Day/night temperature environment affects cell elongation but not division in *Lilium longiflorum* Thunb.

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Abstract

*Lilium longiflorum* Thunb. cv. ‘Nellie White’ plants were grown in different day/night temperature (DT/NT) environments to determine the anatomical basis for differential responses of stem elongation to DT and NT. *Lilium* plants were forced in 1986 and 1987 under 25 and 12 different DT/NT environments, respectively, with temperatures ranging from 14 to 30°C. Parenchyma and epidermal cell length and width were measured in stem tissue (1987) and epidermal cell length and width were measured in leaf tissue (1986). Total cell number per internode and vertical cell number per internode were calculated. Stem parenchyma and stem and leaf epidermal cell length increased linearly as the difference (DIF) between DT and NT increased (DIF = DT — NT), i.e. as DT increased relative to NT. DIF had no effect on stem parenchyma width, stem and leaf epidermal cell width, or cell number per internode. Data suggested that stem elongation responses to DIF are elicited primarily through effects on cell elongation and not division.

Key words: Thermoperiodism, thermomorphogenesis, stem elongation, DIF, cell division, cell elongation, leaf expansion.

Introduction

Early research by Went (1944, 1952) showed that day/night temperature (DT/NT) significantly affects plant height. Plant height increased as DT increased and NT decreased (Went, 1957). However, the way in which temperature affected plant height was unclear as Went did not determine internode number.

Tangeras (1979) and Karlsson (1988) reported that the effect of DT and NT on plant height was due to differences in internode elongation and not number, when DT/NT ranged from 10 to 20°C and 10 to 26°C on *Fuchsia ×hybrida* Hort. ex Vilm. and *Dendranthema grandiflora* L., respectively. Both Tangeras and Karlsson demonstrated that internode elongation increased as DT increased and NT decreased.

Morphological development in some plants is highly correlated with the relationship between DT and NT rather than the actual DT/NT plants are grown at when DT/NT range from 10 to 25°C (Erwin et al., 1989, 1991; Karlsson et al., 1989; Berghage and Heins, 1990; Moe et al., 1991). Specifically, internode length in *Lilium* increased 482% as the difference (DIF) between DT and NT (DIF = DT — NT) increased from −16 to +16°C (Erwin et al., 1989). While Went (1957) suggested that plant stem elongation was primarily influenced by DT, our measurements of internode lengths from photographic plates of *Pisum sativum* L. plants in Went's (1957) original article (Plate IX) showed that internode length was highly correlated with DIF ($r^2 = 0.72$). The effect of temperature on plant morphology was referred to as ‘thermomorphogenesis’ (Erwin et al., 1989).

Leaf expansion is also affected by temperature (Milthorpe, 1959; Dale, 1964, 1965). In *Fuchsia* cv. ‘Dollar Princess’, leaf expansion increased as DIF increased from −12 to +12°C with DT/NT ranging from 12 to 24°C (Erwin et al., 1991). In *Phaseolus vulgaris* L., leaf expansion was greatest when DT and NT were equal, i.e. 0°C DIF (Dale, 1965). Examination of Dale's data (1964) showed that conclusions on temperature effects on leaf expansion were based on a number of temperature regimes which contained either a 30°C DT/NT. Based on Dale's data and conclusions, leaf

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expansion was reduced with 30 °C DT compared to 25 °C. If data from environments with a 30 °C DT are excluded, leaf area increased linearly as DIF increased.

In contrast to Fuchsia and Phaseolus, leaf expansion of Cucumis sativus L. and Lilium did not increase as DIF increased. Instead, Cucumis leaf expansion increased when plants were grown at constant 20 °C (0 °C DIF) compared to fluctuating DT/NT (Erwin and Strefer, unpublished data). Lilium leaf expansion was a function of NT only where leaf expansion decreased as NT increased (Erwin et al., 1989).

The basis for temperature effects on plant stem elongation and leaf expansion are not understood. Indeed, the effect of DT/NT on cell dimensions in stems and leaves is unknown. The objective of our research was to determine how DT/NT affects cell elongation and division in elongating internodes and expanding leaves. In particular, we were interested in identifying whether DIF effects on stem elongation and leaf expansion are due to differences in cell elongation and/or division.

Materials and methods

Lilium longiflorum cv. 'Nellie White' bulbs 17.7-20.3 cm in circumference were planted in 15.2 cm plastic pots on 28 October, 1986 (Expt 1) and 1 November, 1987 (Expt 2) in a soilless medium consisting of equal parts of sphagnum peat, perlite and vermiculite (1:1:1). Base levels of phosphorus and micronutrients were added to the soilless medium when it was mixed. Potted bulbs were then placed in a controlled environment greenhouse for 2 weeks where air temperature was adjusted to maintain a medium temperature of 17 ± 1 °C to encourage root development. Plants were then vernalized in the dark for 6 weeks at 4 °C, after which 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 a night interruption (2 μmol m⁻² s⁻¹ (400–700 nm wavelength)) from 2200–0200 h delivered with incandescent lamps. After the long-day treatment, plants were returned to natural photoperiodic conditions (c. 9 h 15 min photoperiod).

Time of flower initiation in Expts 1 and 2 was established by terminal shoot dissections on randomly selected plant samples starting 13 January, 1986 and 11 January, 1987, respectively. Plant samples were taken every 3 days. Flower initiation was defined as the first visible sign of a reproductive meristem (De Hertogh et al., 1976). Flower initiation was observed on 100% of the plants sampled in Expts 1 and 2 on 22 January, 1986 and on 27 January, 1987, respectively.

**Experiment 1 (1986)**

One hundred and twenty five plants were selected for uniformity after flower initiation (22 January, 1986). Plants were then divided into 5 groups of 25 plants each and were moved to glasshouses maintained at constant 14, 18, 22, 26 or 30 ± 1.5 °C. Within each glasshouse, plants were further divided into 5 groups of 5 plants each. Each group of 5 plants constituted a DT/NT regime treatment group. DT/NT treatment groups were moved among greenhouse sections at 0800 and 1700 h each day to yield a total of 25 different DT/NT regime treatments (5 plants per DT/NT regime group). Each treatment group received the same DT/NT regime from the initiation of the experiment until anthesis. Movement of plants at 0800 and 1700 h required approximately 30 min at each move period.

An opaque curtain was pulled over plants after they were moved at 1700 h and was retracted just prior to 0800 h to provide a 15 h nyctoperiod to parallel the NT treatment. Plants were spaced to provide 900 cm² per plant. The uppermost leaf of each plant was marked with a black dot when plants were moved into the glasshouse to indicate the stage of development of each plant at the inception of the temperature treatments.

Plants were watered as needed to ensure that the medium was always moist for each DT/NT regime. Fertilizer was composed of 14.3 mM calcium nitrate and 5.1 mM potassium nitrate at each watering. Phosphorus and micronutrients were added when soil was mixed and were retained in the medium throughout the experiment. Levels of nutrients were in the recommended ranges for production in soilless media as identified through Spurway analysis throughout the experiment. Electrical conductivity was maintained at 0.75 to 1.0 mS to maintain similar fertility across DT/NT regimes.

**Experiment 2 (1987)**

Seventy two plants were selected for uniformity after flower initiation (27 January, 1987). Plants were divided into 4 groups of 18 plants each and were moved to glasshouses maintained at 15, 20, 25, or 30 ± 1.8 °C. Within each glasshouse, plants were further divided into 3 groups of 6 plants each. Each group of 6 plants constituted a DT/NT regime treatment group. Plants were moved among glasshouse sections at 0800 and 1700 h each day but no plants were placed in the 30 °C glasshouse at night. Therefore, plants were exposed to 12 DT/NT regimes (6 plants per DT/NT regime). Other procedures were as discussed in Expt 1.

**Tissue collection**

Leaf and stem samples were collected at anthesis. A leaf was collected from 5 cm below the base of the inflorescence on 3 plants from each temperature regime treatment (3 of the 5 plants) in Expt 1, i.e. 3 leaves were collected from 3 different plants. A stem segment approximately 2 cm in length was collected between the 5th and 10th internodes below the base of the inflorescence on 4 plants from each treatment (4 of the 6 plants) in Expt 2, i.e. 4 stem segments were collected from 4 different plants. Tissue samples were placed in a formalin based fixative (formaldehyde, glacial acetic acid, and tertiary butyl alcohol) immediately after excision.

**Leaf tissue measurements**

Epidermal cell length and width data were collected on leaf tissue. Leaf tissue was blotted dry and a clear solution of nail polish (Mabelline—01 'crystal clear) was applied to the leaf surface on the nail polish. The tape was then removed along with a replica of the leaf surface on the nail polish. The tape and replica were then mounted on a slide for epidermal cell measurements.

**Stem tissue measurements**

Longitudinal sections were cut using an Oxford Vibratome Model No. 501 for central stem parenchyma cell length and width determinations. Stem epidermal cell dimensions were determined as described above for leaf epidermal cell collection.
Data collection

A Zeiss microscope fitted with a Nikon FX-35A camera and a Nikon UFX exposure meter were used for brightfield observations and photography of all samples. Measurements of cell dimensions were made from photographs. An enlargement of the slide micrometer was used for cell dimension determinations. Measurements of cell dimensions were made with an accuracy of ±0.1 microns.

As mentioned previously, 3 leaves and 4 stem segments were collected for sampling from each DT/NT regime treatment in Expt 1 and 2, respectively. A single photograph was taken from each leaf for leaf epidermal cell length and width determinations. Two photographs were taken from each stem segment: one to identify stem epidermal cell length and width; one to identify central parenchyma cell length and width. Ten measurements were taken at random from each photograph or slide.

Therefore, there were 30 leaf epidermal cell length and width determinations made for each DT/NT regime treatment (30 measurements from 3 plants). There were 40 measurements each for stem epidermal cell length and width and parenchyma cell length and width taken from 4 plants (10 measurements per plant) from each DT/NT regime treatment.

Internode length was calculated by dividing leaf number above the leaf marked at the beginning of the treatment by the change in stem length from the beginning of the treatment. Internode width was measured using a digital micrometer when cell length and width measurements were made.

Cell number per internode and vertical cell number per internode were calculated from internode and cell dimension data (Table 1). Cell number per internode was calculated by dividing the calculated volume of an internode by average calculated parenchyma cell volume. Volumes were calculated using the formula for the volume of a cylinder. Vertical cell number in an internode was calculated by dividing internode length by parenchyma cell length.

Data analysis

The experiments were organized as a 5 x 5 and a 4 x 3 factorial, respectively, for Expt 1 and 2, with DT and NT as the main factors. There were 30 (10 measurements from 3 plants) and 40 measurements (10 measurements from 4 plants) for leaf and stem cell data, respectively. Analysis of variance was conducted for each dependent variable and are reported in each table.

Since the data was 'continuous', analysis using multilinear regression analysis was also appropriate. DT, NT, average temperature, and DIF were evaluated as independent variables to determine DT/NT effects on cell length and width. DIF levels were calculated for each DT/NT treatment. Polynomials, interactions, and combinations of the above-mentioned terms were also evaluated. Selection of regression functions were based on significance of the model and individual parameters, correlation ($r^2$), and visual inspection of the regression fit. Analysis of variance and regression analysis were conducted using the Systat statistical analysis program (Systat Inc., Exanston, IL, USA).

Results

Internode length increased as DT increased and NT decreased (Table 1). For instance, internode length increased from 20 to 36 mm as DT increased from 15 to 25 °C when NT was held at 15 °C. In contrast, internode length decreased from 36 to 18 mm as NT increased from 15 to 30 °C when DT was held at 25 °C.

The effect of DT and NT on internode length could best be described quantitatively as a function of DIF rather than by DT or NT independently. Internode length increased linearly as DIF increased from -10 to +10 °C (Fig. 1).

Internode width decreased slightly as DT increased and NT decreased (Table 1). For instance, internode width decreased from 15 to 11 mm as DT increased from 15 to 25 °C when NT was held at 15 °C. Conversely, internode width decreased slightly from 11 to 10 mm as NT increased from 15 to 30 °C when DT was held at 25 °C.

Stem parenchyma and epidermal cell length increased as DT increased (Table 2). For instance, stem parenchyma cell length increased from 158 to 295 μm as DT increased from 15 to 25 °C when NT was held at 15 °C. Stem epidermal cell length increased from 172 to 388 μm as DT increased from 15 to 25 °C when NT was held at 15 °C.

Stem parenchyma and epidermal cell length decreased as NT increased (Table 2). For instance, stem parenchyma cell length decreased from 295 to 173 μm as NT increased from 15 to 30 °C when DT was held at 25 °C. Stem epidermal cell length decreased from 388 to 219 μm as NT increased from 15 to 30 °C when DT was held at 25 °C.

Leaf cell elongation responded similarly to DT and NT as stem cell elongation (Table 2). Leaf epidermal cell length increased from 354 to 464 μm as DT increased from 14 to 26 °C when NT was held at 14 °C. In general,
leaf epidermal cell length decreased if DT was increased further from 26 to 30°C. Leaf epidermal cell length decreased from 464 to 331 μm as NT increased from 15 to 30°C when DT was held at 26°C.

The effect of temperature on cell elongation in *Lilium* could best be described quantitatively as a function of DIF rather than DT and/or NT. Cell elongation increased linearly as DIF increased, i.e. as DT increased relative to NT. Stem parenchyma and epidermal cell length increased from 95 to 295 μm and from 200 to 388 μm, respectively, as DIF increased from −15 to +10°C (Fig. 2a). Similarly, leaf epidermal cell length increased from 300 to 464 μm as DIF increased from −16 to +16°C (Fig. 3).

Stem parenchyma and stem epidermal cell width were unaffected by DT, NT, or DIF within the temperature range studied (Table 2; Fig. 2b). Leaf epidermal cell width was also unaffected by DIF (Fig. 3).

Calculated cell number per internode was not affected by DT or NT and was not correlated with DIF (Table 3). Similarly, the calculated vertical cell number per internode was not affected by DT, NT, or DIF (Table 3).

### Discussion

Results presented in this paper suggest that DT/NT or DIF effects on stem elongation and leaf expansion in *Lilium* are elicited primarily through effects on cell elongation and not division. The bases for this conclusion are (1) cell length is strongly correlated with DIF, (2) cell width was not strongly correlated with DIF, and (3) cal-

### Table 2. The effect of day (DT) and night temperature (NT) on *Lilium longiflorum* Thumb. cv. ‘Nellie White’ stem parenchyma and epidermal cell length and width

DT and NT ranged from 15 to 25±2°C and from 15 to 30±1°C, respectively, in Expt 2 when stem data were collected. DT and NT ranged from 14 to 30±1.5°C in Expt 1 when data on leaf tissue were collected. Plants were grown under a 9 h photoperiod. Thermoperiod paralleled the photoperiod.

<table>
<thead>
<tr>
<th>NT (°C)</th>
<th>DT (°C)</th>
<th>Stem parenchyma (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Length</td>
<td>158*</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>234</td>
</tr>
<tr>
<td>20</td>
<td>Length</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>120</td>
</tr>
<tr>
<td>25</td>
<td>Length</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>185</td>
</tr>
<tr>
<td>30</td>
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<td>134</td>
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<tr>
<td></td>
<td>Width</td>
<td>114</td>
</tr>
<tr>
<td>Stem epidermal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Length</td>
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</tr>
<tr>
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<td>200</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>236</td>
</tr>
</tbody>
</table>

### Table 3. The effect of day (DT) and night temperature (NT) on *Lilium longiflorum* Thumb. cv. ‘Nellie White’ leaf epidermal cell length and width

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>NT</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td>DT×NT</td>
<td>***</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Numerals represent treatment means based on 40 measurements taken from 4 plants per DT/NT regime.

b Significance of terms as determined in an analysis of variance (alpha=0.05). Significance of terms where *=0.05, **=0.01, and ***=0.001 significance of F parameter.

b Numerals represent treatment means based on 30 measurements taken from 3 plants per DT/NT regime.
The effect of the difference (DIF) between day (DT) and night temperature (NT) (DIF = DT - NT) on *Lilium longiflorum* 'Nellie White' stem central parenchyma (O) and epidermal cell (O) length (a) and stem central parenchyma and epidermal cell width (b). Symbols represent 12 treatment means from 12 D1F values calculated from 12 DT/NT regimes (Table 2). Regression model significance (P) for parenchyma and epidermal cell means was <0.01 and <0.001, respectively. Regression model significance (P) was n.s. for both parenchyma and epidermal cell width.

**Table 3.** The influence of day (DT) and night temperature (NT) on *Lilium longiflorum* cv. 'Nellie White' calculated total cell number per internode and vertical cell number per internode. Calculated amounts are determined by dividing mean calculated total internode volume by mean calculated individual cell volume (original data from Tables 1 and 2). Vertical cell number was calculated by dividing mean internode length by mean individual parenchyma cell length.

<table>
<thead>
<tr>
<th>NT (°C)</th>
<th>DT (°C)</th>
<th>Cell number/internode</th>
<th>Vertical cell number/internode</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>1327° 1358° 1265°</td>
<td>12.7 13.2 12.2</td>
</tr>
<tr>
<td>20</td>
<td>873</td>
<td>1265 927</td>
<td>8.0 12.4 9.6</td>
</tr>
<tr>
<td>25</td>
<td>711</td>
<td>1051 1585</td>
<td>7.6 11.1 18.0</td>
</tr>
<tr>
<td>30</td>
<td>1416</td>
<td>667 30</td>
<td>13.7 8.2 10.0</td>
</tr>
</tbody>
</table>

* Numerals represent treatment values calculated from calculations based on data presented in Tables 1 and 2. 'Significance of terms where *=0.05, **=0.01, and ***=0.001 significance of F parameter.
Tsujita, 1988). Similarly, exogenous applications of GA₃ overcame inhibition of *Campanula isophylla* Morettii, and *Lycopersicon esculentum* L. internode elongation, respectively, when plants were grown in a negative DIF environment (Moe et al., 1991; Erwin and Pierson, 1992). In contrast, application of a GA biosynthesis inhibitor, aminomidal, resulted in a greater percentage decrease on internode elongation of positive-DIF-grown plants than negative-DIF-grown *Lilium* plants (Erwin et al., 1989).

Temperature influences both the levels of bioactive GA levels and the ability of tissue to respond to GA. Endogenous levels of GA₁ increased 1.6-fold when *Triticum aestivum* L. plants were grown at constant 25 versus 10 °C (Pinthus et al., 1989). No data were found which resolved the effect of fluctuating temperatures on endogenous GA levels.

Both light and temperature affect the ability of tissue to respond to GA. Lockhart (1958) demonstrated that dwarfism in *Phaseolus* occurred only in light-grown plants. Pinthus et al. (1989) demonstrated that genotypic differences were conspicuous among genetic dwarf *Rht* mutants of *Triticum* when plants were grown at constant 25 °C, but were only slight or inconspicuous when plants were grown at constant 11 °C. Interestingly, the increase in the endogenous GA₁ level was much greater, i.e. 3-fold, 6-fold, and 9-fold on the *Rht₁*, *Rht₂*, and *Rht₁ + 2* genotypes, respectively, than the 1.6-fold increase in GA₁ in the wild-type when temperature was increased from 10 to 25 °C. Apparently, the response of *Triticum* tissue to endogenous or applied GA is restricted by the upper limits set by the different *Rht* alleles.

Whether GA effects on stem elongation are mediated through effects on cell elongation and/or division is unclear. GA application has been shown to affect both cell elongation and division (Kaufmann, 1965; Raskin and Kende, 1984; Katsumi and Ishida, 1991). Greulach and Haesloop (1958) reported that dwarfism in *Phaseolus* could be overcome with exogenous GA application and that the increase in internode elongation on dwarf *Phaseolus* was due to an increase in cell number per internode. However, Skjedstad (1960) determined that similar application of GA to overcome dwarfism in *Zea mays* L. resulted in an increase in cell number and size. More recently, Keyes et al. (1989) showed that dwarfism induced by the GA response *Rht* alleles in *Triticum* was a result of a reduction in cell length and not number in leaf blades.

Lastly, data (J.E. Erwin, unpublished) suggested that the temperature of the elongating region of a stem and not the roots was critical in eliciting DIF effects on stem elongation. The independence of the shoot tip temperature relative to the root temperature suggested that turgor pressure was not the primary factor through which DIF affects stem elongation. In contrast, *Chenopodium album* L. stem elongation was affected when shoot temper-

ature was altered independently of root temperature (Lecharny et al., 1985). Lecharny et al.'s data (1985) suggested that turgor (associated with root temperature) may play a partial role in eliciting temperature effects on stem elongation. Hence, turgor may play some role in elicitation of DIF effects on stem elongation.

The response of stem elongation and/or cell elongation to DIF is an observation which leads to questions concerning the ecological or evolutionary basis for DIF responses. The dependence of stem elongation on DIF has been demonstrated across a variety of plant species and is not exclusive to *Lilium* (Erwin, 1991). It could be hypothesized that plant stem elongation responses to DIF may be a mechanism by which plants can direct elongation growth during different periods of a year to maximize stem elongation at critical times to compete effectively in a plant community. Such a mechanism would be of significance for temporal orientation during the year as well. Experiments are currently underway to determine the potential ecological significance of stem elongation responses to DIF.

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**References**


