PREDICTING VARIABILITY IN ANTHESIS OF EASTER LILY (Lilium longiflorum Thunb.) POPULATIONS IN RESPONSE TO TEMPERATURE

P.R. Fisher
Department of Plant Biology
University of New Hampshire
Durham, New Hampshire 03824
U.S.A.

J. H. Lieth
Department of Environmental Horticulture
University of California
Davis, California 95616-8587
U.S.A.

R.D. Heins
Department of Horticulture
Michigan State University
East Lansing, Michigan 48824-1325
U.S.A.

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Abstract

Most thermal time models for ornamental plant species are deterministic, i.e. they predict the timing of a phenological event (for example anthesis) for the average of a population. In ornamental plant production, the variability and distribution of harvest, in addition to the mean, is important for planning resources (e.g. labor, cooler space, shipping). The objective was to predict the distribution of anthesis dates for a population of Easter lilies (Lilium longiflorum Thunb.). ‘Nellie White’ grade 8/9 Easter lilies were grown at two research greenhouse locations during 1996 and 1997 under a variety of temperature and cooling regimes. The mean and standard deviation in thermal time from visible bud to anthesis was estimated at 792 ± 62°Cd with a base temperature of -4.5°C. The elongation of flower buds over thermal time was quantified using an exponential function, with an R² of 0.99. As bud length increased, the variability in thermal time to anthesis decreased, i.e. the standard deviation in thermal time to anthesis was negatively correlated with bud length. Combining the model of visible bud to anthesis and the bud elongation function allowed prediction of the distribution of anthesis dates. On a single date, the developmental stage (visible bud, or a particular flower bud length) was recorded for a sample of plants. The combined model was then run for each developmental stage, to predict when each stage would reach anthesis. Predicted anthesis dates for each stage were combined in order to predict the anthesis distribution for the entire population.

1. Introduction

Commercial production of Lilium longiflorum Thunb. (Easter lily) in North America requires precise temperature control so that the crop will flower in time for Easter sales. Due to the commercial importance of this crop, there has been substantial work to quantify the influences of bulb cooling and the greenhouse climate during forcing on development rate of Easter lilies.

The relationship between temperature and development rate has been quantified with ever greater precision in order to reduce commercial financial losses associated with early or late crops (Erwin and Heins, 1990; Fisher and Heins, 1996; Fisher, et al., 1996; Fisher, et al., 1997a; Fisher, et al., 1997b; Healy and Wilkins, 1984; Karlsson, et al., 1988; Lieth and Carpenter, 1990). In contrast to a close relationship between development rate and temperature, Heins, et al. (1982a,b) found that light intensity
between the visible bud and anthesis stages had little or no effect on lily development rate or flower quality.

Existing models quantifying the development of Easter lily are deterministic, i.e. they predict the average growth stage of the crop over time. In commercial production, however, the distribution of anthesis dates (i.e. the proportion of plants harvested on any particular date) of the entire population is most important, rather than the mean or median harvest date. Easter lilies that flower early require additional handling, use cooler space, and may decline in quality, whereas Easter lilies that do not flower by Easter have little market value.

The objective was to quantify the distribution of anthesis dates for Easter lily at different temperatures, by predicting the thermal time to anthesis for earlier stages in development. Prediction and control of the distribution of anthesis dates using greenhouse temperature has the potential to achieve an optimum harvest distribution for available resources including cooler space, labor, and shipping.

2. Materials and Methods

2.1. Experimental designs

‘Nellie White’ Easter lilies (grade 8/9, i.e. with bulbs 20-23 cm in circumference) were grown at two greenhouse locations (Michigan State University, East Lansing, MI (MSU); and University of New Hampshire (UNH)). Timing of visible bud (the date when at least one immature flower bud is first externally visible on a plant without moving leaves) and anthesis (when petals on at least one flower crack open) were recorded daily for each plant, and bud length was measured twice each week. Air temperature was logged with an aspirated thermocouple (MSU) or a thermistor (UNH) placed 30 cm above canopy height. Fifteen-minute average temperatures were averaged over 24 hours to calculate average daily temperature (ADT in °C).

**MSU population:** Bulbs were planted in MetroMix 510 media (Scotts, Marysville, Ohio) in 15-cm diameter pots in October 27 and were placed in a cooler at 4.5°C. Fifty plants were removed from the cooler on December 12 and placed in a commercial greenhouse (Grand Rapids, Michigan) where they were grown at 13-16°C until January 1, 17-18°C until flower initiation in mid-January, and 20-21°C until February 16. Plants were then moved to an MSU greenhouse, and ADT was maintained at 20.3±0.1°C (means are reported ± 95%).

**UNH population:** Bulbs were case-cooled at 4.4°C in redwood sawdust from October 23 1996 until forcing. Bulbs were removed from the cooler and planted in 15-cm diameter pots with Pro-Mix BX (Premier Horticulture, Red Hill, PA) on November 20 (40 bulbs, 4 weeks cooling), December 4 (120 bulbs, 6 weeks pre-cooling), December 18 (40 bulbs, 8 weeks cooling), and January 2 (40 bulbs, 10 weeks cooling). Greenhouse nysctoperiod was maintained at 12 hours until March 23 after which plants were exposed to natural night lengths. Plants were grown at a daily average temperature of 17.3 ± 0.4°C until March 22. Plants that had received 6 weeks cooling were then randomly separated into three groups of 40 plants and were moved into three greenhouses with different ADT (16.4±0.6°C, 18.9±0.2°C, and 20.9±0.4°C) where they remained until anthesis. Plants receiving 4, 8, and 10 weeks cooling were grown at 18.9±0.2°C from March 22 until anthesis.

2.2. Analysis

**Visible bud to anthesis:** Erwin and Heins (1990) investigated the relationship between the number of days from visible bud to anthesis and ADT for ‘Nellie White’ Easter lilies. Five plants per treatment were grown in greenhouses with a 14-hour nysctoperiod at 25 day/night temperature combinations ranging from 14 to 30°C.
The x-intercept method (Arnold, 1959) was used to calculate thermal time from visible bud to anthesis. The effective temperature \( T_e \), in °C, was calculated using Eq. [1], where air temperature \( T \), in °C, above a maximum temperature \( T_{\text{max}} \), in °C, was assumed to have an equal and maximal effect on development rate (the reciprocal of days to anthesis). In the Erwin and Heins (1990) experiment, development rate increased as temperature increased to 26°C, and did not increase from 26°C to 30°C. We therefore assumed that \( T_{\text{max}} \) equalled 26°C.

\[
T_e = \begin{cases} 
T & \text{for } T \leq T_{\text{max}} \\
T_{\text{max}} & \text{for } T > T_{\text{max}}
\end{cases}
\]  

(1)

The linear regressions of mean development rate on \( T_e \) were compared between the Erwin and Heins (1990) and UNH data sets using the analysis of covariance method described by Snedecor and Cochran (1989, p.390). The variances, intercepts, and slopes were not significantly different between data sets. In order to improve the statistical estimation of \( T_b \), we therefore combined data from UNH, Erwin and Heins (1990), and MSU (one temperature only) in one analysis. Linear regression of mean development rate on \( T_e \) allowed estimation of the base temperature and thermal time to anthesis \( (t_a, \text{ in degree days (°C Cd)}) \) for the visible bud stage.

The thermal time from visible bud to anthesis was further analyzed to estimate how many individual plants within a population would flower per degree day. Thermal time was calculated using logged temperature data for each individual plant in the MSU and UNH populations from the equation

\[
t = \sum_{j=1}^{r} \max\{T_{e,j} - T_b, 0\} \Delta h_j
\]

(2)

where \( T_{e,j} \) is the effective average temperature over the period \( j \), and \( \Delta h_j \) is the length of period \( j \) (1/96 day, i.e. 15 minutes). Standard deviation, skewness and kurtosis were analyzed for the individual plant data, using the methods described by Snedecor and Cochran (1989, p.79).

**Bud elongation**: An exponential function was used to describe flower bud length \( B \) (in mm) at thermal time \( t \) (°Cd) under constant temperature conditions, given an initial bud length measurement of \( B_0 \) (mm) at time \( t_0 \)

\[
B = B_0 e^{k(t-t_0)}
\]

(3)

where the parameter \( k \) has units of °C⁻¹d⁻¹. At anthesis, Easter lily flowers consistently open at a maximum bud length \( B_f \) (in mm) of around 160 mm in length (Healy and Wilkins, 1984; Fisher, et al., 1996). Eq. [3] can therefore be modified as

\[
B_f = B e^{k t_a}
\]

(4)

and \( t_a \) can be calculated by

\[
t_a = \frac{\ln \left( \frac{B_f}{B} \right)}{k}
\]

(5)

combined into one large data set containing bud lengths, average temperatures, and days to anthesis. Thermal time to anthesis was calculated for each bud measurement, assuming the same base temperature calculated for the thermal time from visible bud to anthesis. The \( k \) parameter (Eq. [5]) was estimated using the PROC NLIN nonlinear regression procedure in SAS (SAS Institute, 1988).

Bud lengths from Fisher, et al. (1996) were rounded to the nearest 1-cm, and the standard deviation of \( t_a \) was calculated for each bud length. Skewness and kurtosis of the distribution of \( t_a \) were analyzed for each bud length in order to check for normality. A function was fit to the standard deviations at different bud lengths, to quantify the change in variability in \( t_a \) as bud length increased. This variance function was combined with Eq. [5] to predict the mean and standard deviation of harvest dates for each length class.

**Combined model:** The visible bud and bud elongation functions were combined into an overall model to predict the anthesis distribution for the population. The model required data in the form of a frequency distribution of the number of plants at each of seventeen developmental stages ((1) before visible bud; (2) at visible bud; (3) 2-cm buds, (4) 3-cm buds, (5) 4-cm buds, (16) 15-cm buds; and (17) the number of plants at anthesis). These data would be collected at one point in time from a sample of plants in an Easter lily crop. The model also required the expected average temperature until anthesis in order to calculate thermal time. The cumulative proportion \( P_i(t) \) of plants at development stage \( i \) that would reach anthesis by thermal time \( t \) was expressed as a function of the mean (\( \mu \)) and standard deviation (\( \sigma \)) in the thermal time of anthesis for each developmental stage

\[
P_i(t) = f(t, t_i, s_i)
\]

where \( f \) was the normal cumulative distribution function. The number of plants \( n(t) \) in the population that would be predicted by the model to be at anthesis by thermal time \( t \) was predicted by

\[
n(t) = \sum_{i=1}^{17} \frac{n_i}{n} \cdot n_r \cdot P_i(t)
\]

where \( n_i \) was the total number of plants sampled that were at stage \( i \), \( n_r \) was the total number of plants sampled and \( n_r \) was the total number of plants in the population. Flowering time was not predicted for plants at stage \( i=1 \), i.e. that were not yet at the visible bud stage.

3. Results and Discussion

**Visible bud to anthesis:** Linear regression of \( T_e \) versus development rate from visible bud to anthesis (Figure 1) resulted in an \( R^2 \) of 0.84, with estimates of the intercept and gradient parameters of 0.00563 d\(^{-1}\) and 0.00126°C\(^{-1}\) d\(^{-1}\). The thermal time \( t_a \) from visible bud to anthesis was calculated to be 792°Cd from these regression parameters, with a base temperature \( T_b \) of 4.5°C.

The base temperature \( T_b \) was extrapolated well beyond the range of temperature data available, and in this situation the x-intercept method (Arnold, 1959) is very sensitive to incomplete or variable data (Pasian and Lieth, 1996). The appropriate base temperature as described by Arnold (1959) for a linear thermal time model is therefore a parameter statistically estimated by the least squares method for the range in temperatures represented by the experimental data, rather than having physiological meaning. The estimated \( T_b \) compares with a value of 1.1°C for 'Nellie White' leaf unfolding before the visible bud stage (Karlsson, et al., 1988). A reanalysis using the x-intercept method on
data for 'Croft' Easter lily from potting to flower (Smith and Langhans, 1962) yielded a \( T_b \) estimate of - 4.6°C.

The standard deviation in \( t_a \) from visible bud to anthesis for individual plants in the combined MSU and UNH data was 62°Cd. Tests for skewness and kurtosis were not significant at the 98% level for any of the MSU or UNH temperature treatments.

**Bud elongation:** The parameter \( k \) in the bud elongation model (Eq. [5]) was estimated to equal 0.00290 ± 0.00002°C⁻¹d⁻¹ (mean ± asymptotic standard error), with an \( R^2 \) of 0.99 (Figure 2).

As bud length increased, the variability in thermal time to anthesis decreased. This would be expected because the exponential elongation pattern (Fig. (2)) of flower buds predicts that a 1-cm range in small buds, for example between 2.5 and 3.5 cm in length, would have a greater range in maturity than larger flower buds (for example between 12.5 and 13.5 cm). In addition, differences in development rate between plants would be expressed over a longer period for smaller flower buds compared with buds almost reaching anthesis. Inter-plant differences in development rate may be caused by biological or microclimate variability, however the sources of variability were not quantified.

The relationship between bud length and standard deviation in \( t_a \) was approximately linear (Figure 3). Linear regression of bud length versus standard deviation (\( s_r \), in °Cd, Eq. [8]) resulted in an \( R^2 \) of 0.86, with estimates of the \( c \) and \( d \) parameters of 63°Cd and - 0.32°Cd/mm. The pattern of model residuals may have been caused by serial correlation of errors - bud measurements recorded on several dates from the same 50 plants were used in the analysis.

\[
s = c + d B
\]  

(8)

Distributions of \( t_a \) for individual bud length observations within each 1-cm size class did not deviate significantly from normality, except for the 12-cm bud length data which was negatively-skewed and had positive kurtosis.

**Combined model:** The combined visible bud and bud length model (Eq. [7]) was formulated to allow its use as a predictive tool. Given the sampling date and the average temperature until anthesis, the model predicted the distribution in anthesis dates for each developmental stage. The parameter \( t_b \) for the visible bud stage was 792°Cd from these regression parameters, with a base temperature \( T_b \) of -4.5°C, and \( s_r \) was 62°Cd. For each bud length stage from 2-cm to 15-cm, \( t_b \) was estimated from Eq. [5], and \( s_r \) was estimated using Eq. [8]. Normality was assumed for the distribution in \( t_a \) for all developmental stages. For plants already at anthesis at the time of sampling, \( t_b \) and \( s_r \) equalled zero.

Figure 4 illustrates the predictions of the model, assuming 100 plants were at the same stage (visible bud, 4-cm, 8-cm, or 12-cm buds) and were grown at 20°C. For 100 plants that all had 12-cm-long buds, the model predicted that anthesis would occur sooner, and with less variability, than 100 plants with smaller buds or at the visible bud stage.

We developed a sampling protocol whereby at one point in time the frequency of plants within development stages 1 to 17 were recorded on a random sample of the crop and entered into a decision-support system. The decision-support system was programmed using Microsoft Excel under the Microsoft Windows platform. The predicted anthesis distribution was presented in both cumulative and per day graphical formats.

This model has application in Easter lily production since it can be used to plan the harvesting resources required each day, including labor, shipping, and cooler space. In addition, it predicts the cumulative number of plants at anthesis by each date. Finally, if temperature control is possible, the model predicts the temperatures required to meet grower objectives (for example target start and end anthesis dates, and maximum number of plants harvested on any single day).
For species other than Easter lily, the distribution in anthesis could also be predicted by (1) quantifying the observed developmental variability at one point in time for a crop, (2) predicting the mean time to anthesis using a thermal time, photothermal time, or more complex model, and (3) including a variability component in the developmental model.

References

Figure 1: Development rate (reciprocal of days from visible bud to anthesis) versus temperature. The solid line represents the linear regression, assuming $T_{\text{max}}$ equals 26°C.

Figure 2: Exponential model (Eq. [4]) of bud elongation over thermal time fit to data from Fisher et al. (1996)
Figure 3: Bud length versus standard deviation in $t_y$. Circles represent standard deviations from experimental data, and the solid line represents a linear regression (Eq. [8]) fit to the standard deviations.

Figure 4: Predicted distribution of anthesis, assuming 100 plants were grown at 20°C, and on day zero were all at the same developmental stage. In the example distributions, plants had flower buds that were either at the visible stage, 4cm, 8cm or 12cm in length.