medium or low light at both temperatures (Table 3). In general, there was no difference in flower time due to duration. Reduction in flowering time occurred at 3 and 9 days at high temperature, high light but flowering occurred in less than 90 days at 3 and 6 days of low temperature, high light. These results indicate that high light may be solely responsible for flower acceleration, however the duration of light was too short to affect flower time (3,5). The combination of high light and high temperature appeared to control the beginning of the flowering process. Treatments which caused earlier flowering caused shorter peduncle lengths and generally were unmarketable. Reduction of flowering time was not evident in experiment 2 which may have been due to seasonal differences. Greenhouse grown control plants started on December 12 (experiment 2) required 141 days to flower while control plants sown on April 4 flowered in 95 days. The response to temperature, light treatments may have been overcome when plants were grown under the more favorable environmental conditions.

This study indicates that early heat treatment supplemented with irradiation above 300 μE $m^{-2}s^{-1}$ may affect flowering time many months after treatment and demonstrates the potential for early flowering in hybrid geranium. At this point the results appear to be too erratic and inconsistent to understand the role of temperature and light in flower acceleration. Although supplemental light has been shown to be necessary for 4 to 6 weeks to reduce flowering time, the plant appears to be more responsive to light when subjected to high temperatures. All treatments which reduced flowering time resulted in unmarketable plants. This does not negate the potential importance of this study, but underlines the necessity of continued research along similar lines. A short pre-transplant treatment which would reduce flowering time without a reduction in quality could revolutionize the production of hybrid geraniums and possibly other bedding crops.

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Table 1. The effect of four weeks of constant temperature treatment on 10 day old hybrid geranium 'Sooner Red'.

Quantum Flux Density ($\mu E\ m^{-2} s^{-1}$)	Temperature (^{o}C)	Days to Visible Bud	Days to Flower
375	10	91 c[z]	112 c
375	21	68 b	95 b
375	32	33 a	65 a
Control		81 bc	105 bc

[z] Mean separation by Tukey's ω test (.05)

Table 2. The effect of temperature, light combinations and plant age on growth and flowering of hybrid geranium 'Sooner Red'.

Treatment		Plant	Days to	Days to	Flower	No. of	Height	
Temp (^{o}C)	Light (μE $m^{-2}s^{-1}$)	Age (wk)	Visible Bud	Flower	Diameter (cm)	Flowers	Veg (cm)	Total (cm)
32	70	3	115	132	4.5	25	17.5	26.0
		5	120	137	4.3	23	17.3	25.2
		7	121	139	4.3	20	16.8	24.8
32	800	3	85	115	4.3	17	18.8	24.0
		5	78	112	4.6	34	18.0	26.5
		7	109	129	4.2	12	17.5	25.5
35	300	3	61	97	4.5	14	15.0	24.8
		5	80	106	4.2	14	15.2	23.4
		7	93	119	3.6	12	12.0	19.5
35	1000	3,5	died					
		7	109	132	4.0	14	19.7	28.7
35	1500	3,5	died					
		7	102	123	4.0	15	17.1	35.8
Control			124	140	4.5	36	24.5	35.8
HSD (.05)			10	10	0.7	8	3.4	4.8

Table 3. The duration effect of temperature, light treatments on flowering and height of 5 week hybrid geranium 'Sooner Red'.

Temperature (^oC)	Light Level	Duration (days)	Days to Flower	Height Veg (cm)	Height Total (cm)	Peduncle Length (cm)
32	high	3	88.2	16.2	21.4	5.3
		6	93.8	18.2	23.8	5.6
		9	76.3	11.0	14.7	3.7
	medium	3	92.8	18.2	23.9	5.7
		6	93.3	17.0	22.3	5.3
		9	96.5	16.3	22.3	5.9
	low	3	91.8	18.2	23.9	5.7
		6	96.2	14.3	20.7	6.3
		9	99.3	15.0	22.2	7.1
26	high	3	89.3	13.6	22.1	8.5
		6	89.3	15.2	21.4	6.2
		9	91.5	14.7	22.1	7.4
	medium	3	93.6	14.6	23.7	9.1
		6	90.6	14.8	23.4	8.6
		9	91.7	15.8	22.1	6.2
	low	3	94.8	15.3	24.0	8.7
		6	97.0	14.2	18.0	3.8
		9	96.0	15.0	22.3	7.3
Control			95.0	18.9	24.3	6.4
HSD (.05)			6.9	4.3	4.6	5.4