Temperature, but not Growth Regulators, Influences Diurnal Stem Elongation Rhythms in Zinnia

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Abstract. Experiments were conducted to determine the effects of treatment with gibberellic acid (GA) on changes in diurnal growth rhythms caused by maturation and day/night temperature differential (DIF) in zinnia (*Zinnia elegans* Jacq. 'Pompon'). Plants were treated with GA₃ or with the GA biosynthesis inhibitor daminozide under three DIF regimes (+5 DIF: 21 °C DT/16 °C NT; 0 DIF: 18.7 °C constant; –5 DIF: 16.5 °C DT/21.5 °C NT), each with a daily average temperature of 18.7 °C, at two developmental stages: stage 1, the period of vegetative growth before flower bud formation; and stage 3, growth just before anthesis. Instantaneous stem elongation rates (SER) were measured using linear voltage displacement transducers. The DIF regime, as has been previously shown, influenced stem elongation primarily by altering the size of an early morning peak in SER; peak height increased as DIF became more positive. GA₃ increased SER throughout the diurnal period with a proportionately larger effect on nighttime growth. Conversely, daminozide decreased SER more or less equally throughout the diurnal period. Neither GA₃ or daminozide transformed growth patterns to match those of positive or negative DIF plants, but instead simply increased or decreased growth amplitude. Furthermore, neither growth regulator altered the basic diurnal SER pattern at any DIF, or influenced the observed shift to greater nighttime growth as plants matured from stage 1 to stage 3. The results suggest that neither the effects of DIF, or the age-related shift in diurnal growth distribution can be explained by changes in total availability of GA in the plant. Chemical name used: mono (2,2-dimethylhydrazide) butanedioic acid (daminozide).

The effect of day/night temperature differentials (DIF) on plant morphology has been widely studied (Erwin et al., 1989; Erwin and Heins, 1995; Myster and Moe, 1995). Today, DIF is used as an alternative to chemical growth regulators to control stem growth in greenhouse crops, but the physiological mechanisms underlying the DIF response have only recently come under scrutiny. Several investigations have attempted to relate DIF effects to gibberellin (GA) availability within the plant. Applications of GA₃ (Erwin and Pierson, 1992; Moe, 1990; Tangeras, 1979), GA₄₊₇ (Langton et al., 1997; Zieslin and Tsujita, 1988), and

GA₁ (Myster et al., 1997) negated the effects of a negative DIF in several greenhouse species. In other studies, applications of a GA biosynthesis inhibitor have nullified the effect of a positive DIF (Moe, 1990; Tangeras, 1979). These results suggest that day/night temperature may regulate stem elongation through alteration of endogenous GA levels.

The influence of DIF on stem elongation was first studied by comparing stem heights of plants after several weeks of growth in specific DIF regimes. Recently, the application of high-resolution measurement techniques using electronic displacement transducers has allowed closer scrutiny of short-term DIF effects in a range of species (Bertram and Karlsen, 1994a, 1994b, 1995; Tutty et al., 1994). In a previous study (Neily et al., 1997), we have characterized the influence of DIF and growth stage on diurnal patterns of stem elongation in zinnia and snapdragon (*Antirrhinum majus* L.) using high-resolution techniques. Plants subjected to positive or negative DIF regimes displayed distinctive patterns of stem elongation, especially characterized by an increase (positive DIF) or decrease (negative DIF) in the size of an early morning peak (EMP) in

SER. In zinnia the period of maximum stem elongation also shifted from daytime to nighttime as plants matured. We hypothesized that, if DIF acts through an alteration of total GA concentration within the plant, a negative DIF pattern may be transformed into a typical positive DIF pattern by applying exogenous GA, and a positive pattern transformed into a negative one by applying an inhibitor of GA biosynthesis. We report here the results of experiments in which we tested this hypothesis by examining the diurnal stem elongation patterns of zinnia subjected to specific DIF regimes at two stages of growth, and treatment with either GA₃ or daminozide, a late-stage inhibitor of GA biosynthesis (Brown et al., 1997).

Materials and Methods

Plant material and cultural conditions. Seedlings of zinnia, cv. 'Pompon', were planted in 10-cm pots containing a peatlite growing medium (Pro-mix; Premier Horticulture Ltd., Rivière-du-Loup, Qué.). Controlled-release fertilizer (Nutricote Type 100; 14N–6.2P–11.3K; Chisso-Ashai Fertilizer Co., Tokyo) was premixed into the medium at a rate of 2.0 g per pot. Plants were grown in a controlled-environment (CE) chamber in which a photosynthetic photon flux (PPF) of 350 µmol·s^{–1}·m^{–2} (LI-COR quantum sensor, model LI-188B, Lincoln, Nebr.) was maintained at the uppermost leaf surface for 13 h each day. Chamber temperature was maintained at 18.7 °C ± 0.5 °C. New plants were placed in this chamber on a biweekly basis in order to maintain a constant supply of plant material. Plants remained in this chamber until they reached two predefined stages of development (as previously described by Neily et al., 1997): stage 1 (vegetative) was defined as vegetative growth, preceding the formation of a flower bud, and stage 3 (pre-anthesis) as the period just before flowering when color first appeared in the bud. One week prior to plants reaching one of these two developmental stages, they were sprayed to runoff with either GA₃ (Sigma Chemical Co., St. Louis) (250 mg·L^{–1}), daminozide (B-Nine; Plant Products, Brampton, Ont.) (6000 mg·L^{–1}), or water (control).

Measurement of stem elongation rate (SER). Three CE chambers, each containing a high-resolution measuring device, were randomly assigned one of three DIF regimes (+5 DIF: 21 °C day/16 °C night; 0 DIF: 18.7 °C constant; –5 DIF 16.5 °C day/21.5 °C night) each with a daily average temperature of 18.7 °C. A PPF of 350 µmol·s^{–1}·m^{–2} with a red/far-red ratio of 2:1 (SKR100, 660/730 nm sensor; Skye Instruments, Llandrindod Wells, Wales) was maintained at the uppermost leaf surface and supplied for 13 h each day. The sprayed plants were placed in the appropriate CE chamber and each plant was attached to a high-resolution measuring device 24 h prior to initiating measurements.

Stem elongation rate was measured using linear voltage displacement transducers (LVDTs) (models 200DC-D and 250 DC-E,

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both with small diameter cores; Schaevitz Engineering, Pennsauken, N.J.) in an apparatus modified from Kristie and Jolliffe (1986) and previously described in detail by Tutty et al. (1994). A datalogger (model CR7; Campbell Scientific, Logan, Utah) simultaneously recorded LVDT output voltages, and plant and air temperatures were sensed by shielded copper-constantan thermocouples. Readings were recorded and averaged for the last minute of a 5-min interval, once every 5 min. Stem positions at successive 5-min intervals were recorded and converted to rates of stem elongation as described previously (Neily et al., 1997). The stem elongation for each plant was measured during three successive diurnal periods.

Data analysis. The experimental design was a completely randomized $2 \times 3 \times 3$ (growth stage $\times$ DIF $\times$ growth regulator) factorial with four replicate samples. Replicates consisted of individual plants grown to the required growth stage. Each factor had a proportional effect in which stem elongation was either increased or decreased by a multiplicative factor. Therefore, a multiplicative model was used in the analysis of variance by logarithmically transforming the data before analysis. The log-means were then back-transformed for presentation. Percent standard errors of the means were calculated from the antilog of the appropriate log SEM (Snedecor and Cochran, 1980).

Results and Discussion

DIF effects on plants not treated with growth regulators. Under control conditions (0 DIF and no growth regulator applied), SER displayed a 24-h rhythm that varied with growth stage and conformed closely with patterns previously described for vegetative (stage 1) and pre-anthesis (stage 3) zinnia (Neily et al., 1997; Fig. 1). In stage 1, most growth occurred during the first 4 h of the light period during an EMP, following which SER declined until shortly before the day/night (D/N) transition. In stage 3 (Fig. 2), the EMP was still evident but growth rates during the night were much greater than at stage 1; SER peaked shortly after the D/N transition and again before the N/D transition.

In plants not treated with growth regulators, DIF affected daily stem elongation at both stage 1 and stage 3 (Figs. 1, 2, 3). Growth increased under positive DIF conditions, but decreased under negative DIF, as found in studies on other taxa (Erwin et al., 1989; Karlsson et al., 1989; Moe, 1990). We have shown that DIF acts principally by modifying the SER pattern during the day in zinnia (Neily et al., 1997). This finding and the presence of an EMP in SER that is either enhanced under positive DIF conditions or diminished as DIF decreases were confirmed in the present study. Changes in size of the EMP mediated by temperature conditions were responsible for the overall temperature effects on growth, and only daytime growth was affected by DIF (Figs. 1, 2, 3).

Effects of GA_3 and daminozide on SER patterns at 0 DIF. Stem elongation in stage 1 and stage 3 zinnias grown under 0 DIF condi-

Fig. 1. Patterns of stem elongation (SER) for zinnia in relation to DIF and growth regulator treatments at growth stage 1 (vegetative). For the control, arrows highlight early morning peaks, and shaded areas indicate growth at night. D = day, N = night, DT = day temperature, NT = night temperature, DIF = day temperature minus night temperature. Traces are averages for four plants for each growth regulator/DIF combination.

Fig. 2. Patterns of stem elongation (SER) for zinnia in relation to DIF and growth regulator treatments at growth stage 3 (pre-anthesis). For the control, arrows highlight early morning peaks, and shaded areas indicate growth at night. D = day, N = night, DT = day temperature, NT = night temperature, DIF = day temperature minus night temperature. Traces are averages for four plants for each growth regulator/DIF combination.

tions and treated with GA_3 was ≈ 4 - and 2-fold, respectively, that of nontreated controls (Fig. 3). Effects of GA_3 on stem growth of zinnia have been previously described (Sawhney and Sawhney, 1976; Shedeed et al., 1986); however, our results also indicate that the GA_3 effects occur without significant change in SER pattern (Figs. 1 and 2). Daminozide-treated plants at 0 DIF showed SER that were about two-thirds and one-half those of the controls, at stage 1 and stage 3, respectively

(Fig. 3), but again the basic diurnal pattern of growth remained unchanged. The effects of supplemental GA_3 on SER were greater during the night than during the day (Fig. 3). For example, elongation during the night for stage 1 plants under 0 DIF conditions was increased >5 -fold by GA_3 , whereas growth during the day showed only a 3-fold increase. This suggests that the supply of GA_3 may limit growth more at night than during the day, a proposition that is partly supported by recent evidence

that, in some plants, GA levels fluctuate rhythmically throughout the diurnal cycle and tend to be lowest at night (Foster and Morgan, 1995; Talon et al., 1991).

Effects of DIF on plants treated with growth regulators. Overall, the effects of DIF on SER were superimposed on those due to supplemental GA₃ and daminozide in plants at both stages (Figs. 1 and 2). Total stem elongation increased with an increase in DIF under all treatment conditions, and in both GA₃- and daminozide-treated plants an increase in size of the EMP largely accounted for differences in daily growth. Changes in growth during the day were responsible for the differences in diurnal stem elongation (Fig 3); growth at night was not significantly affected by DIF.

Exogenously applied GA or GA-synthesis inhibitors can negate the effects of DIF on stem elongation (Moe, 1990; Zieslin and Tsujita, 1988), possibly because DIF changes GA biosynthesis. Our results suggest that DIF effects are not dependent on increasing or decreasing the GA pool size in the plant, since application of neither GA₃ nor the GA-synthesis inhibitor, daminozide, could reproduce diurnal growth patterns characteristic of positive or negative DIF plants. Thus, exogenous GA₃ did not increase the size of the EMP and daminozide did not eliminate or reduce it. Furthermore, the effects of DIF were still evident in the presence of high levels of GA₃, making it unlikely that DIF acts through modification of total GA levels in the plant. This does not, however, preclude the interaction of DIF and GA in influencing stem elongation. DIF may affect tissue sensitivity to GA (Pinthus and Meiri, 1979; Singh and Paleg, 1984) or the conversion of nonactive GAs to bioactive GAs, such as GA₁ (Graebe, 1987; Ihlebek et al., 1995). Recent studies have provided further support for the latter effect and for an influence of DIF on the formation of other precursors of GA₁ (Grindal et al., 1998; Jensen et al., 1996; Langton et al., 1997). If similar mechanisms occur in zinnia, then the DIF-dependent growth patterns could reflect GA₁ availability.

Stage of development altered the distribution of growth between day and night periods, confirming the results of an earlier study (Neily et al., 1997). During stage 1, growth of plants under 0 DIF conditions occurred predominantly during the day, but by stage 3 most growth occurred at night (Table 1). Application of GA₃ also tended to shift growth from daytime to nighttime. The age-related shift in timing of growth occurred in plants with both an excess (GA₃-treated) and a deficiency (daminozide-treated) of GA. We therefore conclude that a diurnal change in total GA availability is not responsible, although the effect could be due to a GA interconversion as discussed previously. The DIF regime also influenced diurnal growth distribution; the percentage of stem elongation occurring during the day increased as DIF became more positive, and once again the effects were independent of growth regulator treatment. Previous studies have indicated that the diurnal distribution of growth is by no means uniform among taxa. In *Dendranthema*, for example,

Fig. 3. Accumulated total, day, and night stem growth for zinnia over three consecutive diurnal periods during stages 1 and 3. Plants were treated with growth regulators (GR) GA₃ (○), daminozide (□), or nontreated control (Δ) at two stages of development and exposed to three day temperature–night temperature (DIF) regimes: +5 DIF: 21 °C DT/16 °C NT; 0 DIF: 18.7 °C constant; –5 DIF: 16.5 °C DT/21.5 °C NT; daily average temperature: 18.7 °C. The photoperiod was 13 h. Percent standard error of the mean for comparisons between individual levels of a factor are 15.1 (total), 13.3 (day), and 19.9 (night). Standard error of the mean for comparisons between individual levels of a factor = $\bar{x} \times \%SEM/100$. Annotations indicate significant effects at $P \leq 0.05$.

Table 1. Percentage of diurnal stem growth, occurring during the day period only, in zinnia treated with growth regulators (GR) at two stages of development and exposed to three DIF regimes. SEM² = 3.09.

DIF ^a	Day period growth (%) ^b							
	Stage 1: vegetative				Stage 3: pre-anthesis			
	GA ₃	Control	Daminozide	Mean	GA ₃	Control	Daminozide	Mean
+5	50.2	65.8	61.7	59.2	43.6	53.6	48.3	48.5
0	47.1	61.2	58.9	55.7	36.4	40.9	45.2	40.8
–5	44.7	52.6	48.6	48.6	32.1	39.1	38.4	36.5
Mean	47.3	59.9	56.4		37.4	44.5	44.0	

²SEM = standard error of the mean.

^bDay growth/total growth*100%.

^aDifference between day temperature (DT) and night temperature (NT); +5 DIF: 21 °C DT/16 °C NT; 0 DIF: 18.7 °C constant; –5 DIF: 16.5 °C DT/21.5 °C NT. Daily average temperature: 18.7 °C. Photoperiod: 13 h.

most stem elongation occurred at night (Bertram and Karlsen, 1994a; Tutty et al., 1994), but in petunia, stem elongation was greater during the day (Bertram and Karlsen, 1994b). To the best of our knowledge, zinnia is the only species in which a shift in growth distribution mediated by DIF and developmental stage has been reported.

Clearly, the effects of DIF are not satisfactorily explained by a direct influence of temperature on total GA availability, since neither reducing endogenous GA (by treatment with an inhibitor) or increasing GA concentrations (by application of exogenous GA₃) simulated characteristic SER patterns of negative or positive DIF plants. Nevertheless, recent work has shown conclusive links between DIF and GA metabolism (Jensen et al., 1996; Langton et al., 1997). We have shown that, in zinnia, DIF acts primarily through the modification of diurnal patterns of stem elongation, particularly that occurring in the early morning. The question of whether this modification is a direct result of alterations in levels of one or more bioactive GAs, or is unrelated to GA metabolism, remains to be resolved.

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