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Quantifying Temperature-controlled Leaf Unfolding Rates in 'Nellie White' Easter Lily

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Abstract. **The rate of leaf unfolding was determined for Easter lily (*Lilium longiflorum* Thunb.) 'Nellie White' grown at day and night temperatures ranging from 14° to 30°C. In this temperature range, rate of leaf unfolding was a linear function of average daily temperature; i.e., the effect on rate of leaf unfolding for day temperature was the same as for night temperature. The function determined was: leaves unfolded per day = −0.1052 + (0.0940 × average daily temperature). Isopleth plots were developed to describe day and night temperatures required for specific rates of leaf unfolding under 8-, 10-, and 12-hr day temperature periods.**

Greenhouse forcing of vernalized Easter lilies traditionally has been divided into three time phases. These phases are a) placement of vernalized potted bulbs in the greenhouse to flower initiation, b) flower initiation to visible bud, and c) visible bud to opening of the first flower (5).

One or more of the phases must be manipulated in order to time the lily crop properly, since the date of Easter can vary by 35 days over years. The plants usually are placed in the greenhouse in mid- to late December, and flower initiation occurs at the end of phase I in mid- to late January (5). Phase I normally does not vary greatly in length, as the recommended temperatures during this phase fall into a narrow range (23). The length of the third phase is usually controlled at 30 to 35 days under commercial greenhouse conditions (5). The second phase is then adjusted by temperature manipulations to properly time the lily crop (23).

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The wholesale marketing period for the Easter lily is 10 days prior to Easter. Plants not in flower by Easter have little market value; therefore, timing is extremely critical. Blaney et al. (3) and Wilkins and Roberts (24) introduce the leaf-counting technique as a method to improve lily timing during phase II. When using the leaf-counting technique, the required leaf unfolding rate is calculated at the beginning of phase II based on plants reaching visible bud 30 to 35 days prior to the desired flowering date. The actual rate of leaf unfolding is then monitored and temperature is adjusted to produce the desired leaf unfolding rate. Initial temperatures selected are normally based on grower experiences with previous lily crops.

Information on quantitative relationships between the environment and rate of lily leaf unfolding is limited (9, 17, 18, 22). Photosynthetic photon flux (PPF) and photoperiod have been shown to be minor factors affecting leaf unfolding rates and time of development (10, 11). Grueber et al. (9) reported on leaf unfolding rates for lilies forced in growth chambers for a wide range of temperatures; however, no functional relationship was presented. Wang and Roberts (22) reported that leaf unfolding rates were proportional to air temperature in the range of 13° to 24°C day temperature (DT) and 10° to 18° night temperature (NT). In a subsequent study, an 18° constant temperature was reported to be optimal, although higher temperatures were not investigated (17). Smith and Langhans (18) concluded that NT had a greater effect on leaf unfolding rate than DT, but both were important in determining time to flower. The larger response to NT was probably due to the long nights (15.5 hr) maintained in their experiment. The authors did not report leaf unfolding rates in relation to temperature.

Stem elongation in Easter lily is highly correlated with the difference between DT and NT in the range from 10° to 30°C (6, 12). Decreasing the difference between DT and NT from a large positive value (+15°) to a large negative value (−15°) resulted in progressively shorter internodes. Modifying traditional DT and NT relationships from higher DT than NT to a lower DT than NT can be a technique for controlling stem elongation in the Easter lily. This technique, however, nullifies previous grower experience with regulating leaf unfolding rates. Therefore, the purpose of the present study was to develop a quantitative model of temperature-driven leaf unfolding rates in Easter lily using practical combinations of DT and NT.

Materials and Methods

Lily bulbs 17.7 to 20.3 cm in circumference were potted 28 Oct. 1985 in 15.2-cm plastic pots filled with a 1 sphagnum peat : 1 perlite : 1 vermiculite (by volume) medium. The potted bulbs were then placed in the greenhouse, where a soil temperature of 17° ± 1°C was maintained to encourage root development. After 14 days, the bulbs were placed at 5° ± 0.5° for 6 weeks. The plants were then returned to the greenhouse under a 20° constant DT and NT and natural photoperiod until emergence. Plants were exposed to a PPF of 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ from incandescent lamps from 2200 to 0200 HR for 7 days after emergence to ensure programming of bulbs to flower (23). Plants were maintained under natural photoperiodic conditions (lat. 42°N, − 9 hr, 15 min light span on 1 Jan.) until the experimental treatments started.

Time of flower initiation was established by terminal dissections on random samples from a population of 200 plants. Floral initiation was defined as any visible sign of the vegetative meristem differentiating into floral primordia. The first sample with 100% differentiation occurred on 22 Jan. On that date, 125 plants were selected for uniformity and placed in one of five greenhouse sections maintained at a constant temperature of 14°, 18°, 22°, 26°, or 30°C. Plants were moved among greenhouse sections every day at 0800 and 1800 HR to provide five levels of DT and five levels of soft NT. Each of the resulting 25 treatments had five plants. Movement of all plants normally required 30 min, and the plants were randomized in the greenhouse sections during each move. An opaque curtain was pulled immediately after the plants were moved at 1800 HR and was retracted just prior to 0800 HR to provide a 14-hr scotoperiod to parallel the night temperature period. Plants were spaced to provide 900 cm^2 per pot and otherwise grown using standard cultural recommendations (23).

Greenhouse temperatures were controlled using a greenhouse climate control computer (Oglevee Computer Systems, Connellsville, Pa.) and monitored by a datalogger (Digistrip III, Kaye Instruments Co., New Bedford, Conn.) using iron/constantan thermocouples. Temperatures in each greenhouse section were measured every 10 sec by the datalogger and averaged to provide hourly mean temperatures. All plants were within 2

Table 1. Setpoints and actual average temperatures for each leaf counting period.

Setpoint night temp (°C)	Setpoint day temperature (°C) 14	18	22	26	30
		24 Jan. to 2 Feb.			
14	13.7	15.2	17.0	18.7	20.7
18	15.4	17.0	18.9	20.5	22.5
22	18.1	19.7	21.5	23.2	25.2
26	20.0	21.6	23.4	25.0	27.1
30	23.0	24.6	26.4	28.0	30.1
		3 to 12 Feb.			
14	13.7	15.1	16.5	18.2	20.2
18	15.8	17.2	18.5	20.2	22.3
22	17.9	19.3	20.6	22.3	24.4
26	20.0	21.4	22.7	24.4	26.5
30	22.6	24.0	25.8	27.0	29.1

Table 2. Mean number of *Lilium longiflorum* 'Nellie White' leaves unfolded per day in response to day and night temperature treatments. Plants were exposed to day and night temperatures for 10 and 14 hr, respectively.

Setpoint night temp (°C)	Setpoint day temp (°C) 14	18	22	26	30
		24 Jan. to 2 Feb.			
14	1.0	1.2	1.2	1.3	1.2
18	1.3	1.3	1.5	1.7	1.7
22	1.7	2.1	2.1	2.2	2.1
26	1.9	2.0	2.1	2.0	2.4
30	2.1	2.2	2.5	2.5	2.4
Significance					
DT	***				
NT	***				
DT × NT	NS				
		3 to 12 Feb.			
14	1.1	1.3	1.4	2.1	2.4
18	1.4	1.7	1.9	2.3	2.4
22	1.5	1.7	1.7	2.0	2.6
26	1.6	1.9	1.7	2.1	2.5
30	2.0	2.1	2.0	2.4	2.8
Significance					
DT	***				
NT	***				
DT × NT	NS				

Regression analysis within each leaf counting period for the function $Y = b_0 + b_1$ *(average daily temperature)*

	24 Jan. to 2 Feb.		
b_0			−0.2073
b_1			0.0972
	3 Feb. to 12 Feb.		
b_0			0.0009
b_1			0.0908
Comparison of slopes	F = 0.69	(df = 1,246)	NS
Comparison of intercepts:	F = 6.00	(df = 1,247)	*

*, ***, NS Significant at the 5% and 0.1% levels and not significant, respectively.

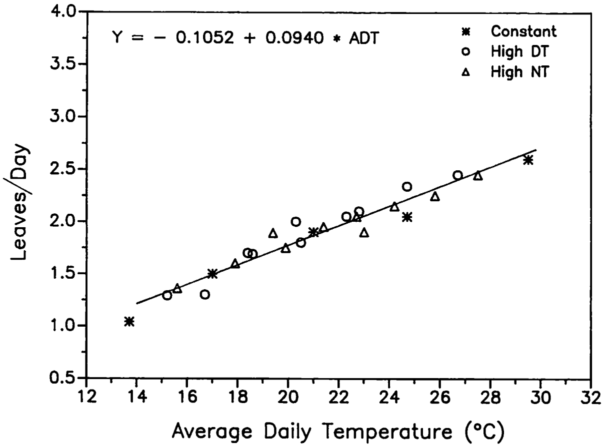

Fig. 1. Number of *Lilium longiflorum* 'Nellie White' leaves unfolded per day with a higher day than night temperature, with a higher night than day temperature, and with the same day and night temperatures. The regression equation was calculated using treatment means averaged over leaf counting periods and average daily temperature (ADT) as the independent variable.

m of the temperature sensors during the experiment. Average DT and NT for each data collection period were calculated from the hourly means and used in the analysis (Table 1).

Each plant was marked to indicate the number of leaves unfolded at the beginning of the experiment. A leaf was considered unfolded when the entire length of the leaf had visibly separated from the main stem. Newly unfolded leaves were recorded every 5 days. Data collection was terminated when visible bud was reached.

Data analyses were performed initially by combining the data recorded every 5 days into 10-day periods. Measurement errors were reduced when averaged over 10 days compared to a 5-day period, since unfolded leaves were recorded to a whole leaf. The functional relationship between temperature and leaf unfolding rates was determined by multiple linear regression analysis (14). Statistical analysis of differences between slopes and elevations among regression equations followed the procedure by Snedecor and Cochran (19). Isopleth plots were created by using the selected function and the Surfer graphing program (8).

Results

The mean numbers of leaves unfolded per day during the leaf counting periods are given in Table 2. We initially determined that DT and NT acted independently on the rate of leaf unfolding. Analysis of variance (ANOVA) within each leaf counting period showed DT and NT to be highly significant factors, whereas the interaction between DT and NT was not significant (Table 2). Forward multiple linear regression analyses on data from the two leaf counting periods were then conducted using linear and quadratic DT and NT terms and interaction terms. The resulting two equations only contained DT and NT as separate independent variables. These analyses indicated that DT and NT acted independently on rate of leaf unfolding.

Leaf unfolding rates over time were studied by comparing the regression equations developed for the two leaf counting periods. Analysis of variance could not be used to compare leaf unfolding rates between periods as actual greenhouse temperatures varied for similar treatments (Table 1). Since DT and NT acted independently, average daily temperature (ADT) was used as the independent variable in this comparison. The slopes of the equations were not significantly different when compared with a two-tailed F-test, whereas the intercepts were significantly different at the 5% level (Table 2). The results of these tests indicated the two equations were statistically parallel with different elevations. Calculation of predicted leaf unfolding rates in the 14° to 30°C range showed a maximum difference in predicted leaf unfolding rate of 0.12 leaves/day. This difference is horticulturally insignificant, and leaf unfolding rate was therefore considered constant during phase II.

The difference in the length between the DT (10-hr) and the NT (14-hr) required further analysis to determine if temperature

Table 3. Regression coefficients calculated to predict number of *Lilium longiflorum* 'Nellie White' leaves unfolded per day using the function $Y = b_0 + b_1$ (average daily temperatures).

Coefficient	Treatments with higher DT[z]	Treatments with higher NT[y]	Treatments with same DT and NT
b_0	−0.3380	0.1001	−0.1566
b_1	0.1072	0.0844	0.0932
r^2	0.95	0.94	0.98
Comparison of slopes			
High DT vs. high NT	F = 2.37	(df = 1196)	NS
High DT vs. constant temp	F = 1.00	(df = 1146)	NS
High NT vs. constant temp	F = 0.59	(df = 1146)	NS
Comparison of intercepts			
High DT vs. high NT	F = 1.58	(df = 1197)	NS
High DT vs. constant temp	F = 5.45	(df = 1147)	*
High NT vs. constant temp	F = 3.95	(df = 1147)	NS

[z]Day temperature.
[y]Night temperature.
*, NSSignificant at the 5% level and nonsignificant, respectively.

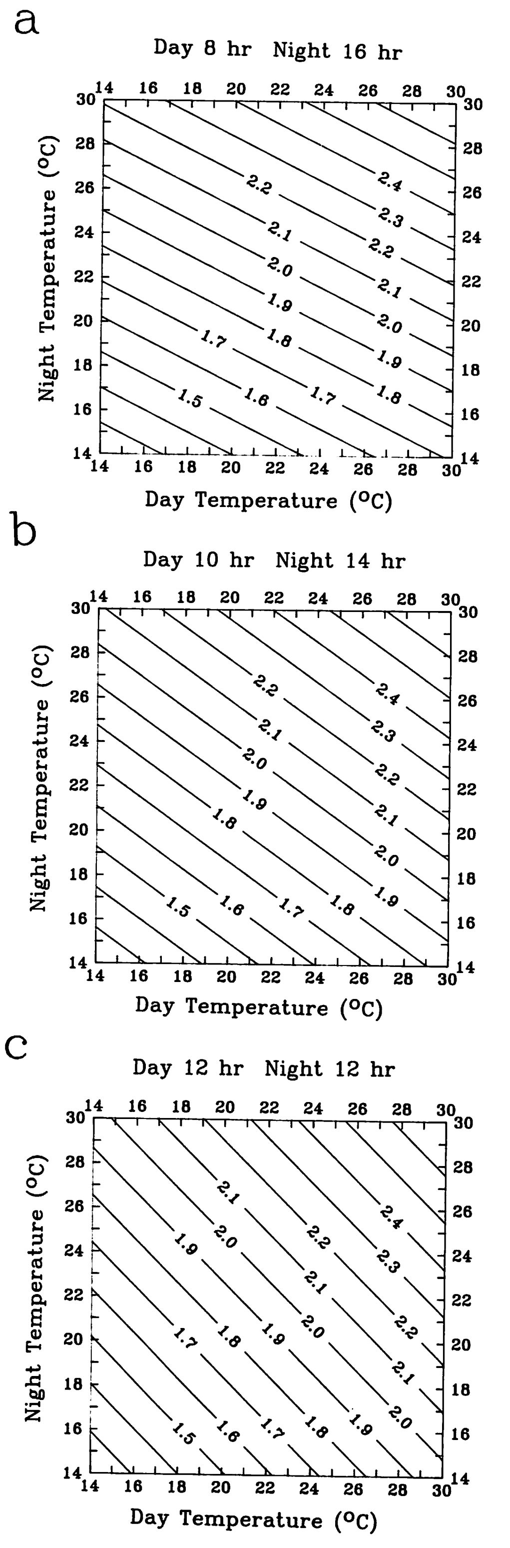

Fig. 2. Isopleth plots showing number of leaves unfolded for combinations of day and night temperatures between 14° and 30°C in *Lilium longiflorum* 'Nellie White' at (**a**) 8 hr, (**b**) 10 hr, and (**c**) 12 hr of day temperature.

delivered to a plant during the night had a greater effect on leaf unfolding rate than the same temperature delivered during the day. The treatments were separated into groups with higher DT than NT, higher NT than DT, and constant DT and NT for regression analysis using ADT as the independent variable (Fig. 1). The analysis resulted in regression equations with no significant difference in slopes and only one instance of the intercepts being significantly different (Table 3). These results suggested that the method of daily temperature delivery to a plant did not significantly influence the rate of leaf unfolding.

Since DT and NT did not exhibit different effects on leaf unfolding rates, the ADT would be expected to summarize the leaf unfolding response to temperature. A regression analysis using the ADT term as the independent variable resulted in an equation with the same r^2 and level of significance as the equation with DT and NT as separate independent variables. The two equations were equal predictors of leaf unfolding rates. ADT also was found to be highly significant ($P < 0.001$) in a separate ANOVA using data from both leaf counting periods. This analysis indicated ADT was an appropriate predictor of leaf unfolding rate.

Regression analysis was conducted with ADT as the independent variable using raw data (250 values), treatment means for each leaf counting period (50 values), or treatment means averaged over the two leaf counting periods (25 values) with ADT as the independent variable. The three resulting regression equations predicted leaf unfolding rates with similar slopes (maximum deviations among slopes was 0.0004) and intercepts (maximum deviation among intercepts was 0.0092). The predicted leaf unfolding rate per day varied by a maximum of 1/100 of a leaf among the three equations in the 14° to 30°C temperature range. Coefficients of determination for the three equations were 0.71 (raw data), 0.73 (mean of treatments), and 0.94 (means averaged over leaf counting periods). The following functional relationship developed from treatment means averaged over leaf counting periods was selected to describe the leaf unfolding response to temperature in Easter lily (Fig. 1):

$$\text{Leaves per day} = -0.1052 + (0.0940 \times \text{ADT}).$$

Discussion

Average temperature responses for leaf unfolding rates have been determined for several plant species. Leaf emergence in sunflower was linearly related to mean temperature in the range of 22° to 32°C DT and 12° to 22° NT (16). A positive linear relationship between leaf emergence and temperature was also found for wheat (7). The rate peaked at 25° but declined as the temperature rose to 30°. Leaf appearance in maize was "nearly linear" in the range of 12° to 26° (20). Likewise, lily leaf unfolding rate was linear in the 14° to 30° range.

Degree days are often used to predict growth when ADT is the major factor influencing rate of plant development (20). Degree days are calculated by totaling daily temperatures greater than a base temperature below which no development occurs. Even though a curvilinear relationship between temperature and rate of development is often observed, linear relationships are assumed in most thermal unit systems (1, 4, 13, 15, 21). Although temperature response curves tend to be curvilinear, the degree day or average temperature concept is useful when the temperature range encountered has an approximate linear relationship to plant development.

A 1°C increase in ADT will unfold 0.094 additional leaves per day in Easter lily, compared to 0.022 leaves for sunflower (16), 0.067 for maize (20), and 0.020 for pea (2). The calculated temperature at which the number of lily leaves unfolded per day equaled zero was 1.12°. However, since extrapolation of the function outside the range of experimental data was necessary to find this value, and the intercepts were significantly different between the two leaf counting periods, the true base temperature may vary from this calculated value. A degree day relationship for lily leaf unfolding was considered inappropriate, since the base temperature was not known with precision and since greenhouse temperatures can be maintained with reasonable accuracy when forcing lilies.

Comparing leaf unfolding rates predicted by the developed model with data from the literature is difficult. Smith and Langhans (18) forced 'Croft' lilies under 25 DT/NT combinations, but leaf number and leaf unfolding rates were omitted from the paper. Although Wang and Roberts (22) reported the rate of leaf unfolding to be proportional to air temperature, an ADT cannot be calculated from their paper, since the lengths of day and night were not reported. The total number of leaves unfolded in 1 month was reported by Roberts et al. (17) for the constant temperatures of 6°, 12°, and 18°C. Assuming a 30-day month, the calculated leaf unfolding rates for their work were 0.63, 0.93, and 1.33 leaves/day, respectively. The predicted rates using our model are 0.46, 1.02, and 1.59 leaves/day. Note the values calculated for 6° and 12° are outside of our experimental range. Leaf unfolding rates published by Grueber et al. (9) for lilies grown under temperatures in the range 15° to 27° were similar to the leaf unfolding rates predicted using our model. The deviations between our predictions and their observed values were −0.09 to 0.29 leaves/day.

A commercial model system for lily forces was created by developing isopleth graphs (Fig. 2). The number of leaves subtending the flower and necessary leaf unfolding rate to achieve visible bud on a certain date for timing can be determined immediately after flower initiation. Using the model, the DT and NT required to obtain the desired leaf unfolding rate with DT periods of 8, 10, or 12 hr can be found in Fig. 2. The selection of any daylength is possible, since the lily responded to ADT. A wide range of DT and NT combinations can be chosen to achieve a particular leaf unfolding rate. As an example, when 1.6 leaves/day must be unfolded, combinations vary from 24.5° DT/14°C NT to 14° DT/21.5° NT for a 10-hr DT period and 14-hr NT period. Choosing different temperature combinations along this isoquant line would influence plant morphology greatly (6, 12), while not altering leaf unfolding rate.

Leaf unfolding in Easter lily was found to be a function of ADT, with DT and NT having equal effects. The developed quantitative relationship between temperature and leaf unfolding rate was incorporated into a model system allowing determination of available temperature combinations for a desired rate of leaf unfolding. This model system is a tool to assist lily producers in accurate timing of lily crops for Easters at different dates.

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