

THERMOMORPHOGENESIS IN LILIUM LONGIFLORUM¹

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ABSTRACT

Stem elongation and leaf orientation in *Lilium longiflorum* Thunb. were influenced more by the difference (DIF) between day temperature (DT) and night temperature (NT) than absolute DT or NT from 14 to 30 C. Plant height and internode length increased 129 and 382%, respectively, as DIF (DT-NT) increased from -16 to 16 C as compared to only 15 and 58% when either DT or NT was increased from 14 to 30 C, respectively. Leaf orientation, defined as the angle between a line perpendicular to the stem and the line from the leaf base to the leaf tip, increased 43° (leaves became more upright) as DIF increased from -16 to 16 C. In contrast to plant height, internode length, and leaf orientation, leaf and flower length were influenced more by absolute temperature than DIF. Leaf and flower length decreased 32 and 14%, respectively, as NT increased from 14 to 30 C. DT had little effect on either leaf or flower length. The influence of DIF on stem elongation suggested that thermomorphogenesis was not a function of total plant carbohydrate or carbohydrate translocation. Instead, DIF appeared to influence the endogenous gibberellin content or the response of plant tissue to gibberellin. Similarities between thermomorphogenic plant responses and photomorphogenic plant responses suggested that these two processes may be related with respect to their perception and/or transduction.

GROWTH is thermoperiodic in many plant species (Dorland and Went, 1947; Went, 1953; Viglierchio and Went, 1957; Hellmers and Sundahl, 1959; Groves and Lang, 1970; Erwin and Heins, 1985; Karlsson and Heins, 1986). For instance, plant height is greater when plants are grown with day temperatures (DT) warmer than night temperatures (NT) in a wide range of plant species including *Lycopersicon* (Went, 1944; 1945), *Phaseolus* (Viglierchio and Went, 1957), *Chrysanthemum* (Karlsson and Heins, 1986), and *Capsicum* (Dorland and Went, 1947). Other plant characteristics which respond to diurnal changes in temperature are flower size (Karlsson and Heins, 1986), leaf shape (Fischer, 1954; Njoku, 1957), and leaf orientation (Erwin and Heins, 1985).

Plant height in *L. longiflorum* Thunb. cv. Ace was influenced by DT and NT (Wilkins, 1973). Lily plants grown with a 32 C DT and 16 C NT from the visible bud stage to anthesis were 149% taller than plants grown with a 16 C DT and 32 C NT (Wilkins, 1973). The cv. Nellie White responded similarly to temperature, with respect to plant height as cv. Ace (Erwin and Heins, 1985). In addition, leaf size, flower size, and leaf orientation were also in-

fluenced by DT and NT with cv. Nellie White (Erwin and Heins, 1985). In contrast, plant height of *Lilium longiflorum* Thunb. cv. Croft was not greatly influenced by DT or NT between 10 and 27 C (Smith and Langhans, 1961).

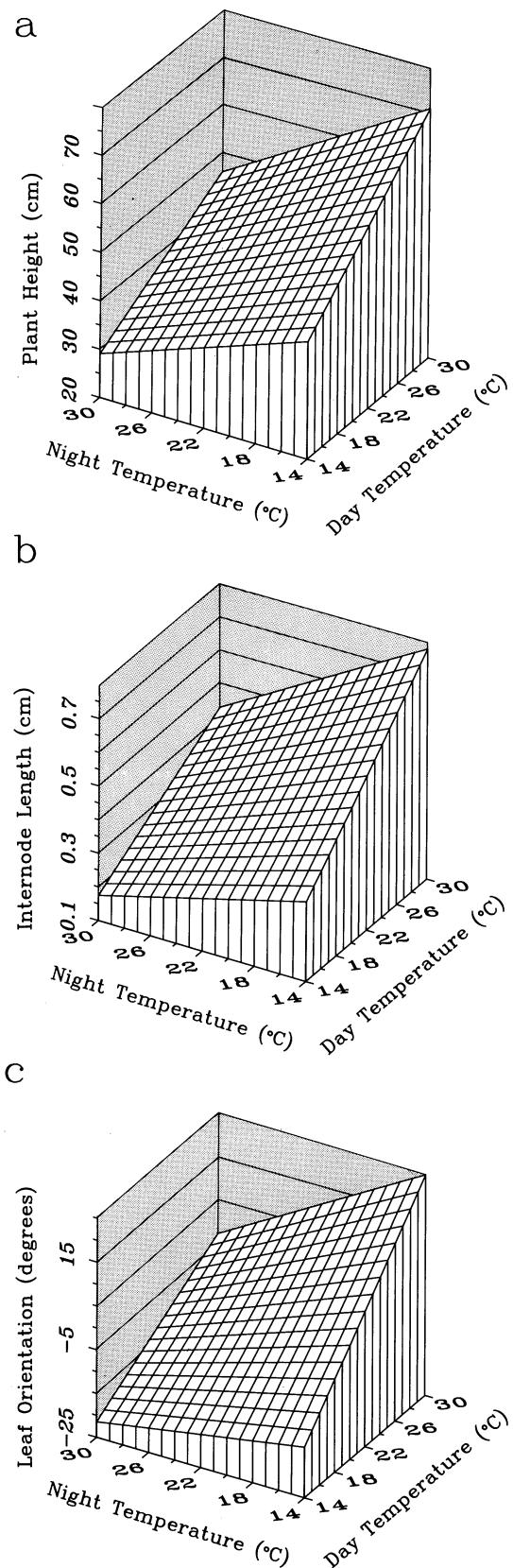
Morphological responses to temperature will be referred to as *thermomorphogenic* in this paper. The term is derived from the Greek derivatives *therme*, meaning heat; *morphos*, the quality of having form; and *gignesthai*, to be born. Hence, *thermomorphogenesis*, the effect of temperature on plant morphogenesis. The term thermomorphogenesis is consistent with the term photomorphogenesis which describes the effect of light on plant morphogenesis.

The objective of this study was to quantify thermomorphogenic responses in *Lilium longiflorum*. In the process of determining morphogenic responses to temperature, we wished to gain some insight into what processes may control thermomorphogenic responses.

MATERIALS AND METHODS—*Lilium longiflorum* Thunb. cv. Nellie White bulbs 17.7–20.3 cm in circumference were planted in 15.2 cm plastic pots on 28 October 1985 in soilless medium consisting of equal parts of sphagnum peat, perlite, and vermiculite (1:1:1). Potted bulbs were placed in a controlled environment greenhouse for two weeks where air temperature was adjusted to maintain a medium temperature of 17 C ± 1 C to encourage root development. Plants were then vernalized in the dark for 6 wk at 5 C. Following vernalization,

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all plants were placed in a greenhouse under natural photoperiodic conditions with constant 20 C DT and NT. Upon shoot emergence, plants received a long day treatment for 7 days consisting of night interruption lighting from 2200 to 0200 hr delivered with incandescent lamps at 2 micromol sec⁻¹ m⁻² (400–700 nm wavelength). After the long day treatment, plants were returned to natural photoperiodic conditions (ca. 9 hr, 15 min light span).

Time of flower initiation was established by terminal shoot dissections on randomly selected plant samples starting 13 January 1986. Plant samples were taken every 3 days. Flower initiation was defined as the first visible sign of a reproductive meristem (De Hertogh, 1976, figure 2c). Flower initiation was observed on 100% of the sample on 22 January. One hundred twenty-five plants were then selected for uniformity based on plant height and leaf number and moved to greenhouses with temperature setpoints of 14, 18, 22, 26, or 30 C. Actual average temperatures during the experiment did not vary by more than 1.8 C from the desired temperature setpoints. Plants were moved among greenhouse sections at 0800 and 1800 hr each day to yield a total of 25 DT/NT treatment combinations. Movement of plants required approximately 30 min. An opaque curtain was pulled over the plants after the plants were moved at 1800 and was retracted just prior to 0800 to provide a 14-hr scotoperiod to parallel the night temperature treatment. Plants were spaced to provide 900 cm² per plant.

During 1987, a group of *Lilium longiflorum* Thunb. cv. Nellie White plants were grown as specified above. At flower initiation, 6 groups of 10 plants each were placed in controlled environment greenhouses maintained at 15, 20, and 25 C. Each group of plants was rotated among greenhouses to yield a total of 9 DT/NT temperature treatments. Within each temperature treatment the plants were divided into 2 groups of 5 plants each. One group was grown as a control. The other group received two applications of 0.25 mg ancymidol (alpha-cyclopropyl-alpha-(4-methoxyphenyl)-5-pyrim-



Fig. 1. Response surface plots generated from predicted final plant height (a), internode length (b), and leaf orientation (c) on *Lilium longiflorum* Thunb. cv. Nellie White as influenced by day and night temperature. Surfaces were based on the regression functions: (a) $1.48602 \cdot DIF + -0.0416 \cdot DT \cdot NT + 1.91394 \cdot AVG\ TEMP + 25.661$ ($r^2 = 0.84$), (b) $0.0223117 \cdot DIF + -0.000752 \cdot DT \cdot NT + 0.0390916 \cdot AVG\ TEMP - 0.0652671$ ($r^2 = 0.82$), and (c) $1.80309 \cdot DIF + -0.07495 \cdot DT \cdot NT + 4.02815 \cdot AVG\ TEMP - 55.18$ ($r^2 = 0.68$), respectively.

idinemethanol) 7 and 14 days after flower initiation to the plant apex. Ancymidol was applied using a Labsystems Finnpipette Dispenser (20–200 µl) as ten 100 µl droplets.

Data were collected at anthesis (terminal flower) on total plant height, leaf number, aborted and non aborted flower number, flower length, leaf length, and leaf orientation. Plant height was defined as the height of the plant from the soil line to the tip of the uppermost pedicel. Internode length was calculated by dividing stem length by leaf number. Leaf number was constant, since plants had initiated flowers prior to placement in the experimental environments. Leaf orientation was defined as the angle between a line perpendicular to the stem and a line from the leaf base to the leaf tip. A 0° leaf orientation indicated a horizontal leaf orientation. Similarly, a positive angle of leaf orientation indicated hyponastic, or upward leaf orientation, and a negative angle of leaf orientation indicated an epinastic, or downward leaf orientation.

Data were statistically analyzed as a 5×5 factorial design with DT and NT as the main factors for the 1986 data. Data were statistically analyzed as a split plot design with DT and NT as the main factors and ancymidol concentration as the subplots in the 1987 data. The ANOVA subroutine of the "Statistical Package of the Social Sciences" (Nie, 1975) was used for analysis of variance. The "All Possible Subsets Regression (P9R)" and the "Stepwise Regression (P2R)" subroutines of the "Biomedical Statistical Software Package" (Dixon, 1983) were used for multilinear regression analysis.

RESULTS AND DISCUSSION—DT and NT influenced plant height in opposite ways. Plant height increased 64% as DT increased from 14 to 30 C with NT held at 14 C. Plant height decreased 29% as NT increased from 14 to 30 C with DT held at 14 C. (Fig. 1a; Table 1).

DT and NT also interacted to influence plant height. The influence of NT on final plant height increased as DT increased. Increasing NT from 14 to 30 C decreased plant height 12.5 cm (29%) when the DT was 14 C and 21.1 cm (30%) when the DT was 30 C. In contrast, the influence of DT on final plant height decreased as NT increased. Increasing DT from 14 C to 30 C increased plant height 27.8 cm (64%) when the NT was 14 C and 19.2 cm (61%) when the NT was 30 C. The percent increase in plant height due to increasing DT was not influenced by NT and vice versa.

The relationship between DT and NT influenced final plant height to a greater extent than

TABLE 1. *Influence of day and night temperature on Lilium longiflorum cv. Nellie White plant height, internode length, and leaf orientation*

Night temperature (C)	Day temperature (C)				
	14	18	22	26	30
Plant height (cm)					
14	43.8	54.6	62.2	68.4	71.6 ^a
18	40.5	45.8	57.0	60.8	63.5
22	31.8	42.4	44.4	50.8	50.8
26	30.2	39.0	41.2	43.6	51.2
30	31.3	33.8	41.0	42.2	50.5
Internode length (cm)					
14	0.31	0.48	0.60	0.68	0.82
18	0.25	0.38	0.56	0.64	—
22	0.23	0.31	0.46	0.48	0.54
26	0.22	0.27	0.41	0.45	0.46
30	0.17	0.20	0.36	0.38	0.49
Leaf orientation (degrees) ^b					
14	-5.9	-5.0	11.6	18.9	26.3
18	-10.0	-17.9	5.8	11.9	14.7
22	-24.2	-10.2	-<>> Y	2.7	6.1
26	-20.7	-13.3	-1.4	-2.6	8.3
30	-16.7	-13.7	-12.5	-7.3	1.7

^a Values represent the experimental means. The greatest SD was 7.2 cm, 0.1 cm, and 15.2 degrees for plant height, internode length, and leaf orientation, respectively.

^b Angle of the leaf, in degrees, between a line perpendicular to the stem and a line connecting the leaf tip to the leaf base.

DT, NT, or average temperature. Plants grown with a NT warmer than DT were consistently shorter than plants grown with equal DT and NT while plants grown with the NT cooler than the DT were taller (Table 1). Plant heights were similar when the relationship between DT and NT was the same (Table 1). For example, the plants shown in Fig. 2 were all grown with a NT 4 C warmer than the DT. All the plants had similar plant heights at anthesis (40.5, 42.4, 41.2, and 42.2 cm, respectively) despite the very different average temperatures associated with each temperature treatment. Similar final heights occurred on other plants with similar relationships between DT and NT. Plant height increased as DT increased relative to NT. Plants grown with a 14 C DT/30 C NT were 40.3 cm shorter than plants grown with a 30 C DT/14 C NT temperature regime. The importance of the relationship between DT and NT on plant height is consistent with results of Lecharny, Schwall, and Wagner (1985) who suggested that the difference in temperature between the day and night was critical in determining the rate of stem elongation and/or phase resetting of the stem elongation circadian rhythm in *Chenopodium rubrum*.

The relationship between DT and NT was

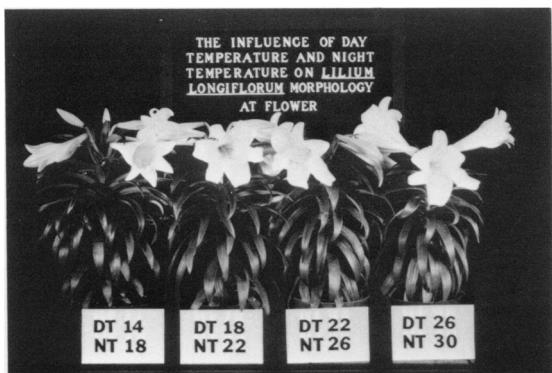


Fig. 2. Appearance of *Lilium longiflorum* Thunb. cv. Nellie White at anthesis when grown under four temperature regimes with day temperatures (DT) 4°C cooler than night temperatures (NT). Plants grown at higher average temperatures flowered earlier than plants grown at cooler average temperatures. As plants grown at higher temperatures reached anthesis, they were placed in a cooler (4°C) until plants grown at cooler temperatures reached anthesis. Stem elongation did not occur in the cooler. When all plants had reached anthesis, the photograph was taken.

described in regression analysis as the difference in temperature between DT and NT, i.e., DT minus NT (DIF). The DIF term was useful in that it described the difference between DT and NT and carried a sign to indicate whether DT or NT was greater. The importance of DIF in determining plant height was shown when it was evaluated independently as a linear function of plant height; DIF accounted for 78% of the variability in plant height among treatment plants.

TABLE 2. Influence of day and night temperature on *Lilium longiflorum* cv. Nellie White leaf and flower length

Night temperatures (C)	Day temperature (C)				
	14	18	22	26	30
Leaf length (cm) ^a					
14	18.2 ^b	21.0	20.8	20.2	19.6
18	17.0	18.0	19.5	18.4	19.1
22	16.6	16.7	16.7	17.8	16.8
26	15.6	14.7	15.7	15.3	15.0
30	12.4	13.3	13.8	14.0	13.6
Flower length (cm) ^c					
14	17.7	17.8	17.9	18.4	16.5
18	17.5	18.6	17.7	17.2	17.2
22	17.6	17.4	17.7	16.9	16.5
26	16.5	16.8	16.7	16.7	16.4
30	15.3	15.8	16.8	16.3	16.0

^a Length from the point of attachment of the leaf to the leaf tip.

^b Values represent the experimental means. The greatest SD was 1.1 cm for both leaf and flower length.

^c Length from the point of attachment of the petal to the petal tip.

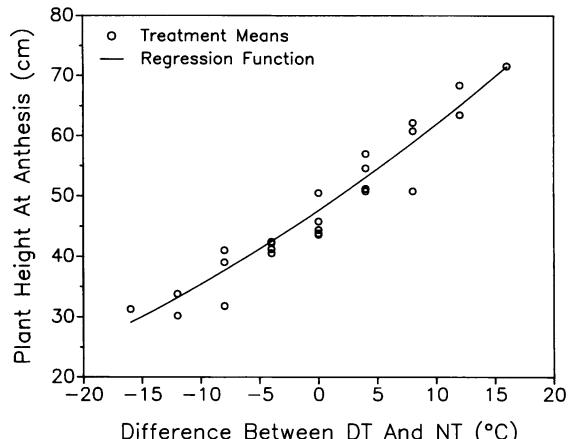


Fig. 3. Relationship between *Lilium longiflorum* Thunb. cv. Nellie White plant height at anthesis and the difference (DIF) between the day temperature (DT) and night temperature (NT). Squares represent mean plant heights for each temperature treatment. The solid line represents the regression function $1.4860 \cdot DIF - 0.0416 \cdot DT \cdot NT + 1.9139 \cdot AVG\ TEMP + 25.661$ ($r^2 = 0.84$). The regression line also represents the effect of both the day temperature by night temperature interaction and the effect of average temperature on final plant height.

Internode length responded to DT and NT in a similar fashion as plant height (Fig. 1b; Table 1). As DIF increased from -16 to 16°C, internode length increased 382% (0.65 cm). No difference in internode length was observed between internodes which matured early in plant development as opposed to late in plant development.

As with plant height and internode length, the relationship between DT and NT influenced leaf orientation of *Lilium longiflorum* (Table 1) to a greater extent than absolute DT and NT. An increase in DIF from -16 to 16°C increased leaf orientation 43°.

Leaf and flower length were influenced more by absolute DT and NT than DIF. Leaf length was primarily influenced by NT (Fig. 4). As NT increased from 14 to 30°C with a 14°C DT, leaf length decreased 32% (5.8 cm) (Table 2). DT had little influence on leaf length. These results contrast results of Friend and Pomeroy (1970) on *Triticum* where leaf length first increased as temperature increased from 10 to 25°C then decreased with temperatures above 25°C. The differences in response of *Lilium* and *Triticum* leaf length to temperatures above 25°C may be due to different temperature optima for leaf growth in these two species.

Both DT and NT influenced flower length (Table 2). NT had a greater effect on flower length than DT. As NT increased from 14 to 30°C with DT held at 14°C, flower length de-

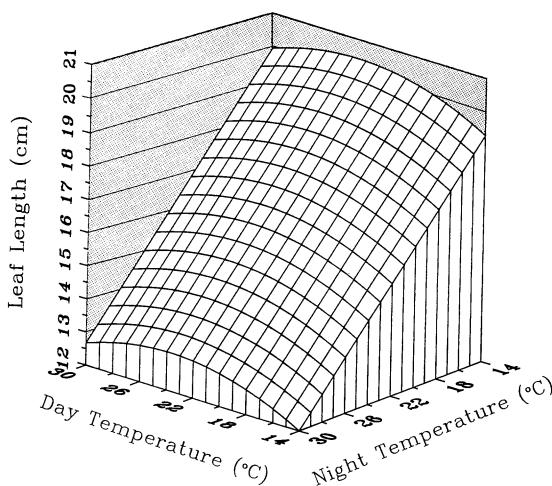


Fig. 4. Response surface generated from predicted final leaf length of *Lilium longiflorum* cm. Nellie White as influenced by day and night temperature. Predicted leaf length was based on the regression function $0.581132 \times DT + -0.012254 \times DT^2 + -0.425519 \times NT + 19.49$ ($r^2 = 0.72$).

creased 39% (2.4 cm). In contrast, as DT increased from 14 to 30°C with NT held at 14°C, flower length decreased 15% (1.2 cm).

The results presented in this paper define thermomorphogenic responses in *L. longiflorum*. Went and Bonner (1943) suggested that thermomorphogenic stem elongation in *Lycopersicon esculentum* resulted from an alteration in the carbohydrate status in the elongating region of the stem. Thermomorphogenic stem elongation responses of *Dendrothema grandiflora* (Chrysanthemum) (Karlsson and Heins, 1986) and *Lilium* are similar. The similar thermomorphogenic responses of these two species showed that a supplemental carbohydrate source such as the *Lilium* bulb does not influence stem elongation. The effect of temperature on *Lilium* stem elongation was not affected by increasing or decreasing the irradiance which plants were grown under between 50 and 400 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (Erwin and Heins, unpublished data). The lack of differential thermomorphogenic responses of *L. longiflorum* to irradiance and the lack of a differential response to temperature between *Dendrothema* and *Lilium* suggested that total carbohydrate availability within the plant is not the primary factor responsible for the stem elongation response to temperature as Went and Bonner (1943) had suggested.

Our results would be compatible with Went and Bonner's (1943) work if carbohydrate availability were limited by translocation. Translocation has been shown to increase exponentially in *Glycine* as temperature in-

TABLE 3. The effect of ancymidol and the day/night temperature regime on *Lilium longiflorum* Thunb. plant height at anthesis

Temper- ature regime (°C)	DIF	Plant height (cm)	Plant height 0.50 mg ancymidol spray	Percent reduction
25/15	10 ^a	48.2 ^b	39.1 ^c	19 ^{*d}
20/15	5	46.5	36.5	22 ^{**}
15/15	0	36.0	30.4	16 ^{**}
20/20	0	35.8	31.2	13 [*]
15/20	-5	29.3	29.7	0 ns
15/25	-10	29.1	30.1	-3 ns

^a Numerals represent day temperature minus night temperature.

^b Numerals represent the treatment mean.

^c Ancymidol was applied as two spray applications of 0.25 mg each. Applications were made 7 and 14 days after flower initiation.

^d Significant at $P = 0.05$ (*); $P = 0.01$ (**); not significant (ns).

creased from 5 to 40°C (Marowich, Richter, and Hoddinol, 1986), i.e., translocation responds to absolute temperature. If carbohydrate translocation were the limiting factor in determining the stem elongation response to temperature in *Lilium*, stem elongation should have increased or decreased as DT and NT increased with a constant DIF. As is seen in Fig. 2, this was not the case. In addition, flower bud abortion should have been negatively correlated with plant height since flower bud abortion is sensitive to carbohydrate depletion (Einer and Box, 1967). This was not the case ($r^2 = 0.03$). It is, therefore, unlikely that thermomorphogenic stem elongation responses are a result of carbohydrate availability and/or carbohydrate translocation.

It is more likely that the effect of DT and NT (DIF) on *Lilium* stem elongation is mediated through differences in hormone synthesis or action, probably gibberellin. Preliminary results of experimentation studying the effect of temperature on stem elongation responses to a gibberellin biosynthesis inhibitor, ancymidol (Moore, 1979), suggested that there is a strong interaction between DIF and the endogenous levels of gibberellin within *Lilium* (Table 3). The effect of the ancymidol application on stem elongation decreased as DIF decreased. In addition, application of GA₄₊₇ can overcome the inhibition of stem elongation induced by a negative DIF (N. Zieslin, personal communication).

Morphological characteristics of *Lilium* grown with a large positive DIF were similar to morphological characteristics of plants with low phytochrome photoequilibria, i.e., grown

under far-red light (Holmes and Smith, 1977). Similarly, the morphology of *Lilium* grown with a negative DIF were similar to morphological characteristics of plants with high phytochrome photoequilibria, i.e., grown under red light. Also, thermomorphogenic behavior appears to be much greater in plants which are highly photoperiodic (e.g., *L. longiflorum*, *Dendrothema grandiflora*, *Euphorbia pulcherrima*, *Fuchsia hybrida*) than plants which are "day neutral" (e.g., *Tulipa hybrida*, *Narcissus pseudonarcissus*, *Cucumis sativa*) (Erwin and Heins, unpublished data). The similarity in thermomorphogenic and photomorphogenic responses suggested that these two processes may be related with respect to their perception and/or transduction. Investigations have been initiated to determine the possible interactions between phytochrome and thermomorphogenic behavior in plants.

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