ABSTRACT

DUBOIS, JEAN-JACQUES BERNADETTE. Plant Level Response of Seven Herbaceous Perennials to Diurnal Temperature Cycling and Sub–diurnal Temperature Variation. (Under the direction of Dr. Frank A. Blazich.)

Climate warming has not affected day and night symmetrically: daily temperature range (DTR) is narrowing, as daily average temperature (DAT) increases. The potential impacts of combined DAT and DTR variation on plants are unknown. Four experiments were conducted to assess such impacts. In the first two, plants of seven herbaceous perennial taxa were exposed to 18 combinations of day and night temperature for 50+ days. Effects on total plant dry weight (DW) were analyzed with the aid of a thermodynamic model, modified to include two temperature dimensions. Results showed that the effects of temperature on DW cannot be accounted for by variation in either DAT or DTR alone, and that their magnitude is equivalent. The greatest effect of variation in DTR on DW was on plants growing closest to their optimal DAT. Time–to–event data analysis methods were used to determine the relative effects of DAT and DTR on anthesis and death in two taxa. Effects of day and night temperature could be separated for both events, but were almost entirely subsumed into DAT. Although effects of DTR were significant, they only became meaningful at the extremes. Results suggest changes in DTR are a lesser concern for anthesis and survival than for gross productivity. Two experiments were then conducted to assess the effect on DW of temperature variation within each of the two phases of the diurnal cycle. Plants of *Delphinium* × *cultorum* and *Rudbeckia fulgida* var. *sullivantii* L. were grown for 40 days under 28 temperature regimes all resulting in cycles of 12-h days/12-h nights at average either 15/15°C, or 25/15°C. There were 14 regimes for each combination, during which temperature varied between 5 and 35 °C every 4 hours. Temperature varied during the light period in the first experiment, and the dark period in the second. Few significant differences in DW were found among regimes for either taxon, and they did not produce a consistent pattern. Results indicate that over season–long durations, effects of sub–diurnal variation on gross productivity are far less consequent than those of diurnal variation.

Plant Level Response of Seven Herbaceous Perennials to Diurnal Temperature Cycling and Sub–diurnal Temperature Variation

by

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Chair, Advisory Committee

DEDICATION

To Amy. Of course.

And to Bernard and Hélène Dubois.

Biography

Jean-Jacques Dubois was born in Namur, Belgium in 1962, and graduated from the Université Catholique de Louvain in 1987, with a License en Philosophie. He moved to the United States the same year, and until 1992, owned an antique furniture restoration business in New Haven, Connecticut, and Boston, Massachusetts. After holding positions in hospital administration, and moving to Raleigh, North Carolina, he enrolled in the undergraduate program in Horticultural Science at North Carolina State University, in Fall 1995. Following suggestions of Dr. F.A. Blazich and other faculty members, he applied to and was accepted into the graduate program in the same department in Fall 1997. He received an MS degree in Fall 1999, and decided to remain in the graduate program in Horticultural Science to pursue a PhD degree.

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The research that follows would not have been possible without the long–lasting support of Dr. F.A. Blazich. Few faculty members do more than he does for the graduate students in their responsibility, and none is as willing as he to follow his students' lead in how much, or how little assistance and direction he will provide. He manages to be more available than any advisor, while maintaining the educational objective of always promoting independence and self–sufficiency. This feat alone makes him one of the very best there is.

Additional support was provided by the other members of the advisory committee, Drs. C.D. Raper Jr., W.H. Swallow, and D.J. Werner. Dr. J.F. Thomas, Director, NC State University Phytotron, advised on translating our treatment designs into growth chamber schedules, and Ms. J.M. Smith was always available for assistance in writing SAS code.

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INTRODUCTION

Temperature is one of the main determinants of plant adaptation. While cold temperature constrains geographical range through survival, high temperature limitations are gradual, from suboptimal growth to outright mortality. Examination of temperature patterns over large land masses such as North America shows that daily average temperature (DAT) and diurnal temperature range (DTR) vary independently of one another, yet experimental evidence of distinct effects of these two factors on overall plant growth has been the object of little investigation.

The effects of variation in the diurnal temperature cycle have taken on special relevance with the confirmation that climate warming has not affected day and night symmetrically. Over most land areas worldwide, DTR has narrowed, as DAT increased (Easterling et al. 1997; Karl et al. 1991; Karl et al. 1993; Kukla, Karl & Riches eds. 1994). Nighttime minima have risen at a rate greater than daytime maxima. Continuation of this trend in the future is considered “very likely” by the IPCC (2001). Suggestive research by Went (1944a; 1944b; 1957) and Camus & Went (1952) has been invoked to indicate the need to assess the potential impact of this asymmetry, particularly on vegetation (Mooney, Koch & Field 1994). However, it appears that gross responses of plants to variation in the amplitude of the diurnal temperature cycle has received little attention.

It is well demonstrated that increasing late night temperature above temperature of the beginning of the light period can alter morphology in many greenhouse crops by reducing internode length, thus imparting a more compact appearance to greenhouse–grown plants. While this effect has been reported in some very disparate genera and families, studies conducted in this context focused on plant morphology and flowering rather than accumulated biomass (Erwin & Heins 1995; Myster & Moe 1995). A survey of the literature reveals that none of the few studies where mass data were reported, as either fresh or dry weight (DW), comprised enough temperature combinations to permit a conclusion regarding the response of plant biomass production to more than a narrow range of temperature differentials. Although a few ecosystem warming experiments have made use of nighttime warming schemes, the rationale of such experiments does not require more than a very small number of treatments (Arft et al. 2000; Llorens, Peñuelas & Estiarte 2003; Rustad et al. 2001;Shaver et al. 2000; Turnbull, Murthy & Griffin 2002).

Two experiments, reported in Chapters 1 and 2 hereafter, were thus conducted to examine the effects of simultaneous variation in DAT and DTR over a broad range of day and night temperatures, in multiple combinations. The primary objective of these two experiments was to evaluate how warranted investigations of the impact of changes in DTR on plants may be, and provide an empirical basis to that end. Plants of seven taxa of herbaceous perennials belonging to four families were subjected to treatment temperatures over periods comparable in duration to their growth season in temperate climates. Total DW was the principal productivity metric for the seven taxa, and survival and anthesis were recorded in the two taxa that underwent either event in nonnegligible proportions. In contrast with measurement of lower level processes, measured at shorter time scales, this permitted any relevant acclimation and compensatory mechanisms at both the leaf and plant level to take effect.

Chapter 1 provides an account of results pertaining to the effects of variation in the diurnal temperature cycle on gross productivity in seven taxa. Chapter 2 presents anthesis and survival data for the two taxa that underwent either event in sufficient proportion. Treatment–

related mortality was sufficient to assess the relative effects of day temperature, night temperature, DAT, and DTR in *Delphinium* × *cultorum* Voss 'Magic Fountains' ('Magic Fountains' delphinium), as was the fraction of plants that underwent anthesis within the period of observation in *D.* × *cultorum* and *Stokesia laevis* L. 'White Parasols' ('White Parasols' Stokes' aster). Time–to–event data analysis methods were used to analyze these data. These methods preserve power when the event of interest does not occur within the period of observation in some experimental units (Cox & Oakes 1984; Kalbfleisch & Prentice 1980), as was the case with the two experiments reported.

In all but the most strictly controlled experimental settings, however, sub–diurnal variations are present, and often take a highly predictable form (Cesaraccio et al. 2001; Fernandez, 1992). Moreover, protected culture facilities (greenhouses and growth chambers) generally allow at least some control of temperature within the day and night periods. However, the physiological processes that are ultimately integrated into net growth respond to temperature changes in the temporal scale of seconds or minutes. While Chapters 1 and 2 establish that the time scale for empirical integration of heat input at the organismal level should be no longer than one of the two phases of the diurnal cycle, it remains unknown whether that scale should be shorter. To clarify this, two additional experiments were conducted to determine the effects of sub–diurnal temperature variation on gross productivity of two herbaceous taxa with contrasting responses to day/night temperature combinations. Plants of *D.* × *cultorum* and *Rudbeckia fulgida* var. *sullivantii* L. 'Goldsturm' ('Goldsturm' blackeyed Susan) were grown in growth chambers for 40 days, under 28 temperature regimes all resulting in diurnal cycles of 12-h days/12-h nights at average temperatures of either 15/15°C, or 25/15°C. There were 14 regimes for each combination, during which temperature changed every 4 hours, in varying permutations of temperatures ranging from 5 °C to 35 °C. Light period temperature was variable in the first experiment, and dark period temperature in the second. Chapter 3 reports results from those experiments.

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CHAPTER 1

Plant–level Response to Changes in the Diurnal Temperature Cycle:

Empirical Evidence from Seven Herbaceous Taxa.

I. Gross Productivity

Plant–level response to changes in the diurnal temperature cycle:

empirical evidence from seven herbaceous taxa.

I. gross productivity

running title: response to temperature cycling part I

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ABSTRACT

Climate warming has not affected day and night temperature symmetrically: daily temperature range (DTR) is narrowing, as daily average temperature (DAT) increases. The potential impacts of that particular aspect of climate change on plants are not known. Plants of seven herbaceous perennial taxa were exposed to 18 combinations of day and night temperature for 50+ days in two experiments, and effects on total plant dry weight were analyzed with the aid of a widely used thermodynamic model, modified to include two temperature dimensions. The broad range of temperatures used made apparent the three dimensionally peaked character of the relation of plant growth and daily heat input. Results showed that the magnitude of the effects of variation in DTR and in DAT on plant productivity are equivalent, and changes in DAT cannot be considered in isolation. Impact of changes in one temperature dimension depends on initial position relative to both dimensions, but plants exposed to stressfully high DAT are least sensitive to changes in DTR. Conversely, the greatest effect of variation in DTR on gross productivity might be expected on those plants growing closest to their optimal DAT.

Key–words: climate change; daily average temperature; diurnal temperature range; gross plant productivity; plant growth; temperature dependence model; vascular plants; *Delphinium*; *Campanula*; *Rudbeckia*; *Stokesia*.

INTRODUCTION

The asymmetry of climate warming with respect to the diurnal temperature cycle has been noted frequently since first identified in 1991 (Karl *et al.* 1991; Karl *et al.* 1993; Kukla, Karl & Riches eds. 1994; Easterling *et al.* 1997). Over most land areas worldwide, diurnal temperature range (DTR) has narrowed, as daily average temperature (DAT) increased. Nighttime minima have risen at a rate greater than daytime maxima. Continuation of this trend in the future is considered "very likely" by the IPCC (2001). It is unknown at present whether this particular aspect of climate change has any relevance with respect to plant adaptation. Suggestive research by Went (1944a; 1944b; 1957) and Camus & Went (1952) has been invoked to indicate the need to assess the potential impact of this asymmetry, particularly on vegetation (Mooney, Koch & Field 1994). However, it appears that gross responses of plants to variation in the amplitude of the diurnal temperature cycle has received little attention.

The hypothesis advanced by Went (1957) was that diurnal temperature cycling, as opposed to constant temperature, is intrinsically beneficial to plant productivity. Contrary to what is sometimes accepted, it lacks empirical support: the data presented by Went (1944a; 1944b; 1957) and Camus & Went (1952) comprise stem length and fruit yield, whose correlation with total plant productivity is likely too weak to be meaningful, and the experiments reported made use of 8-h day/16-h night temperature combinations, rendering the analysis ambiguous. The duration of exposure to day and night temperature being unequal, heat input and temperature are not equivalent for the two periods. The use of either leads to entirely divergent conclusions. Dry weight (DW) was chosen by some critics (Dale

1964; Haroon, Long & Weybrew 1972; Friend & Hellson 1976; Patterson 1993) as the principal growth metric, in experiments where the diurnal temperature cycle was divided into two 12-h periods, making instantaneous temperature and heat input equivalent as basis for interpretation. The objective, however, was limited to establishing whether or not temperature cycling , as opposed to constant temperature, leads to increased productivity. No attempt was made to characterize the complete response of growth to a full set of combinations, even though such data may be gleaned from figures in some of these reports.

It is well demonstrated that increasing late night temperature above temperature of the beginning of the light period can alter morphology in many greenhouse crops by reducing internode length, thus imparting a more compact appearance to greenhouse–grown plants. While this effect has been reported in some very disparate genera and families, studies conducted in this context focused on plant morphology and flowering rather than accumulated biomass (Erwin & Heins 1995; Myster & Moe 1995). A survey of the literature reveals that none of the few studies where mass data were reported, as either fresh or DW, comprised enough temperature combinations to permit a conclusion regarding the response of plant biomass production to more than a narrow range of temperature differentials.

Finally, although a few ecosystem warming experiments have made use of nighttime warming schemes, the rationale of such experiments does not require more than a very small number of treatments. They do not justify contrasting natural conditions with conditions other than warmer ones, thereby leaving aside the commonalities that may be present in the response of all plants to temperature change (Arft *et al.* 2000; Shaver *et al.* 2000; Rustad *et al.* 2001; Turnbull, Murthy & Griffin 2002; Llorens, Peñuelas & Estiarte 2003). These experiments obviously generate important data regarding ecosystem processes under warmer conditions, but remain predictably inconclusive with respect to the responses of single species. Productivity is increased in some species, diminished in others. This outcome is made all the more likely by the use of local provenances for experimental plant material that is essential in this context: among contemporaneous populations of distinct species in any given locality or region, it is not unreasonable to suppose that each will be situated differently in relationship to the boundaries of its unique environmental tolerances. Unidirectional change in conditions might thus be expected to affect some of the species positively, some negatively.

How current mechanistic models of plant systems would respond to variation in DTR is uncertain. A handful of simulation studies to date have addressed this question specifically (Moot *et al*. 1996; Rosenzweig & Tubiello 1996; Dhakhwa *et al.* 1997; Dhakhwa & Campbell 1998; Constable & Retzlaff 2000). It is assumed that the magnitude of the time interval over which temperature inputs enter models—typically hourly—, together with the detail in which contributing physiological processes are characterized, are sufficient to warrant the congruence of model and plant behavior in response to variations in both DAT and DTR. Although likely, this congruence cannot be verified, if only for lack of empirical reference points for the effects of varying DTR. Keeping in mind the extensive validation that widely used models have undergone, experimental data isolating these two factors would provide a useful check of model behavior, especially near temperature tolerance limits. Short of this, such data would provide some indication of the potential pertinence of simulations addressing this aspect of climate change specifically.

The following two experiments examined the effects of simultaneous variation in DAT and DTR over a broad range of day and night temperatures, in multiple combinations. The primary objective of these experiments was to evaluate how warranted investigations of the impact of changes in DTR on plants may be, and provide an empirical basis to that end. Plants of seven taxa of herbaceous perennials belonging to four families were subjected to treatment temperatures over periods comparable in duration to their growth season in temperate climates. Total DW was the principal productivity metric for the seven taxa, and survival and anthesis were recorded in the two taxa that underwent either event in nonnegligible proportions. In contrast with measurement of lower level processes, measured at shorter time scales, this permitted any relevant acclimation and compensatory mechanisms at both the leaf and plant level to take effect. We determined whether DAT is sufficient to account for the effects of temperature on plant productivity, anthesis, and survival, and what benefits may be realized by separating the diurnal cycle into at least two data. Productivity data are reported in the present paper; anthesis and survival of two taxa are the object of Chapter 2. Particular attention was paid to quantifying the relative effects of day and night temperature.

Comparisons were aided by fitting the temperature dependence model of Johnson, Eyring & Williams (1942) to the productivity data, with two temperature dimensions in place of one. In the context of plant adaptability and tolerance, data and models that extend to stressful conditions have distinct advantages. The thermodynamic model of Johnson *et al.* (1942), especially as augmented by Hultin (1955), then Sharpe and DeMichele (1977), has proven especially apt for characterizing the temperature dependence of biological processes, in very disparate organisms and at multiple scales, whenever broad temperature ranges are considered. As intended by its authors, the model makes it possible to relate the shifts and asymmetries commonly observed in those conditions, to the thermodynamics of enzyme processes.

MATERIALS AND METHODS

Response of four taxa (Expt. 1). Two hundred and seventy seedlings each of *Delphinium* × *cultorum* Voss ‘Magic Fountains’ (‘Magic Fountains’ delphinium) and *Rudbeckia fulgida* var. *sullivantii* L. ‘Goldsturm’ (‘Goldsturm’ blackeyed Susan), 108 plantlets of *Campanula takesimana* × *glomerata* ‘Kent Belle’ (‘Kent Belle’ campanula), and 72 plantlets of *Stokesia laevis* L. ‘White Parasols’ (‘White Parasols’ Stokes’ aster) were planted in 2.84 L containers filled with a substrate of 8 composted pine bark : 1 sand (by vol.) amended with 1.19 kg m^{-3} dolomitic lime. *Delphinium* × *cultorum* and *R. fulgida* were grown from commercially obtained seed, while *C. takesimana* × *glomerata* and *S. laevis* were propagated vegetatively (clonally). Following 20 d of acclimation in a heated greenhouse, the plants were transferred to six, 9 m^2 growth chambers (A–chambers) (Thomas, Downs & Saravitz 2004) at the Southeastern Plant Environment Laboratory [SEPEL (N.C. State Univ. Phytotron)], set at constant temperatures of 10, 15, 20, 25, 30, or 35 °C, and treatments were initiated. The experiment was in a completely randomized design with a factorial arrangement of eighteen, 12-h day/12-h night temperature combinations, or 18 combinations of DAT and day–night temperature difference or diurnal temperature range (DTR) (Table 1.1, Fig.1.1). These combinations represent the broadest range to have been sampled in comparable experiments. The 12-h photoperiod was provided by a combination of cool–white fluorescent lamps and incandescent bulbs, supplying a photosynthetic photon flux of 640 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level as measured by a LI–COR 1800 spectroradiometer (LI–COR, Lincoln, NE).

Temperature combinations were achieved by moving plants to appropriate chambers at the beginning and end of the 12-h photoperiod. Care was taken to move plants grown under constant temperature with all others, and to randomize the position of all plants within each chamber at each transfer. Plants were fertilized at every irrigation with a complete nutrient solution providing N at 7.57 $mol \cdot m^{-3}$. Electrical conductivity and pH of the substrate solution were measured every 7 d, and plant nutrition adjusted accordingly to maintain constant pH and nutrient concentration. Ninety plants each of *D.* × *cultorum* and *R. fulgida* —five per treatment—were harvested after 13, 34, or 56 d. Fifty four plants of *C. takesimana* × *glomerata* —three per treatment—were harvested after 13 or 55 d, and 36 plants of *S. laevis*—two per treatment—after 34 or 55 d. At each harvest, leaf count and leaf area were recorded in addition to leaf, stem, flower, and root DW, after at least 96 h of drying at 70 °C.

Response of five taxa (Expt. 2). Two hundred and seventy seedlings each of *D.* × *cultorum* 'Magic Fountains', *R. fulgida* 'Goldsturm', *C. takesimana*, *C. glomerata*, and *S. laevis*, all smaller than plantlets in Expt. 1, were planted in 0.75–L containers filled with the same substrate as in Expt. 1. Following 20 d of acclimation in a heated greenhouse, the plants were transferred to six, 9 m^2 growth chambers (A–chambers) at the Southeastern Plant Environment Laboratory, set as previously, and treatments were initiated. Growth chambers were maintained as described for Expt. 1. The experiment was in a randomized complete block design with the same treatments as in Expt. 1 (Table 1.1, Fig.1.1), and apparent size of the plantlets at treatment initiation as blocking factor. Plants were grown as in Expt.1. Ninety plants of each taxon—five per treatment—were harvested after 13, 34, or 55 d. At each harvest, leaf, stem, flower, and root DW were recorded as described for Expt. 1. In both experiments, 12 plants of each taxon were harvested at treatment initiation to provide an estimate of initial metrics.

Model description. The model of Johnson *et al.* (1942) originates with the conceptualization of biological "reactions" as consisting of at least two sub–reactions: one accelerating the process, the other inhibiting it, both increasing with temperature, at rates characteristic of each. In the original form of the model (Eqn 1), the numerator characterizes response to temperature in the absence of inhibition, while the denominator accounts for the decrease in response under high temperatures (Johnson *et al.* 1942).

$$f(T) = \frac{c\ T\ e^{\frac{-\Delta H_a}{RT}}}{1 + e^{\frac{\Delta S}{R} - \frac{\Delta H_d}{RT}}} \qquad \text{Eqn 1}$$

Each of the two, or three, sub–processes is characterized using the following thermodynamic parameters: enthalpy of activation (ΔHa), change in entropy associated with deactivation (ΔS), and enthalpy of deactivation (ΔHd). Temperature (T) is measured on the Kelvin scale, and R is the gas constant.

Hultin (1955) showed that the same formulation can be used when a nonlinearity is present at low temperature, and Sharpe and DeMichele (1977) extended the model to include the possibility of both low and high temperature inhibition thresholds, as in Eqn 2. The value of the dimensionless proportionality constant *c* changes between the single and double inhibition equations.

$$f(T) = \frac{c\ T\ e^{\frac{-\Delta H_a}{RT}}}{1 + e^{\frac{\Delta S_{low}}{R} - \frac{\Delta H_{d\ low}}{RT}} + e^{\frac{\Delta S_{high}}{R} - \frac{\Delta H_{d\ high}}{RT}}} \qquad \text{Eqn 2}$$

A somewhat more biologically suggestive parameterization has been achieved by remarking, after Hultin (1955), that $\Delta Hd / \Delta S = T_{½}$, the temperature at which the process—or the single limiting enzyme—has a 0.5 probability of being active, and making the attendant substitutions. In addition, taking into consideration that, at temperatures where no inhibition occurs, the response of interest is characterized fully by the numerator, it is possible to define the new parameter ρ_{Tref}, as the value of the response at some reference temperature T_{ref}, chosen within the uninhibited range (Schoolfield, Sharpe & Magnuson 1981):

$$\rho_{Tref} = c\ \ T_{ref}\ \ e^{\frac{-\Delta H_a}{RT}} \qquad \text{Eqn 3}$$

This in turn allows the elimination of *c* in favor of the more meaningful quantity, ρ_{Tref}, by rearranging Eqn 3 and replacing *c* in Eqn 1 or 2. It should be noted that the uninhibited range corresponds to that range in which thermal time methods are applicable.

These reformulations notwithstanding, the exact referent of thermodynamic expressions used at the organism level has not been clarified, and it is unlikely that the value of the parameters of the equations can be estimated by procedures other than empirical. This was noted by

Johnson *et al.* (1942), and Johnson, Eyring & Stover (1974), who specified that applying their model beyond simple molecules, for example to protein processes, already presupposes that systems of processes behave as single processes: parameters should be viewed as characteristics of the system rather than of any particular enzyme. The need for the more restrictive postulation of a single limiting step has been suggested, but only displaces the indefiniteness, from the referent of expressions to the nature of the enzyme, and becomes more implausible with each up–scaling step. Thus, although using the terminology of thermodynamics is customary, a more descriptive interpretation of the parameters is justified, particularly if the purpose of estimating these parameters is primarily comparative.

RESULTS

Data

Results of both experiments were analyzed separately, but are summarized in common. The conclusions reached in analyzing total plant DW were true of leaf area, leaf, stem, and root DW, and of flower DW where flower DW data was sufficient. Total DW data only are reported henceforth. With seven taxa grown under 18 day/night temperature combinations, the two experiments comprised 162 treatments. After 13 d, plants in the slowest growing of the 162 treatments (taxon/day temperature/night temperature) had grown 0.46 to 2.72 times initial plant DW, depending on taxon (Table 1.2). Maximum observed growth after 13 d ranged between 2.10 and 4.52 times initial size. After 55 d, the smallest observed growth was between 1.29 and 8.24 times initial DW, and the largest, between 7.58 and 48.90. Within the time boundaries of the experiments, some treatments with higher day or night temperatures were lethal to *D.* × *cultorum*. For that taxon, the lowest gains reported at 13 and 55 d are those of the smallest living treatment. These data imply that, all taxa considered, the fastest growing treatment was between 1.55 and 5.30 times larger than the slowest one after 13 d, and between 3.05 and 19.30 after 55 d. As a result, the magnitude of the responses observed was wholly suitable to support meaningful conclusions. In all taxa, the range of temperatures used in these experiments was sufficiently broad to expose a distinct optimum in the response of productivity, with growth decreasing at both lower and higher temperatures. Significantly, these peaked responses were present in two temperature dimensions, with little evidence of any ridge, resulting in three dimensional peaks (Figs. 2 & 3).

With respect to the effects of differential changes in day and night temperature, it is readily evident that changes in one dimension affect plant growth in a manner that is entirely dependent on the initial position of the plant within a taxon's response space. For example, the effect of a 5 °C increase in DTR would depend both on taxon, and on initial day/night combination. Those two temperature dimensions can be chosen as any two of the following factors: day temperature, night temperature, DAT, and DTR, given that any pair among those four factors is a linear combination of the remaining pair. Although all pairwise combinations hold the same information, they are not all so suited to graphical representation as the day temperature/night temperature or DAT/DTR pairs.

Effects of all factors were significant in all taxa, experiments, and harvests, as were their interactions. In addition, for those two taxa that were present in both experiments— *D.* × *cultorum* and *R. fulgida*—, there were strong interactions between experiment and all other factors. All pairs among the four temperature factors under consideration being linear combination of the remaining pair, any pair would explain the same proportion of observed variability, although each single factor does not. Over both experiments and all taxa, the smallest proportion of variability two factors accounted for was 58%, for *S. Laevis* in the second experiment (Table 1.3). For that taxon and experiment, the corresponding proportions for single factors were 27%, 25%, 32%, and 15%, for day and night temperature, DAT, and DTR, respectively. The largest proportion of variability explained by two factors was 97%, for *R. fulgida* in the second experiment. Corresponding proportions for single factors were 43%, 35%, 64%, and 6%. The same disparity was found between the explanatory value of single factors and sets of two factors for top DW, and root DW.

Model fitting and revision

From an empirical standpoint, the distinctive strength of the temperature dependence model of Johnson *et al.* (1942) is its ability to characterize the asymmetries and rapid changes that are often present in the response of biological processes to variation in temperature when considered over a broad range. The fit of either the four– or six– parameter model (Eqn 1 and Eqn 2) to the effect of either day or night temperature alone was exceptional for all taxa, when the other temperature dimension was held fixed. When both dimensions were taken into account, the multiplicative combination of two, four–parameter terms, one each for the effects of day and night temperature (Eqn 4), was sufficient to render some of the asymmetries in each of the two dimensions, as attested by

$$f(T_{day}, T_{night}) = C \left(\frac{T_{day} \; e^{\frac{-\Delta H_{a\ day}}{RT_{day}}}}{1 + e^{\frac{\Delta S_{day}}{R} - \frac{\Delta H_{d\ day}}{RT_{day}}}} \right) \left(\frac{T_{night} \; e^{\frac{-\Delta H_{a\ night}}{RT_{night}}}}{1 + e^{\frac{\Delta S_{night}}{R} - \frac{\Delta H_{d\ night}}{RT_{night}}}} \right) \qquad \text{Eqn 4}$$

some satisfactory fits (Table 1.4). However, this model failed to capture the particular interaction responsible for the observation that in most taxa, optimum temperature for either day or night decreased as the temperature of the other part of the diurnal cycle increased (Figs. 2 & 3). Introducing an inhibitory effect of day temperature within the effects of night temperature accomplished this objective (see denominator of night term in Eqn 5). The model presented in Eqn 5 was also best suited to top DW and root DW data, which are not presented.

$$f(T_{day}, T_{night}) = C \left(\frac{T_{day} \; e^{\frac{-\Delta H_{a\,day}}{RT_{day}}}}{1 + e^{\frac{\Delta S_{day}}{R} - \frac{\Delta H_{d\,day}}{RT_{day}}}} \right) \left(\frac{T_{night} \; e^{\frac{-\Delta H_{a\,night}}{RT_{night}}}}{1 + e^{\frac{\Delta S_{night1}}{R} - \frac{\Delta H_{d\,night1}}{RT_{day}}} + e^{\frac{\Delta S_{night2}}{R} - \frac{\Delta H_{d\,night2}}{RT_{night}}}} \right) \qquad \text{Eqn 5}$$

Under some conditions of day temperature, meaningful growth appeared possible at night temperatures lower than those sampled in these experiments (Figs. 2 & 3), and inhibitory shifts that might be present under those conditions could not therefore be captured in the model. If such shifts were not sampled, the resulting inclusion of fewer inhibition terms than would theoretically be possible could have reduced somewhat the precision of the estimation of the parameters that were included. This is likely to be a greater hindrance in *D.* × *cultorum*, for which growth continued at both the lowest day and night temperatures sampled, and which experienced high mortality at higher temperatures.

With respect to the effects of differential changes in day and night temperature, it is readily evident that changes in any one dimension affected plant growth in a manner entirely dependent on the position of the plant within a taxon's response space. For example, the effect of a 5 °C increase in DTR would depend both on taxon, and on initial day/night combination. In addition, the effects of changes in DTR in most taxa were inversely proportional to the proximity of DAT to its optimum. Increasing responsiveness to variation in DAT with proximity to optimal DTR was even more pronounced, as reflected in the fact that DAT explained a greater proportion of variability in seven out of nine cases (Table 1.3). Finally, as noted previously, intergeneric and interspecific differences within each experiment were not meaningfully greater or smaller than the differences observed between the successive iterations of *D.* × *cultorum* and *R. fulgida*.

DISCUSSION

These experiments demonstrate that changes in DTR have the same potential to alter productivity as changes in DAT, and that neither one should be considered in isolation. The effects of variation in DTR on plant productivity are equivalent in magnitude to those of variation in DAT, but as the presence of interaction between experiment and temperature factors made apparent, other factors can modify the value of driving parameters. The empirical values of all parameters obtained from these experiments, including optimal day and night temperature, should therefore not be generalized to other environments. The results presented herein, however, establish that consideration of the effects of change in DTR in the predictive assessment of the impact of climate change on plants is warranted, and that with respect to daily heat input, a minimum of two data are necessary. Any two of four temperature factors (DAT, DTR, day, and night temperature) would convey the same information. It is when any of those factors is at its optimum that change in the orthogonal one had the greatest impact.

In the particular conditions of these experiments, optimal temperature conditions entailed a nonzero difference between day and night temperature for all but *D.× cultorum*. This may seem to confirm Went's hypothesis (Went 1957), but it is very unclear, and difficult to prove, that such would be the case in all environments. Furthermore, given that few plants, if any, are ever growing in environments that match their intrinsic optimal requirements, it may be more relevant to note that the relative deficit resulting from moderately sub– or supra–optimal temperature in one part of the diurnal cycle (i.e. day or nighttime), can be partially offset by increasing or decreasing temperature in the other part. In other words, the optimal value of DTR changes with every changing value of DAT, and vice versa, even though growth would only attain its theoretical maximum at some unique combination of both. For a given taxon, constant temperature is likely to be preferable at some values of DAT, and cycling at others. The import of combining broad ranges of day and night temperature is to expose the three dimensionally peaked character of the relation of plant growth and daily heat input, more than to ascertain the inherent superiority of constant or cycling temperature.

Examination of the data readily illustrates the necessity of carefully circumscribing conclusions based on small numbers of day/night temperature combinations, as in many plant height control, and ecosystem warming experiments. In view of the overall relationship of temperature and plant DW (Figs. 2 & 3), it is clear that data issued from sampling of three constant temperatures and two cycling combinations, for example, while unquestionably appropriate in many contexts, would not be conclusive with respect to the general relationship of plant productivity and temperature cycling. Our experiments also strongly underscore the advantage of warming experiments that include changes in both DAT and DTR. Those experiments could be further enhanced by including assessment of within population variability with respect to response to changes in DAT and DTR among their objectives.

Incidentally, additional experiments by the authors (Dubois, 2004) did not find any meaningful effect of sub–diurnal temperature variation. There was little or no difference in DW among plants of *D.*× *cultorum* or *R. fulgida* grown for 40 d under 28 different combinations of 4-h temperature periods that all produced day/night averages of either 25/15 or 15/15 °C. This suggests that, where season–long effects on productivity are concerned, there may be little benefit to using more than two temperature data per 24-h.

The empirical data produced in these experiments reflect the integration of all plant processes at the organismal level, and over a seasonal time scale. Characterizations based on scaling–up of cell or organ processes should be expected to produce similar observations when extended to similar durations. Our results emphasize the suitability of checking the behavior of mechanistic models under a variety of scenarios involving changes in both DAT and DTR. In particular, the presence of marked shifts or thresholds outside of the milder range of conditions suggests that special caution should be exercised in extending in to stress conditions models that do not provide for such behavior. In special reference to models of annual crops, it should be noted that the data of Friend and Helson (1976), which included *Triticum aestivum* L. (wheat) and *Zea mays* L. (corn), showed no significant difference between annual and perennial taxa.

In addition, results presented herein demonstrate that the temperature dependence model of Johnson *et al*. (1942) can be used to characterize organism level temperature dependence adequately, and that it can be adapted to two temperature dimension cases. The observation that DAT alone often held explanatory power somewhat greater than DTR, day temperature, or night temperature alone, was conveyed in the three–dimensional model by introduction of an effect of day temperature within the effects of night temperature. The sampling space defined by these experiments did not apparently extend to the extremes of daytime heat combined with nighttime cold that would have exposed a precipitous decrease in productivity down to the values observed when temperature was highest in both periods. It is difficult to assess whether such an extension of the day/night combinations sampled would change the overall explanatory value of DAT, and with what additional effects it might then be possible to augment the three–dimensional model. It does not mean, however, that predictive worth is compromised in localities that experience relative extremes of combined high day and low night temperature, but rather that it may not be as robust for those populations growing near the limit of their own tolerance relative to that combination.

Given relatively genetically uniform plant material—be it a cultivar or a single wild provenance—, any unidirectional change in either DTR or DAT affects productivity in a manner that depends on the position of this population within its own response space, under initial conditions. In any single location, diminution of DTR, like increase in DAT, would result in decreased growth for some taxa, and increased for others. Furthermore, whether a reduction in DTR would temper the effects of an increase in DAT, or worsen them, may not be predictable without knowledge of the response of the particular cultivar or provenance to a broad range of temperature combinations. From the standpoint of gross productivity, changes in one temperature dimension stand to have the greatest impact on those populations of a given taxon growing in localities where values of the orthogonal dimension are closest to the optimum for that taxon. Within a population, the greatest change in productivity might be expected for those plants that are best adapted to local value of the orthogonal dimension, not on those at the margin. Assessing adaptability to climate change would seem to require that within and between population variability be investigated both in tolerance to increased DAT, and to diminished DTR.

Finally, gauging the need for inclusion of the effects of changes in DTR in the predictive assessment of the effects of climate change on plants does not amount to contrasting the effects of change in DAT alone, and those of change in DTR alone. The scenarios to be compared are a change in DAT alone, and the same change in DAT, modified by a concomitant reduction in DTR. The first would correspond to a 45 degree translation, from lower to higher values of DAT in the response surface of Fig. 1.2 & 1.3, the second to a longer translation with an angle smaller than 45 degrees, between the same values of DAT. This implies again that the difference between the effects of these two scenarios on plant productivity becomes larger as plants are growing closer to their optimal DAT. Putatively, the difference in gross productivity between climate warming that affects only DAT, and climate warming that affects both DAT and DTR, is greatest not at the confines of a species geographical range, but at the center. At the fringes of the range, either scenario would have the same effect.

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Table 1.1. Eighteen 12 h day/12 h night temperature combinations used in Expts. 1 and 2, and the corresponding 18 combinations of daily average temperature (DAT)/diurnal temperature range (DTR).

T_{day} (°C)	10	10	10	15	15	15	20	20	20	25	25	25	30	30	30	35	35	35
T_{night} (°C)	10	20	30	15	25	35	10	20	30	15	25	35	10	20	30	15	25	35
DAT (°C)	10	15	20	15	20	25	15	20	25	20	25	30	20	25	30	25	30	35
DTR (°C)	0	−10	−20	0	−10	−20	10	0	−10	10	0	−10	20	10	0	20	10	0

Table 1.2. Summary of total plant DW data of Expts. 1 and 2.

Taxon	Time (d)	Initial DW (g)	Lowest treatment DW (g ± SE)	Highest treatment DW (g ± SE)	Ratio of highest to lowest DW	Increase from initial to lowest treatment DW	Increase from initial to highest treatment DW
				Experiment 1			
Campanula glomerata × takesimana	0	2.66					
	13		3.50±0.08	6.56±0.68	1.87	1.32	2.47
	55		10.56±1.78	33.93±3.41	3.21	3.97	12.76
Delphinium × cultorum	0	2.49					
	13		3.11±0.39	6.37±0.58	2.05	1.25	2.56
	56		6.82±1.53	28.35±3.84	4.16	2.74	11.39
Rudbeckia fulgida	0	2.20					
	13		4.26±0.19	6.60±0.42	1.55	1.94	3.00
	54		13.58±0.84	51.24±3.51	3.77	6.17	23.30
Stokesia laevis	0	5.64					
	13						
	55		8.02±1.46	42.76±3.54	5.33	1.42	7.58
				Experiment 2			
Campanula glomerata	0	0.43					
	13		1.18±0.10	1.96±0.08	1.66	2.72	4.52
	56		5.61±1.13	20.36±1.02	3.63	12.92	46.90
Campanula takesimana	0	2.15					
	15		1.94±0.22	4.52±0.38	2.33	0.91	2.10
	57		9.14±0.33	27.87±1.67	3.05	4.26	12.99
Delphinium × cultorum	0	0.80					
	13		0.37±017	1.96±0.30	5.30	0.46	2.45
	55		1.03±0.53	19.79±1.15	19.30	1.29	24.80
Rudbeckia fulgida	0	0.86					
	13		1.81±0.13	2.76±0.12	1.52	2.09	3.19
	55		7.39±0.56	42.32±0.73	5.73	8.54	48.90
Stokesia laevis	0	1.32					
	15		2.14±0.48	3.47±0.62	1.62	1.62	2.63
	58		4.14±1.30	23.28±1.06	5.63	3.13	17.65

Table 1.3. Explanatory value of temperature variables (percentage of variability explained) in Expts. 1 and 2.

Taxon	Day temp.	Night temp.	Daily avg. temp.	Diurnal temp. range	Two temp. data
			Experiment 1		
Campanula glomerata × takesimana	37	24	31	32	77
Delphinium × cultorum	10	45	35	43	63
Rudbeckia fulgida	44	34	57	14	88
Stokesia laevis	43	32	37	15	81
			Experiment 2		
Campanula glomerata	27	22	47	8	84
Campanula takesimana	38	23	36	19	85
Delphinium × cultorum	28	24	61	27	83
Rudbeckia fulgida	43	35	64	6	97
Stokesia laevis	27	25	32	15	58

Table 1.4. Summary of model fit (R^2) for the modified Johnson *et al.* (1942) temperature dependence model.

Taxon	Seven parameters	Nine parameters
	Experiment 1	
Campanula glomerata × takesimana	0.762	0.916
Delphinium × cultorum	0.936	0.979
Rudbeckia fulgida	0.775	0.902
Stokesia laevis	0.957	0.958
	Experiment 2	
Campanula glomerata	0.752	0.917
Campanula takesimana	0.868	0.959
Delphinium × cultorum	0.712	0.937
Rudbeckia fulgida	0.678	0.871
Stokesia laevis	0.854	0.911

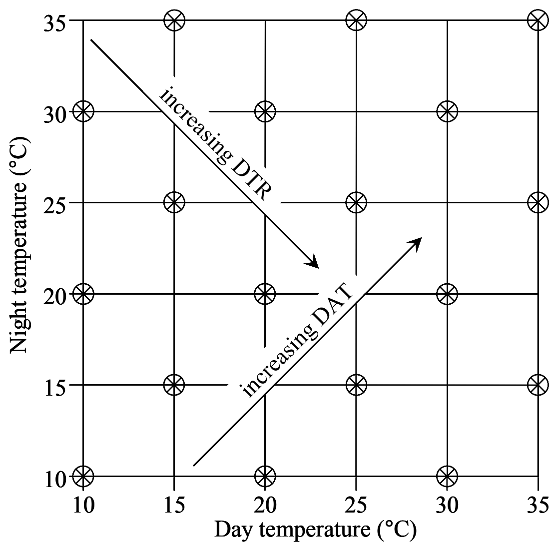


Figure 1.1. Eighteen 12-h day/12-h night temperature combinations used in Expts. 1 and 2. To each combination corresponds a marked intersection of day and night temperature.

Figure 1.2. Total plant DW of *Campanula* taxa after 54 to 58 d. Values are scaled to percentage values of the largest value observed (grey contours) or modeled (black contours).

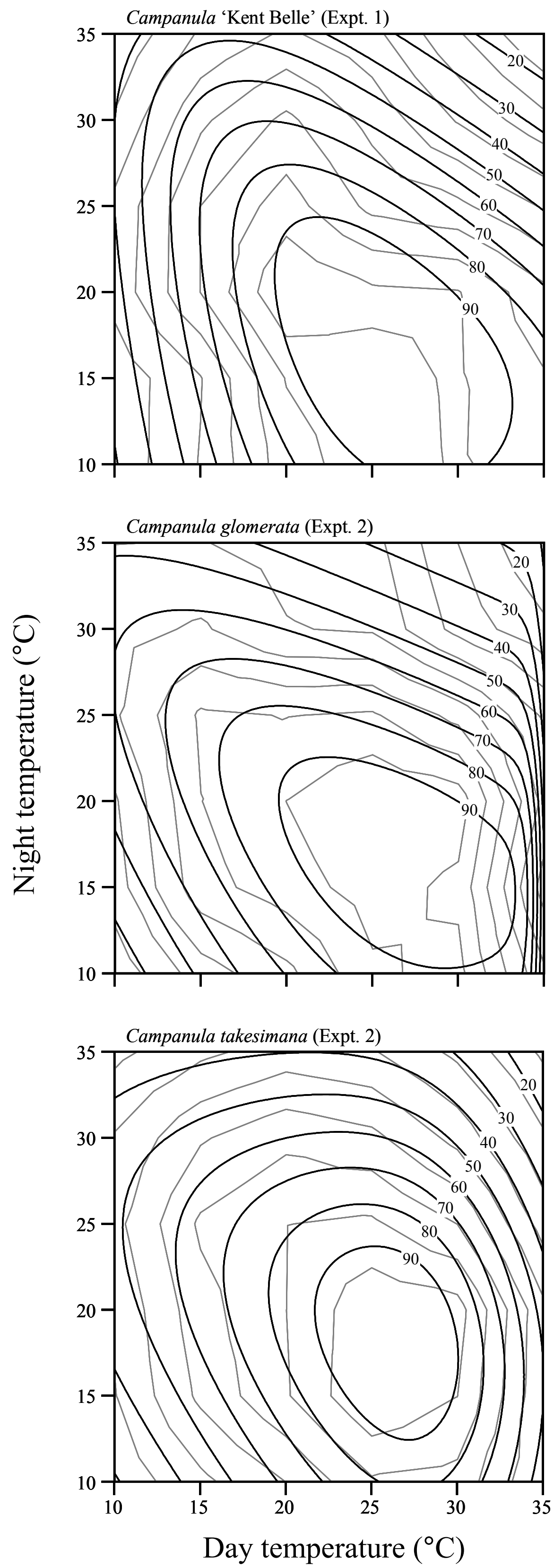
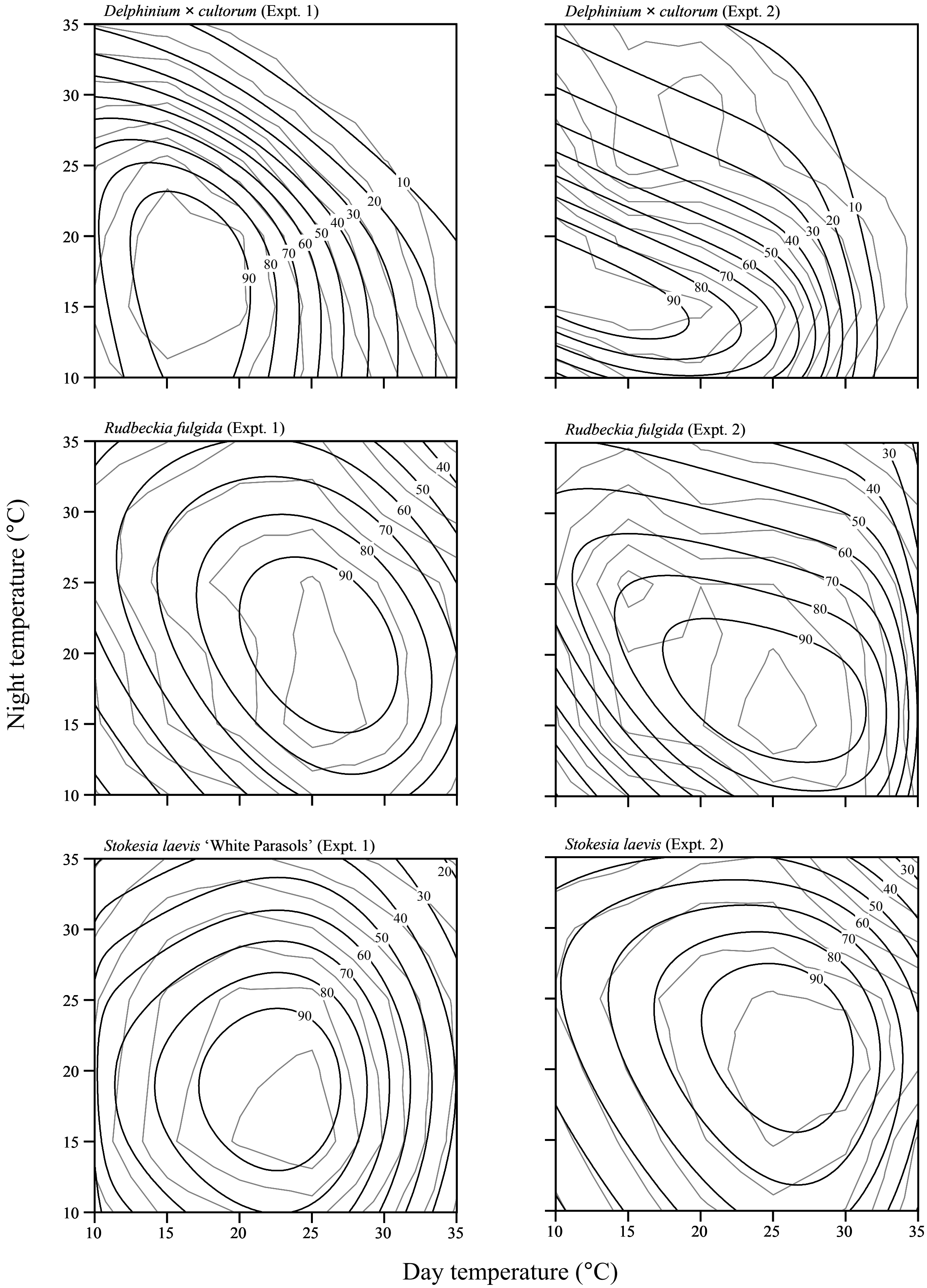

Campanula 'Kent Belle' (Expt. 1)
Campanula glomerata (Expt. 2)
Campanula takesimana (Expt. 2)
Night temperature (°C)
Day temperature (°C)
10
15
20
25
30
35
20
30
40
50
60
70
80
90

Figure 1.3. Total plant DW of Delphinium, Rudbeckia, and Stokesia taxa after 54 to 58 d. Values are scaled to percentage values of the largest value observed (grey contours) or modeled (black contours).



Delphinium × cultorum (Expt. 1)
Delphinium × cultorum (Expt. 2)
Rudbeckia fulgida (Expt. 1)
Rudbeckia fulgida (Expt. 2)
Stokesia laevis 'White Parasols' (Expt. 1)
Stokesia laevis (Expt. 2)
Night temperature (°C)
Day temperature (°C)

CHAPTER 2

Plant–level Response to Changes in the Diurnal Temperature Cycle:

Empirical Evidence from Seven Herbaceous Taxa.

II. Anthesis and Survival of Two Taxa

Plant–level response to changes in the diurnal temperature cycle:

empirical evidence from seven herbaceous taxa.

II. anthesis and survival of two taxa

running title: response to temperature cycling part II

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ABSTRACT

Over most land areas, night temperature is rising faster than day temperature. The relevance of variation in daily temperature range (DTR) to plant adaptability being unknown, two experiments were conducted to provide an empirical basis, on which to assess the support for investigations of the impact of this specific aspect of climate change. Plants of seven perennial taxa were exposed to 18 combinations of day and night temperature for 50+ days, and it was found that the impact of variation in DTR on total plant dry weight is comparable to that of variation in daily average temperature (DAT). Two taxa underwent anthesis and death at varying rates under different experimental temperatures, and time–to-–event data analysis methods were used to determine the relative effects of DAT and DTR. In both anthesis and survival data, effects of day and night temperature could be separated, but were almost entirely subsumed into DAT. Although effects of DTR were significant, its action only became meaningful at the extremes of its value. Results suggest the consequences of changes in DTR on anthesis and heat–related plant death are a lesser concern than their implications for gross productivity.

Key-words: climate change; daily average temperature; diurnal temperature range; time–to–event; vascular plants; *Delphinium*; *Stokesia*.

INTRODUCTION

From 1951 to 1990, the increase of daily minimum temperatures outpaced that of maxima (Karl et al. 1991; Karl et al. 1993; Kukla, Karl & Riches eds. 1994; Easterling et al. 1997). As daily average temperature (DAT) continues to increase, further narrowing of diurnal temperature range (DTR) is considered "very likely" by the IPCC (2001). The consequences of this narrowing for plants are unknown at present. Two experiments were therefore conducted to assess empirically whether the potential impact of changes in DTR warrants investigation. The first part of this report gave an account of results pertaining to the effects of variation in the diurnal temperature cycle on gross productivity in seven taxa. The following presents anthesis and survival data for the two taxa that underwent either event in sufficient proportion. Treatment–related mortality was sufficient to assess the relative effects of day temperature (T_{day}), night temperature (T_{night}), DAT, and DTR in *Delphinium* × *cultorum* Voss 'Magic Fountains' ('Magic Fountains' delphinium), as was the fraction of plants that underwent anthesis within the period of observation in *D.* × *cultorum* and *Stokesia laevis* L. 'White Parasols' ('White Parasols' Stokes' aster).

Event data are often summarized and analyzed as the duration necessary for some fixed proportion (e.g., 50%, 90%, or 100%) of a sample to undergo the event (e.g., germinate, flower, or dehisce), or as the proportion that undergoes the event by some meaningful point in time (e.g., 10 d, 30 d, or one season). The role of heat input in developmental processes and the events that mark them is characterized customarily using thermal time, through which the occurrence of events can be made dependent upon both temperature and time of exposure (Ritchie & NeSmith 1991).

In order to assess relative effects of DAT and DTR, however, it is preferable that the range of temperatures to which plants are exposed, be broad enough to capture responses at their extremities. In consequence, it is to be expected that a nonnegligible proportion of plants will not undergo the events of interest within a practicable period. In addition, the amount of information produced by the two experiments was maximized by filling growth chambers to full capacity at initiation of treatments, and randomly removing a preset proportion of plants of each taxon in one of three partial harvests, before canopy closure could be reached. Many plants were thus removed from observation before undergoing either anthesis or death, and would have to be considered missing, should general linear models be used in analyzing these data. Time–to–event data analysis methods, in contrast, were developed for the analysis of this class of data, and preserve power when the event of interest does not occur within the period of observation in some experimental units (Kalbfleisch & Prentice 1980; Cox & Oakes 1984). Thermal time approaches to phenological prediction and time–to–event data analysis methods both place time directly within the predictive function for events. In both perspectives, the consequences of an increase in the duration of exposure are similar (but not identical), to those of an increase in temperature. The present data made time–to–event data analysis methods preferable.

In the experiments described herein and in Chapter 1, plants were grown under a broad range of T_{day} and T_{night}, in multiple combinations, and for a period > 50 d. Results, reported in Chapter 1, demonstrate that heat input cannot be aggregated into fewer than two daily values with respect to its season–long effects on gross plant productivity. A minimum of two data per 24-h period is necessary to account for the relationship of temperature and dry weight (DW), and investigation of the impact of changes in DTR on gross plant productivity may thus be warranted. The objective of the following study was to examine whether the same is true of anthesis and survival.

MATERIALS AND METHODS

Anthesis of *D. × cultorum* and *S. laevis,* and survival of *D. × cultorum* (Expt. 1).

Materials and methods for Expt. 1 have been described in Chapter1. Briefly, plants were cultivated under 18 day/night temperature combinations, as summarized in Table 1. Two hundred and seventy seedlings of *D. × cultorum* and 72 plantlets of *S. laevis* were subjected to those combinations. Plants of *D. × cultorum* were grown from commercially obtained seed, mean number of expanded leaves at treatment initiation was 13.08 (SD = 2.39), and mean plant dry weight (DW) was 2442 mg (SD = 637 mg). No floral buds were visible. Plants of *S. laevis* were propagated vegetatively, and small but clearly visible floral buds were present on all plants. Mean number of expanded leaves at treatment initiation was 25.3 (SD = 4.8), and mean plant DW was 5645 mg (SD = 1647 mg). All plants were examined daily, and every flowering and death was recorded. Dead plants were removed immediately. Anthesis was recorded for *D. × cultorum* when the buds of a third of the inflorescence were fully opened, and when at least one flower was fully expanded for *S. laevis*. Plant death was recorded when plants were fully wilted and had lost all color. Plants were assigned randomly to one of two (*S. laevis*) or three (*D. × cultorum*) partial harvests before treatments were initiated, and were harvested whether or not they had flowered. Ninety plants of *D. × cultorum*—five per temperature combination— ,minus those that had died by the time of harvest, were destructively harvested after 13, 34, or 56 d to collect productivity data. Thirty six plants of *S. laevis*—two per treatment—were harvested after 34 or 55 d. For the purpose of time–to–event data analysis, one datum was recorded for every plant, consisting of either the day the event was observed, or the day of harvest if a plant had not flowered or died by the time of harvest. Each datum was paired with an indicator of whether the time recorded was the time of the event, or the time when the plant was withdrawn from observation without having undergone the event. Thus, there were 270 data for anthesis of *D.* × *cultorum*, and 270 for death, and 72 data for anthesis of *S. laevis*. Eight plants of *D.* × *cultorum* both flowered and died within the period of observation. The range of temperatures applied was such that, under some of the 18 combinations, anthesis or death were extremely unlikely within a practicable duration. The resultant censoring was compounded by the removal of a large proportion of plants through intermediate harvests.

Survival of *D.* × *cultorum* (Expt. 2).

Materials and methods for Expt. 2 have been described in Chapter 1. Briefly, plants were cultivated under the same 18 day/night temperature combinations as in Expt. 1 (Table 1). All plant material for this experiment was smaller and less mature than in Expt. 1. Death of *D.* × *cultorum* was the only event that occurred in a nonnegligible proportion of the sample. This event was recorded as described for Expt. 1. Plants of *D.* × *cultorum* were grown from commercially obtained seed. Mean number of expanded leaves at treatment initiation was 8.13 (SD = 0.71), and mean plant DW was 798 mg (SD = 137 mg). No floral buds were visible.

Statistical methodology.

Normal theory general linear models, namely analysis of variance and linear regression, are used customarily in analyzing time–to–event data in plant biology, despite these data generally violating the assumption of normality. The tallying of a changing cumulative proportion over time that is often used in collecting and processing event data, reflects the understanding of event occurrence as a function of both time, and modifying factors, presently temperature. Events accrue with time, and the rate of accrual changes with both time, and those covariates. Setting fixed proportions at which the dependence of duration upon the factors of interest is analyzed, or setting fixed points in time at which to analyze proportions, are two ways to make abstraction of time. Although data may be recorded at many points between initiation and termination of an experiment, only one point in time is taken into consideration at once; the course of the process over time is left unexamined. Thus, less information than is available is used, and the greater the frequency of observations, the more information must be ignored. This diminishes the ability to draw conclusions, curtailing power in testing and prediction, which may become impossible at times when data collected are, in reality, sufficient. Additional loss of information stems from censoring: observations are said to be censored, when an experimental unit is removed from observation before having undergone the event, whether through accidental loss, because of intermediate harvests, or termination of a study. Event time for a censored unit is known to be at least as great as time of removal, but that partial information cannot be taken into consideration by general linear models. The bias resulting from having to regard those observations as missing is commensurate to the proportion of units affected, often to the point of invalidating attempts at analyzing data that were collected.

Time–to–event data analysis methods are well established in medicine, engineering, and actuarial science. They take nonnormality into account, provide for description and analysis of the entire course of accrual over time, and incorporate the partial information of censored observations properly (Kalbfleisch & Prentice 1980; Cox & Oakes 1984; Meeker & Escobar 1998; Hosmer & Lemeshow 1999). Nonparametric methods, primarily product–limit (Kaplan-Meier) estimation, are preferred for preliminary presentation and analysis of data. The product–limit estimate is the observed cumulative event proportion, increasing at every time one or more events are recorded, and adjusted for censored observations. The resulting step function constitutes the most accurate representation of observed progression over time. Parametric methods characterize the relationship of time, event probability, and influencing factors fully. Estimation of parameters and prediction of durations necessary for any proportion of a sample to undergo the event, and of proportions undergoing the event by any point in time, are thus possible. Using semi–parametric methods, estimation of parameters is possible, and prediction at any level of the covariates, but not at times other than those at which events did occur. Because of their ease of interpretation, parametric methods were preferred in analyzing data reported herein.

Parametric models can be expressed in a form evocative of the ordinary linear model, as follows: $t_i = e^{\beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \sigma \varepsilon_i}$, or $\ln t_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \sigma \varepsilon_i$, with t_i the event time of an experimental unit, and x_i s the explanatory variables (covariates). Besides the exponentiation, the differences with the familiar model are the σ multiplier of random error ε (scale parameter), and the necessity to determine the most appropriate distribution for ε. The value of the parameters $\beta_1, \ldots, \beta_k$ provide a direct quantification of the effects of the covariates. In the case of continuous variables such as temperature, the practical interpretation of coefficients is as follows: holding other factors constant, every unit increase in x_i results in an e^{β_i} change in event time. If median time to anthesis at 20 °C is 15 d, it will be ($15\,e^{\beta_i}$ d) at 21 °C, and the same is true for any percentile other that the 50th. In other words, the dependence of event time on the factors of interest is given by $\ln t_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_k x_{ik} + \sigma\varepsilon_i$, and the smaller β_i, the smaller the effect of x_i. A value of 0 for one of the β_i s implies that the corresponding factor x_i has no effect. Models can be compared using a likelihood ratio test, conducted using twice the positive difference between the log–likelihoods of two models, which follows a chi square distribution. Differences to be tested may be between distributions of ε, between combinations of variables in the exponent, or different relationships among them. For a given set of data, the value of model log–likelihood relative to that of other models, is indicative of the explanatory value of the variable or combination of variables included, with comparatively larger values corresponding to a greater contribution of those factors

RESULTS

Anthesis of *D.* × *cultorum* (Expt. 1).

Thirty percent of plants of *D.* × *cultorum* underwent anthesis prior to dying or being harvested. Flower size and reproductive competence were never taken into account, and stunting was substantial where DAT was highest. In eight of 18 day/night temperature combinations, all plants flowered within 56 d, conditionally on not having been removed from observation by harvesting or death. Among the 81 plants whose anthesis was observed, eight died prior to harvest. Because anthesis does not preclude death, but the converse is true, the two events are termed 'semi–competing risks'; death constitutes simple censoring with reference to flowering. Among the 189 plants censored with respect to anthesis, 137 were censored through harvesting, and the other 52 by death. No flowering was observed in the four temperature combinations with DAT ≥ 30 °C, but 27 deaths occurred before harvest among those 60 plants (Figs. 1 & 3).

For anthesis data of *D.* × *cultorum*, the lognormal distribution was found to be the most appropriate. The log–likelihood of the null model, i.e., the model that did not include any explanatory variable, was −68.25. No single variable improved model log–likelihood above the null model when included alone and in the first degree. T_{day} alone, in the first and second degree ($t_{anth} = e^{\beta_0 + \beta_1 T_{day} + \beta_2 T_{day}^2}$, with t_{anth} time to anthesis), yielded a log–likelihood of −57.34, and the same model for T_{night} alone produced −61.72. Both are significantly better than the null model ($P < 0.0001$). The log–likelihood of the full quadratic model, however, comprising both T_{day} and T_{night} in the first and second degree, and a multiplicative interaction term, was −41.69, a significant improvement over either of those two single factor models ($P < 0.0001$). It must be noted, though, that the log–likelihood of the model including only first and second degree terms of DAT ($t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2}$) was −49.89, smaller than that of the full quadratic model (P=0.001), but largely superior to that of all other single factor models. Indeed, DTR alone, in either the first or the first and second degree (log–likelihood = −67.60), brought no improvement to the null model.

To compare the effect of DAT and DTR on anthesis, the following model combined parsimony and high log–likelihood: $t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR^2}$, whose log–likelihood (−42.99), was equivalent to that of the full quadratic model (P=0.273), even though it comprises two variables fewer. All three factors (DAT, DAT^2, DTR^2) being significant, the most meaningful comparison is between the magnitude of their effects, as estimated by the parameters. In addition, it can also be compared to the magnitude of the effects of T_{day} and T_{night} in the full quadratic model. The relevant models in this comparison are thus:

$$\ln t_{anth} = 6.4608 - 0.1534T_{day} + 0.0026T_{day}^2 - 0.1241T_{night} + 0.0019T_{night}^2 + 0.0018T_{day}T_{night},$$

and $\ln t_{anth} = 6.4134 - 0.272DAT + 0.0062DAT^2 + 0.0007DTR^2$. Given that both models account for observations equally well, it is readily apparent from the comparison of parameters that the effects of T_{day} and T_{night} on anthesis can be distinguished, as demonstrated by the first model, but that when DAT is used, it captures most of the effect of temperature: the action of DTR is significant, but hardly meaningful relative to that of DAT. The relative action of DAT and DTR, and how much fit is improved by addition of DTR to DAT as explanatory factor, is illustrated in Fig. 1. The entirety of the data is represented by a step curve of observed time to anthesis for every day/night temperature combination. Those are compared with values predicted when DAT alone in the first and second degree is used ($t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2}$), and when DTR is added to the model ($t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR^2}$). The difference in the overall fit of the models is clearest under the more extreme temperature combinations. The relationship between DAT and time to anthesis is illustrated in Fig. 2, for a few values of DTR, and median time only. Although prediction is possible for any value of time and covariates, the plotting does not include extrapolation outside of the range of temperatures where anthesis occurred, and beyond the period of observation.

Survival of *D.* × *cultorum* (Expt. 1).

Twenty two percent of 270 plants of *D.* × *cultorum* died before being harvested (78% were harvested). In five out of 18 day/night temperature combinations, all plants died within the period of observation. No death was observed in the nine temperature combinations with DAT < 25 °C (Fig. 3). Applying a standard parametric model to the data from all 18 groups would imply that regardless of temperatures applied, all plants would eventually be killed by heat, should enough time be left to pass. This is plainly not true. Comparatively to the duration of survival at lethal values, there is a strong possibility of indefinite survival outside of stressfully high day or night temperatures, at least with respect to that specific cause of death. Furthermore, the covariates of interest very likely influence both the occurrence of death, and its timing. Regarding all plants whose death was not observed as censored would tax the precision of estimates, possibly severely. Models that use two separate distributions, one for event occurrence (i.e., whether the event takes place or not), and the other for event time, known as 'cure models', are preferable from a theoretical standpoint (Farewell 1982; Maller & Zhou 1996), but impractical. The penalty on precision that results from using a single distribution can be minimized if data to be analyzed are restricted to the subset of nine temperature combinations where deaths were observed: within that range, the distribution of event occurrence is not as relevant as it is for the totality of the sample, and the assumption that no death is heat–related at DAT < 25 °C is not as implausible. The transition from temperature conditions under which all plants survive, to conditions that would eventually be lethal to all, may not have taken place exactly where DAT was 25 °C, and was probably at least somewhat gradual, but the restriction that no death would be heat–related below a threshold of 25 °C DAT, while imperfect, minimizes the loss of precision that follows from not estimating the two combined distributions. The subset of the data thus defined comprised 135 observations, 55% of which were censored, instead of 78% among 270 plants.

There was no difference in the suitability of the Weibull, lognormal, and gamma distributions for these data, and the lognormal is adopted in the following analysis. The log–likelihood of the null model was −78.89. In comparison, the log–likelihoods of single–factor models with T_{day}, T_{night}, DAT, and DTR in the first degree as only explanatory variable were −56.58, −73.39, −26.21, and −77.61, respectively. Thus, DTR alone had no effect on survival, but the effect of each of the other three factors was significant at $P < 0.001$. The addition of a second degree term was beneficial only in the case of DAT ($P = 0.007$), with a log–likelihood of −22.53 for $t_{surv} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2}$ (with t_{surv} survival time). The log–likelihood of two–factor models, all four of which are equivalent, was −21.26. The two such models of interest are $t_{surv} = e^{\beta_0 + \beta_1 T_{day} + \beta_2 T_{night}}$ and $t_{surv} = e^{\beta_0 + \beta_1 DAT + \beta_2 DTR}$. No interaction term was significant in any model. A better fit ($P = 0.002$) was realized for the combination of DAT and DTR, however, by the following: $t_{surv} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR}$, whose log–likelihood was −17.60, significantly better than all other models considered. In conclusion, the two models most suitable for comparing the magnitude of the effects of T_{day}, T_{night}, DAT, and DTR, are $\ln t_{surv} = 7.1569 - 0.0730 T_{day} - 0.0523 T_{night}$, and $\ln t_{surv} = 0.8301 + 0.3063 DAT - 0.0073 DAT^2 - 0.0094 DTR$. As with anthesis, the effects of day and night temperature on survival of *D.* × *cultorum* can be separated, both are in the same range of magnitude, and most of the overall effect of temperature on survival can be summarized by DAT alone. Comparative fit of the model using only DAT, in the first and second degree, and the model using both DAT and DTR is illustrated in Fig. 3. As with anthesis, the greatest difference between models, indicative of the contribution of DTR, is under situations where the absolute value of DTR is largest. The relationship between DAT and median survival time is illustrated in Fig. 4, for a few values of DTR.

Anthesis of *S. laevis* (Expt. 1).

All 72 plants of *S. laevis* had visible flower buds at initiation of treatment, and all but four plants flowered during the period of observation. Flower quality was not taken into account, and was poor under both high and low DAT. The lower power and precision that resulted from the smaller number of plants, relative to *D.* × *cultorum*, was offset by their greater uniformity, and by the smaller number of harvests. Together with the more advanced stage of flower development, these lead to far lighter censoring. Log–likelihood of the null model was −42.38. Log–likelihood of single–factor models in the first degree for T_{day}, T_{night}, DAT, and DTR, was −23.28, −23.79, 8.25, and −42.38, respectively. Thus, as in anthesis and survival of *D.* × *cultorum*, DTR alone had no effect on anthesis of *S. laevis*. DAT alone, in the first and second degree ($t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2}$), produced a log–likelihood of 22.24, and the addition of DTR in the second degree to this model ($t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR^2}$) increased it to 30.01, a significant improvement ($P < 0.0001$). All terms in the full quadratic model had a significant effect, but the model log–likelihood was not significantly different from that of $t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR^2}$ (P=0.289). Coefficients for those two models are: $\ln t_{anth} = 5.4933 - 0.1158 T_{day} + 0.0017 T_{day}^2 - 0.0882 T_{night} + 0.0115 T_{night}^2 + 0.0005 T_{day} T_{night}$, and $\ln t_{anth} = 5.4934 - 0.2040 DAT + 0.0033 DAT^2 + 0.0005 DTR^2$. The difference in magnitude between the coefficients of DAT and DTR is even greater than for anthesis and survival of *D.* × *cultorum*, even though the effects of T_{day} and T_{night} can be distinguished, and are commensurate with each other. The comparative fit of $t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2}$, and $t_{anth} = e^{\beta_0 + \beta_1 DAT + \beta_2 DAT^2 + \beta_3 DTR^2}$ is illustrated in Fig. 5. Response of median time to anthesis to variation in DAT is illustrated in Fig. 6, for a few values of DTR.

Survival of *D.* × *cultorum* (Expt. 2).

Only 12% of 270 plants of *D.* × *cultorum* died before being harvested. At least one plant in every day/night temperature combination survived to the first harvest. No death was observed in the nine temperature combinations with DAT < 25 °C, and only four plants died before harvest in the five combinations with DAT=25 °C (75 plants). Sixteen deaths were observed before harvest among the 45 plants exposed to DAT=30 °C, and 12 among the 15 plants exposed to DAT=35 °C. As in Expt. 1, the subset defined by DAT < 25 °C was used in analyzing the data, based on the assumption that no death below that threshold would be heat–related. Log–likelihood of the null model was −74.53. Log–likelihood of single–factor models in the first degree for T_{day}, T_{night}, DAT, and DTR, was −65.42, −65.91, −44.17, and −74.53, respectively. The model involving the first degree of T_{day} and T_{night} was the only significant multifactorial model, and its log–likelihood was −44.15. Thus, while a longer period of observation would have yielded sufficient data to quantify the relative effects of DAT and DTR better, the data obtained were fully consistent with the results of Expt. 1. DTR alone had no effect on survival of *D.* × *cultorum*, and DAT absorbed the entire effects of T_{day} and T_{night}.

DISCUSSION

Effects of T_{day}, T_{night}, DAT, or DTR can be differentiated within the overall action of varying temperature on the timing of anthesis in *D.* × *cultorum* and *S. laevis*, and of heat–related death in *D.* × *cultorum*. The contribution of variation in DTR, however, is small relative to that of DAT: the effects of T_{day} and T_{night}, may be distinguished, but are almost entirely subsumed into DAT. Results thus suggest that, in most circumstances, one temperature datum is sufficient to account for the relationship of daily heat input and timing of anthesis or death. This contrasts with the findings from the present experiments regarding response of gross plant productivity (Chapter 1).

In these experiments, temperatures extended from values under which survival of *D.* × *cultorum* was likely indefinite, to where it was precipitously short, and in all directions from the shortest time of anthesis for either taxon. The absolute values of DTR spanned 40 °C, and DAT extended from 10 to 35 °C. Under these conditions, which ensured that the essential characters of the relationship of temperature and events of interest were captured, the impact of varying DTR only became meaningful when that factor reached its extremes. Change in DTR would have to be exceedingly larger than change in DAT for its effect on event time to be within the same magnitude. For noncultivated plants under natural conditions, this suggests that within a local population of a given taxon, all plants are affected by change in DAT, but only those at the extremes of that population's distribution with respect to the response of event time to varying DTR, are affected by change in that factor. The same hypothesis might be extended to local populations within a taxon's overall geographical range: all populations may be affected by changes in DAT, but only populations at the extremes of the taxon's range with respect to DTR would be.

There was, incidentally, little or no difference in size between the effects of day and night temperature on anthesis of *D.* × *cultorum* and *S. laevis* and survival of *D.* × *cultorum*. Despite the plausibility of the hypothesis that plant death under conditions of prolonged heat stress could be related to accumulated assimilatory deficit, whereby losses from nighttime respiration exceed gains from photosynthesis, data from observation of the actual event, do not bear it out. High night does not appear to be any more responsible for plant mortality than high day temperature, and neither is a lack of relative nighttime cooling.

The significance of the results presented in Chapter 1 and those herein may not be entirely alike for cultivated and noncultivated plants. Insofar as ecological stability under rapid environmental change is desirable in natural ecosystems, success in noncultivated plants is correlated at least as closely with biomass productivity, as it is with flowering and survival. Sustained productivity, required under natural competitive conditions, appears controlled by both DAT and DTR. Flowering is a necessary condition of genetic success, and is controlled mostly by DAT. It is not, however, a sufficient condition, and reproductive functioning was not examined. Finally, lethal failure under stress is near entirely accounted for by the action of DAT, but only represents the terminal confine of adaptability. Increasing temperature likely brings about the disappearance of individuals and populations earlier than they would be killed directly by heat. Thus, although attention to the impact of changing DTR on noncultivated plants may not be central where survival and the timing of flowering are concerned, it remains warranted overall.

Food and fiber crops, cultivars of which are often bred and selected for their performance in specific climatic conditions, are commensurately less likely to be found near the boundary of their environmental tolerance. Changes in prevalent temperature conditions would obviously affect these crops, but where anthesis and survival are concerned, effects of change in DAT would justify modifying cultural practices and cultivar choice well before those of change in DTR. Where decline in gross (biomass) productivity, however, would make such alterations necessary, changes in both DAT and DTR would be relevant.

Cultivars of ornamental plants, in contrast, are almost never developed for adaptation to a circumscribed region. Cultivation is often attempted in a broad variety of climates, and despite the greater control of inputs that is sometimes afforded ornamental cultivation, plants are routinely found beyond the limits of their adaptability, albeit only temporarily. It might therefore be among ornamental taxa that examples of changes in DTR affecting survival and the timing of flowering are to be found.

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Table 2.1. Eighteen 12 h day/12 h night temperature combinations used in Expts. 1 and 2, and the corresponding 18 combinations of daily average temperature (DAT)/diurnal temperature range (DTR).

T_{day} (°C)	10	10	10	15	15	15	20	20	20	25	25	25	30	30	30	35	35	35
T_{night} (°C)	10	20	30	15	25	35	10	20	30	15	25	35	10	20	30	15	25	35
DAT (°C)	10	15	20	15	20	25	15	20	25	20	25	30	20	25	30	25	30	35
DTR (°C)	0	−10	−20	0	−10	−20	10	0	−10	10	0	−10	20	10	0	20	10	0

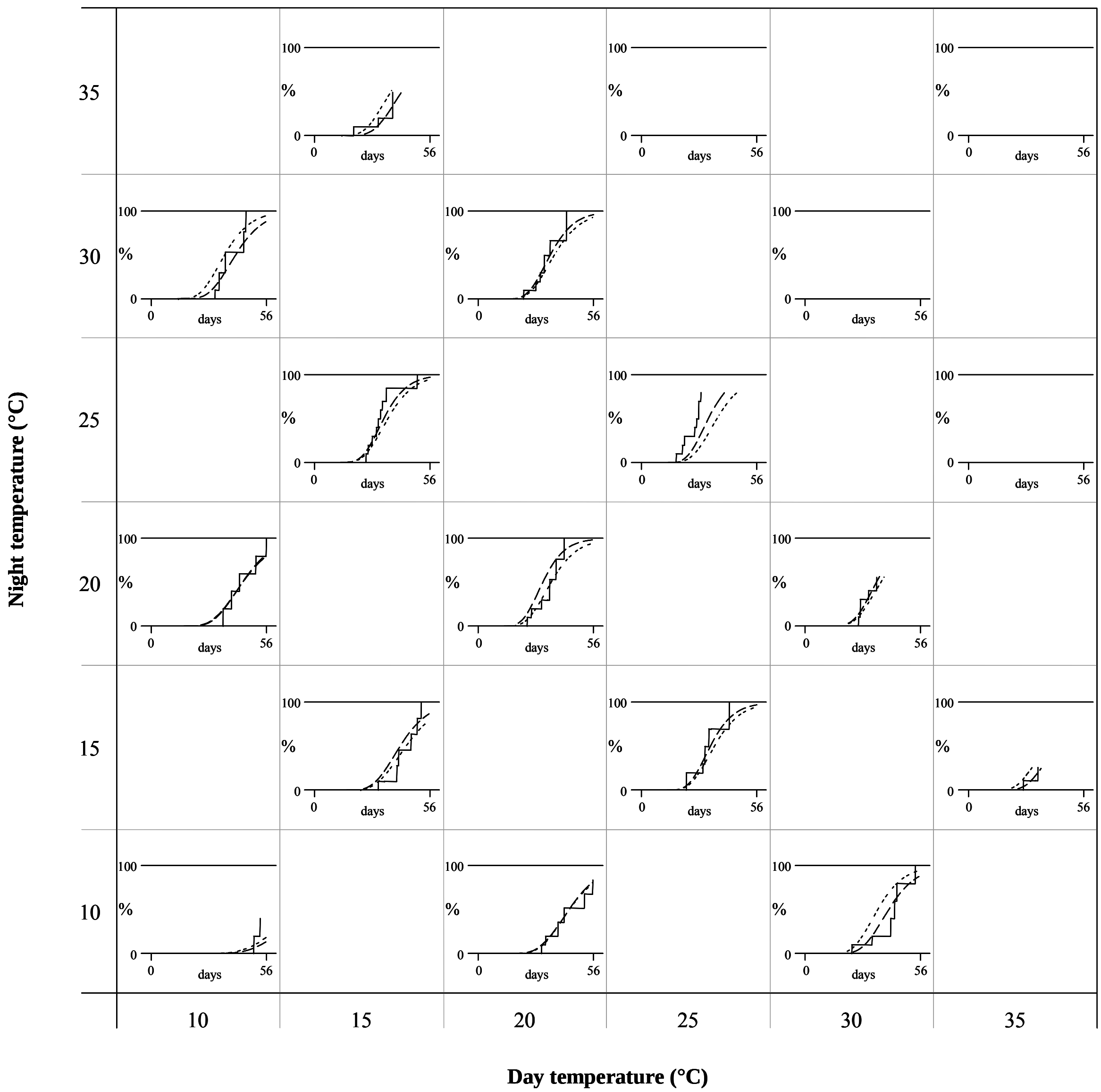

Figure 2.1. Progression of the flowering fraction of *D.× cultorum* over time, under 18 experimental combinations of T_{day} and T_{night}, or DAT and DTR. Data, represented by step curves, are compared with a function of time and DAT only (short dash), and a function of time, DAT, and DTR (long dash)

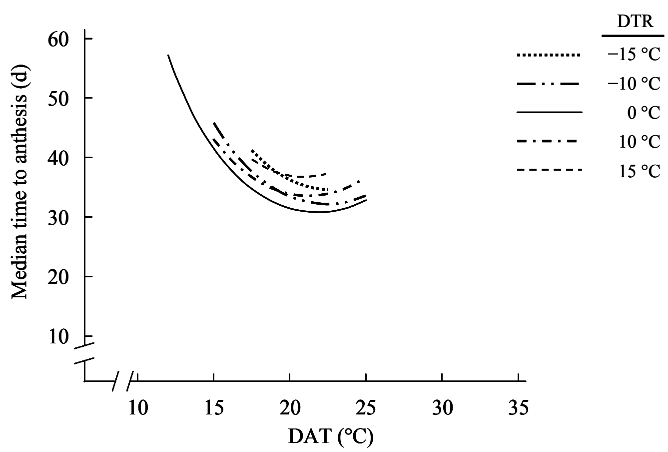

Figure 2.2. Effect of DAT on median time to anthesis of *D.*× *cultorum*, at selected values of DTR

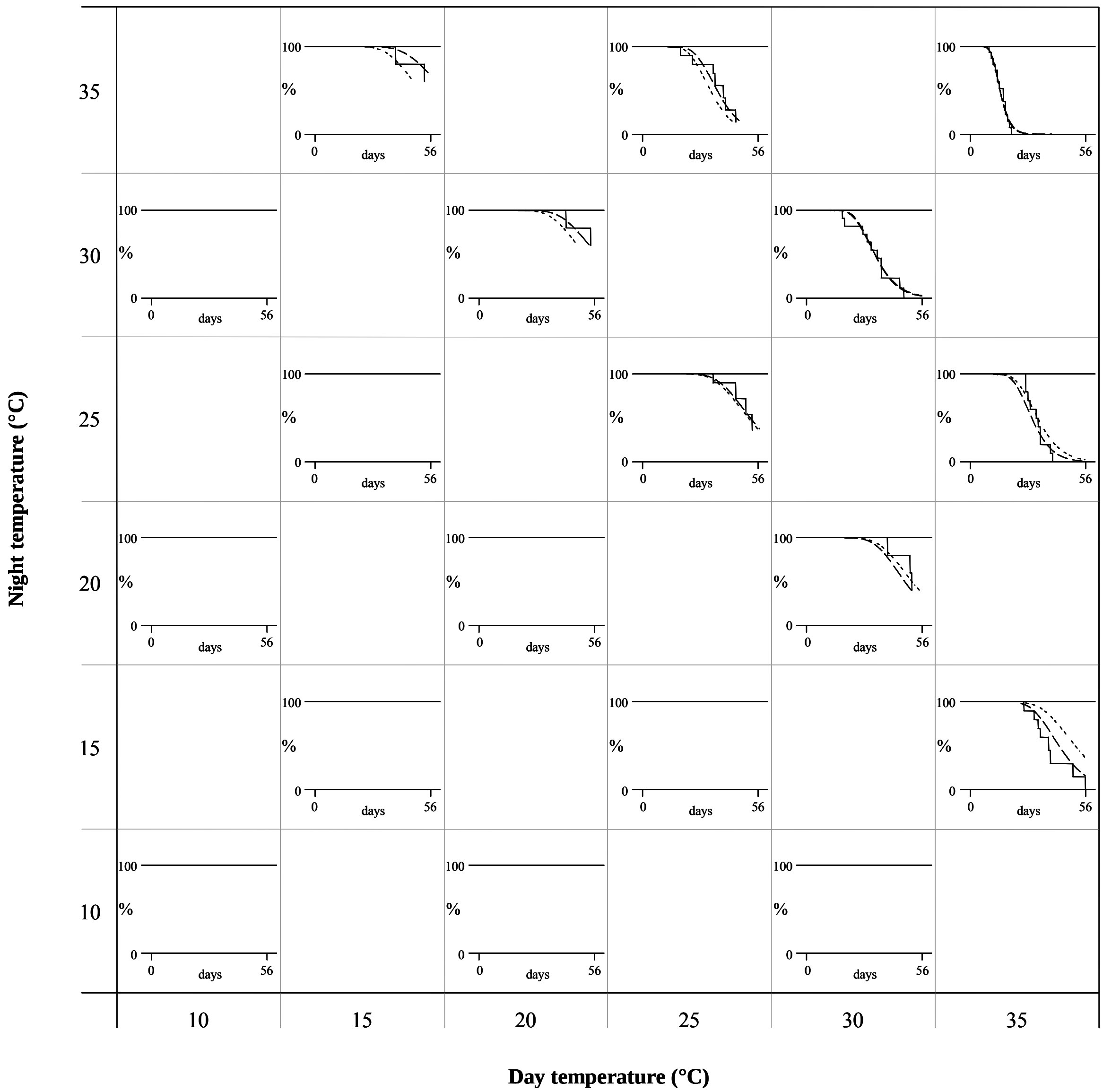

Figure 2.3. Progression of the surviving fraction of *D.× cultorum* over time, under 18 experimental combinations of T_{day} and T_{night}, or DAT and DTR. Data, represented by step curves, are compared with a function of time and DAT only (short dash), and a function of time, DAT, and DTR (long dash)

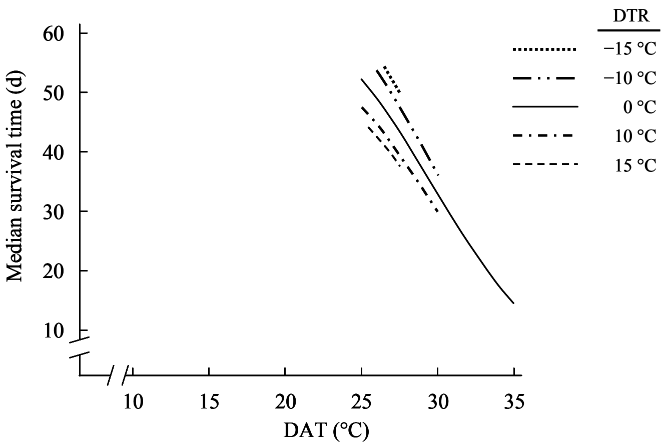

Figure 2.4. Effect of DAT on median survival time of *D.*× *cultorum*, at selected values of DTR

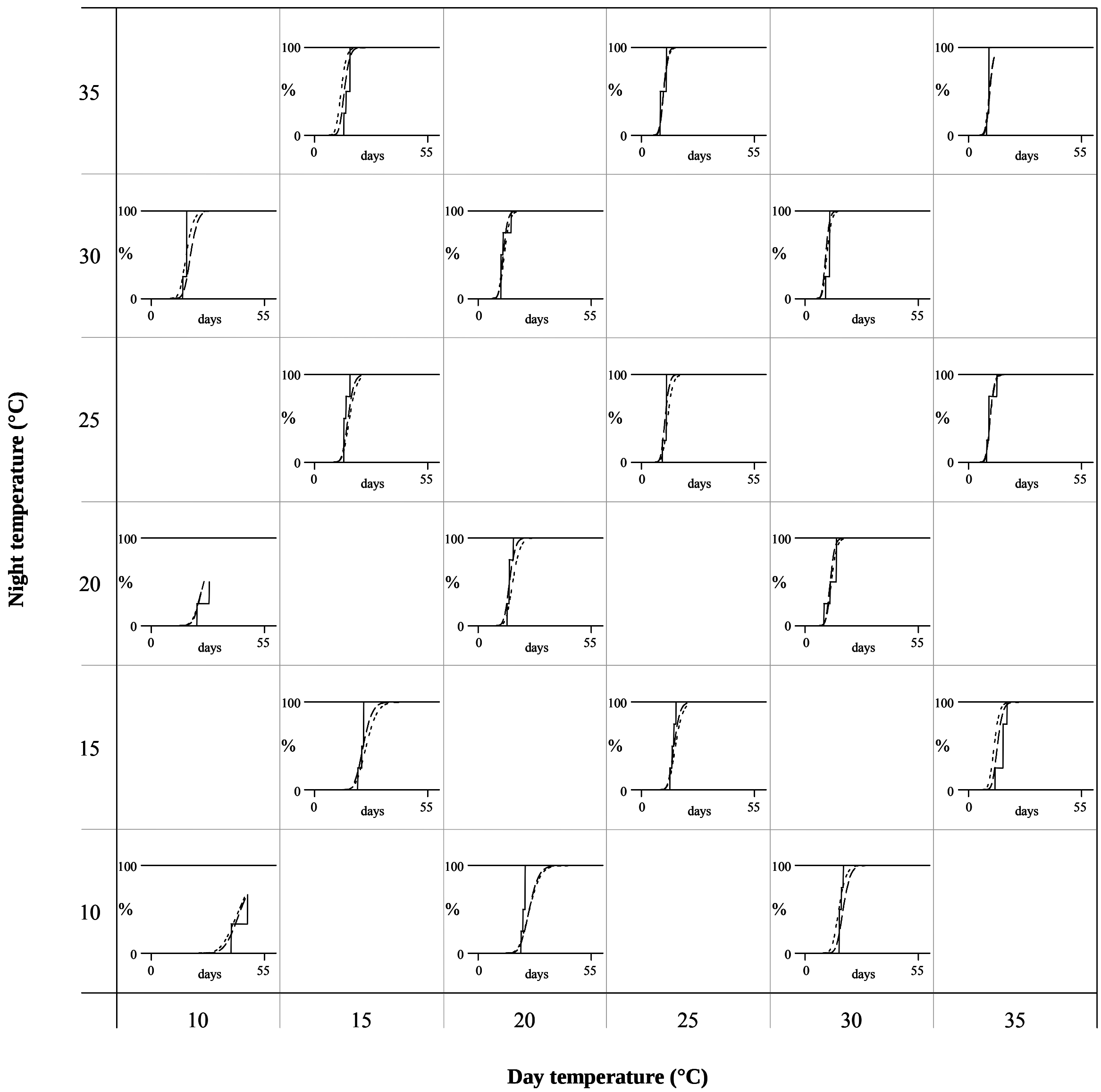

Figure 2.5. Progression of the flowering fraction of *S. laevis* over time, under 18 experimental combinations of T_{day} and T_{night}, or DAT and DTR. Data, represented by step curves, are compared with a function of time and DAT only (short dash), and a function of time, DAT, and DTR (long dash)

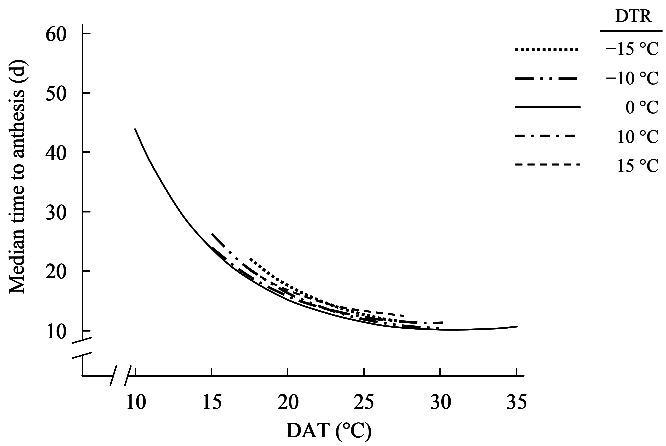

Figure 2.6. Effect of DAT on median time to anthesis of *S. laevis*, at selected values of DTR

CHAPTER 3

Experimental Comparison of the Effects of Diurnal and Sub–diurnal Temperature Variation on Gross Productivity in Two Species of Herbaceous Perennials

Subject Category: Developmental Physiology

Experimental Comparison of the Effects of Diurnal and Sub–diurnal Temperature Variation on Gross Productivity in Two Species of Herbaceous Perennials

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Experimental Comparison of the Effects of Diurnal and Sub–diurnal Temperature Variation on Gross Productivity in Two Species of Herbaceous Perennials

ADDITIONAL INDEX WORDS: daily average temperature, diurnal temperature range, gross productivity, vascular plants, *Delphinium*, *Rudbeckia*

ABSTRACT. Two experiments were conducted to assess the effect on gross plant productivity (dry weight) of temperature variation within each of the two phases of the diurnal cycle. Plants of *Delphinium* × *cultorum* Voss 'Magic Fountains' ('Magic Fountains' delphinium) and *Rudbeckia fulgida* var. *sullivantii* L. 'Goldsturm' ('Goldsturm' blackeyed Susan) were grown in growth chambers for 40 days, under 28 temperature regimes all resulting in diurnal cycles of 12-h days/12-h nights at average temperatures of either 15/15°C, or 25/15°C. There were 14 regimes for each combination, during which temperature changed every 4 hours, in varying permutations of temperatures ranging from 5 °C to 35 °C. Light period temperature was variable in the first experiment, and dark period temperature in the second. Few significant differences in total plant dry weight were found among regimes for either taxon, and those differences did not produce a consistent pattern. Results indicate that over season–long durations, effects of sub–diurnal variation on gross plant productivity are far less consequent than those of diurnal variation. Over season–long durations, the deficit in growth that might result from episodes of nonoptimal temperature within the day or night would remain small, provided that average day and night temperature are close to their optimum.

The practical norm of growing plants under lower night than day temperatures is often accepted in both protected culture and experimental settings. The hypothesis that diurnal temperature cycling—as opposed to constant temperature—is inherently beneficial to plant growth originated with Went (1944a, 1944b, 1957), and Camus and Went (1952), but the empirical data presented in support of the hypothesis at the time was ambiguous at best. Research on control of plant height through the use of higher night temperature than day, known as DIF, has elucidated the effects of day/night temperature difference on plant morphology and flowering (Erwin and Heins, 1995; Myster and Moe, 1995), while experiments by Dale (1964), Friend and Helson (1976), and Dubois (2004) clarified the relation of diurnal temperature cycling and gross productivity.

Results reported by Dubois (2004) demonstrated that the dependence of gross plant productivity on daily heat input cannot be accounted for with fewer than two data per 24-h period. Optimizing daily heat input with respect to growth cannot be achieved without optimizing both daily average temperature, and day/night difference. Moreover, for every nonoptimal value of one factor, there is a different optimal value of the other factor. Since among day and night temperature, daily average temperature, and day/night difference, any pair is a linear combination of the remaining pair, the same is true for any combination among those four factors. The algebraic relationships among the four temperature factors are such that the value of any two fully determines the value of all four. Any new value of day temperature, for example, requires adjustments to daily average temperature, or day/night difference, or night temperature. Although the relative deficit resulting from moderately sub– or supra–optimal temperature in one part of the diurnal cycle can be partially offset by increasing or decreasing temperature in the other part, growth cannot be maximized without optimizing both at once, and in relation to each other. For every value of one of the four factors, in a given taxon and environment, there is a unique, and changing optimal value of each of the other three factors, but it is only when two factors are at their optimum that maximum potential productivity can be reached (Dubois, 2004).

While the effect of temperature variation among day and night portions of the 24-h cycle is well characterized, no systematic experimental inquiry into the effects of variation within each of these periods has been reported. Yet, in all but the most strictly controlled experimental settings, sub–diurnal variations are present, and often take a highly predictable form (Cesaraccio et al., 2001; Fernandez, 1992). Moreover, protected culture facilities (greenhouses and growth chambers) generally allow at least some control of temperature within the day and night periods. Dubois (2004) has shown that a three–dimensional adaptation of the thermodynamic temperature dependence model of Johnson et al. (1942), with one dimension each for day and night temperature, constitutes a suitable empirical characterization of the relationship of daily heat input and dry weight (DW). However, the physiological processes that are ultimately integrated into net growth respond to temperature changes in the temporal scale of seconds or minutes. While Dubois (2004) has demonstrated that the time scale for empirical integration of heat input at the organismal level should be no longer than one of the two phases of the diurnal cycle, it is unknown whether that scale should be shorter. To clarify this, the following research was conducted to determine the effects of sub–diurnal temperature variation on gross productivity of two herbaceous taxa with contrasting responses to day/night temperature combinations.

Materials and Methods

Variable Daytime, Constant Night Temperature (Expt. 1). One hundred and forty transplants each of *Delphinium* × *cultorum* Voss 'Magic Fountains' ('Magic Fountains' delphinium) and *Rudbeckia fulgida* var. *sullivantii* L. 'Goldsturm' ('Goldsturm' blackeyed Susan) were potted 29 July 2002 in 2.8-L containers filled with a substrate of 8 composted pine bark : 1 sand (by vol.) amended with 1.2 $kg \cdot m^{-3}$ dolomitic lime. They were then transferred randomly to six, 3 m^2 growth chambers [B–chambers (Thomas et al., 2004)] at the Southeastern Plant Environment Laboratory (NC State Univ. Phytotron) for 24 d of acclimation under constant 12-h days/12-h nights of 20 °C. The 12-h photoperiod was provided by a combination of cool–white fluorescent lamps and incandescent bulbs, supplying a photosynthetic photon flux (*PPF*) of 640 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level as measured by a LI–COR 1800 spectroradiometer (LI–COR, Lincoln, Nebr.) (Thomas et al., 2004). In this environment, *D.* × *cultorum* had been found to grow best at moderately low day temperatures, with little or no difference between day and night, and *R. fulgida* grew best under moderately high day temperature, with a large day/night difference (Dubois, 2004). Therefore, among the 14 temperature regimes under which all plants were grown in Expt. 1, seven were designed to generate a diurnal cycle of 12-h day/12-h night at average temperatures of 15/15°C, and seven at 25/15°C.

At the end of the acclimation period, plants of both taxa were graded visually and separated into five groups based on apparent size, with two plants of each taxon and size group assigned randomly to one of 14 temperature regimes. Treatments were then initiated by transferring plants to six, 3 m^2 growth chambers [B–chambers (Thomas et al., 2004)] set at 15 °C for 12 h in the dark, and six temperature programs of three successive 4-h periods at

5, 15, 25, or 35 °C during the light period. The experiment was in a randomized complete block design with five blocks and apparent size of the transplants at treatment initiation as a blocking factor. The 14 temperature regimes (A–N) listed in Table 1 were achieved by moving all plants to appropriate chambers at 0, 4, and 8 h from the beginning of the light period. *PPF* during the 12-h light period was 640 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided by a combination of cool–white fluorescent lamps and incandescent bulbs (Thomas et al., 2004). Plants were fertilized daily with a complete nutrient solution providing N at 106 $mg \cdot L^{-1}$. Three 10-min periods of nonphotosynthetically active lighting were provided at 0, 4, and 8 h from the beginning of the dark period to simulate the brief period plants would spend in the facility's lighted hallways during dark period chamber changes in the second experiment. Plants were harvested after 14 or 40 d, five plants per taxon (one per block) per temperature regime, and data recorded. Data included leaf area, measured using a LI–COR 3000 leaf area meter (LI–COR, Lincoln, Nebr.), and shoot and root DW, recorded after at least 96 h of drying at 70 °C. Data were subjected to analysis of variance (ANOVA). Data analysis was generated using SAS® software, Version 8 of the SAS System® for Windows®.

Constant Daytime, Variable Night Temperature (Expt. 2). One hundred and sixty eight transplants of each taxon were potted 21 Oct 2002 as described for Expt. 1, and transferred randomly to six, 3 m^2 growth chambers [B–chambers (Thomas et al., 2004)] set at 20 °C for 27 d of acclimation as described for Expt. 1. Plants of both taxa were then graded visually and separated into six groups based on apparent size, with two plants of each taxon and size group assigned randomly to one of 14 treatments. Treatments were initiated by transferring plants to six, 3 m^2 growth chambers [B–chambers (Thomas et al., 2004)] set at light period temperature of either 15 or 25 °C, and eight temperature programs of three 4-h periods at 5, 15, or 25 °C during the dark period. As in Expt. 1, seven regimes corresponded to a diurnal cycle of 12-h day/12-h night at average temperatures of 15/15°C, and seven at 25/15°C. The experiment was in a randomized complete block design with six blocks and apparent size of the transplants at treatment initiation as a blocking factor. The 14 temperature regimes listed in Table 2 were achieved by moving all plants to appropriate chambers at 0, 4, and 8 h from the beginning of the dark period. *PPF* during the light period was 640 $\mu mol \cdot m^{-2} \cdot s^{-1}$, generated by a combination of cool–white fluorescent lamps and incandescent bulbs. Plants were fertilized daily with a complete nutrient solution providing N at 106 $mg \cdot L^{-1}$. Plants were harvested after 14 and 40 d, six plants per taxon (one per block) per temperature regime, and data recorded as described in Expt. 1. Data were subjected to ANOVA. Data analysis was generated using SAS® software, Version 8 of the SAS System® for Windows®.

Results

Despite the amplitude of temperature fluctuations, and the dissimilitude of regimes, the evidence of an effect of sub–diurnal variation on gross productivity, distinct from the effect of diurnal cycling and from plant–to–plant variability, was weak and inconsistent. Under variable day temperature (Expt. 1), a significant difference ($P \geq 0.05$) among temperature regimes was detected only in *R. fulgida*, and only after 40 d (Table 3). Under variable night temperature (Expt. 2), a significant difference was found after 40 d, in both taxa. The increased power that resulted from the greater number of plants per treatment in the second experiment accounts at least in part for the difference in significance between Expts. 1 and 2.

Where effects of temperature regime could thus be perceived, however, effects were inconsistent. There was no commonality between taxa in the ranking of regimes with respect to plant DW within each experiment, and ranking changed entirely between 14 and 40 d within each experiment and taxon (Fig. 1). No regime showed a clear and meaningful advantage, or disadvantage. In particular, we contrasted the effect of the same day/night average temperature (15/15 or 25/15 °C), achieved through constant temperature in each of the light and dark periods, or through the six corresponding variable regimes (G vs. A–F, and N vs. H–M in Tables 1 and 2) . Out of 16 contrasts, three were significant at $P \geq 0.05$ (Table 3). Treatment differences were even fewer for leaf area and DW of shoots and roots. In the presence of such a lack of discernible response to sub–diurnal temperature variation, further quantifying the few differences that were found could not be justified.

Discussion

In contrast to the effects of temperature variation between day and night, (Dubois, 2004), results presented herein indicate the effects of variation within each of the two phases on gross productivity are weak and inconsistent. Despite the fact that temperature changes act upon the physiological processes that subtend plant growth on a time scale as small as seconds, it appears that the organismal impact of temperature variation is registered on a diurnal scale. From empirical and practical standpoints, the interval of time over which plants integrate heat input is shorter than 24 h, but is unlikely to be shorter than the two periods that constitute the diurnal cycle.

The conclusion that the organismal impact of temperature variation is registered on a diurnal scale does not extend to situations where temperature might reach such values that gross plant functioning is compromised. Temperatures high enough to cause irreversible damage would by definition affect long–term growth negatively. The present study offers empirical evidence that when day and night temperature averages are not so far removed from their respective optima, daily episodes of extreme temperature have little or no effect on growth, comparatively to constantly mild temperature. Thus, when no lasting damage occurs from periods of extreme conditions, a deficit in overall growth may not necessarily result from the stoppage of photosynthesis for part of the light period. This in turn may suggest that under nonstress conditions, a surplus of photosynthate is produced by these taxa in the vegetative phase, and subsequently respired. Adaptive changes in efficiency may therefore take place at the organism level. Given that plants were subjected to the 28 diurnal temperature regimes for a prolonged period, it could be hypothesized that the weakness of the effects of sub–diurnal temperature variation were a result of plant acclimation. However, if such was the case, treatment effects would have been larger after 14 d than after 40 d, or at least similar. The adjustments that resulted in overall growth being relatively unaffected by such diversity in temperature environments, are more likely to have occurred within each diurnal cycle, rather than over the course of several days or weeks.

From an applied perspective, mainly in protected environments such as greenhouses, data herein suggest that, as long as average day and night temperature are properly optimized, wide fluctuations in temperature within each sub–diurnal period have no meaningful effect on gross plant productivity. Although separate optimization of both daily average temperature and day/night temperature difference is beneficial (Dubois, 2004), constant and varying temperature within each part of the diurnal cycle may not have any advantage over each other. Incidentally, patterns of sub–diurnal variation that mimic naturally occurring patterns have no intrinsic superiority. Objectives that might justify the use of temperatures far divergent from average day or night temperature, during part of the two periods, include morphological control (DIF), control of disease organisms, and infrastructure economics. It is well established that in applying DIF, the desired morphological effects are controlled by the temperature difference between the last few hours of the dark period, and the first few of the light period (Erwin and Heins, 1995; Myster and Moe, 1995). Our data indicate that the potential deficit in growth (total plant DW) that may follow from deviating from optimal growing temperatures during the critical interval for DIF, can be avoided by maintaining optimal average growing temperatures for each of the light and dark periods. Likewise, it has been suggested that meaningful prevention of some disease problems in greenhouse crops could be achieved by rapidly raising temperature to supra–optimal values at times of day when prevailing cool and moist conditions foster those problems (Brian E. Whipker, personal communication). The results reported herein suggest that daily episodes of supra–optimal temperature would have little or no effect on eventual growth, provided that temperature is subsequently lowered to sub–optimal values, in order to achieve optimal average for the light, or dark period. Finally, if wide sub–diurnal variation in temperature has little ill effect on growth, reducing resources expended on maintaining temperature as stable as possible through successive heating and cooling should not be a concern, at least with respect to gross productivity.

Maximal growth cannot be achieved without both average day and average night temperature being optimal. Deficit in growth resulting from average temperature deviating from the optimum in one of the two phases of the diurnal cycle can sometimes be minimized by compensating in the other half, but can not be fully offset. Nonoptimal temperature during some interval within either of the two halves, on the contrary, can likely be fully compensated for by adjusting temperature during the rest of the affected half. It appears that ill effects of sub–diurnal temperature fluctuations on plant growth should be a much lesser concern than ill effects of inadequate average day and night temperature.

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Table 3.1. Fourteen temperature regimes used in Expt. 1

		Time (h)					
		Light			Dark		
		0–4	4–8	8–12	12–16	16–20	20–24
	Temp. regime	Temperature (°C)					
Day/night average temperature (15/15 °C)	A	5	15	25	15	15	15
	B	25	15	5	15	15	15
	C	25	5	15	15	15	15
	D	15	5	25	15	15	15
	E	15	25	5	15	15	15
	F	5	25	15	15	15	15
	G	15	15	15	15	15	15
Day/night average temperature (25/15 °C)	H	15	25	35	15	15	15
	I	35	25	15	15	15	15
	J	35	15	25	15	15	15
	K	25	15	35	15	15	15
	L	25	35	15	15	15	15
	M	15	35	25	15	15	15
	N	25	25	25	15	15	15

Table 3.2. Fourteen temperature regimes used in Expt. 2

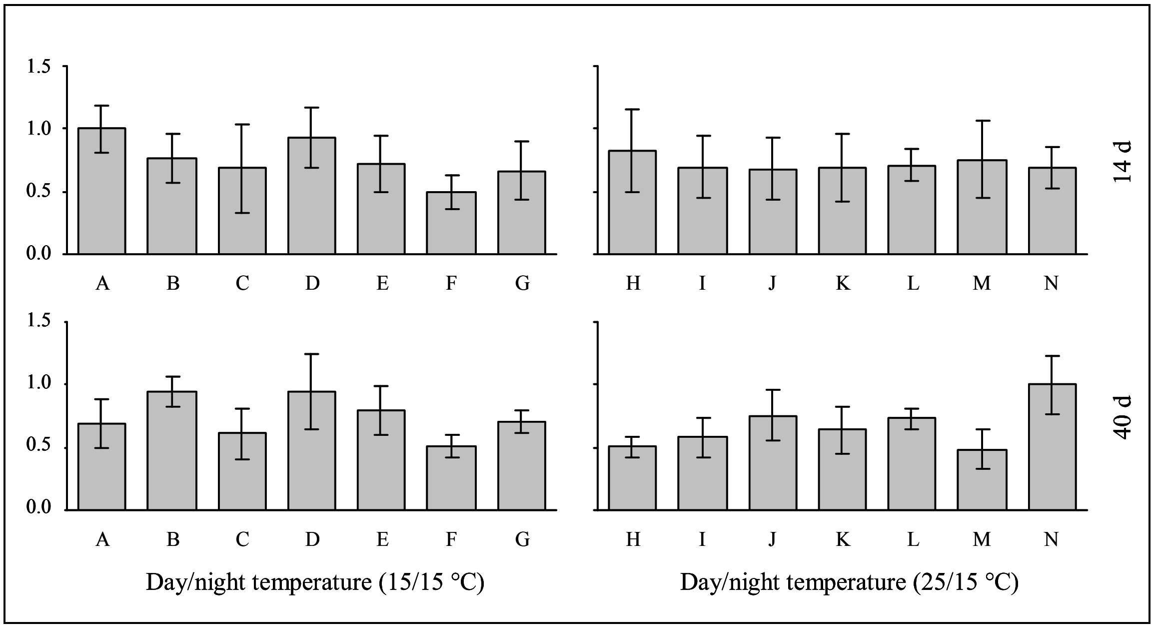
		Time (h)					
		Light			Dark		
		0–4	4–8	8–12	12–16	16–20	20–24
	Temp. regime	Temperature (°C)					
Day/night average temperature (15/15 °C)	A	15	15	15	5	15	25
	B	15	15	15	25	15	5
	C	15	15	15	25	5	15
	D	15	15	15	15	5	25
	E	15	15	15	15	25	5
	F	15	15	15	5	25	15
	G	15	15	15	15	15	15
Day/night average temperature (25/15 °C)	H	25	25	25	5	15	25
	I	25	25	25	25	15	5
	J	25	25	25	25	5	15
	K	25	25	25	15	5	25
	L	25	25	25	15	25	5
	M	25	25	25	5	25	15
	N	25	25	25	15	15	15

Table 3.3. Effects of sub–diurnal temperature variation on total plant DW in *D.* × *cultorum* and *R. fulgida*, for variable day and night temperature, after 14 and 40 d. Overall effect of temperature regime and contrasts between constant temperature regimes and corresponding variable temperature regimes (*P* values) are presented.

Expt.	Time	Effect	Taxon	
			D. × *cultorum*	*R. fulgida*
Variable day temperature (Expt. 1)	14 d	Temperature regime	NS	NS
		Constant vs. variable (15/15 °C)	NS	NS
		Constant vs. variable (25/15 °C)	NS	NS
	40 d	Temperature regime	NS	0.049
		Constant vs. variable (15/15 °C)	NS	NS
		Constant vs. variable (25/15 °C)	0.015	NS
Variable night temperature (Expt. 2)	14 d	Temperature regime	NS	NS
		Constant vs. variable (15/15 °C)	0.032	NS
		Constant vs. variable (25/15 °C)	NS	NS
	40 d	Temperature regime	0.031	< 0.0001
		Constant vs. variable (15/15 °C)	NS	NS
		Constant vs. variable (25/15 °C)	0.011	< 0.0001

[NS] Nonsignificant.

Delphinium × cultorum



Rudbeckia fulgida

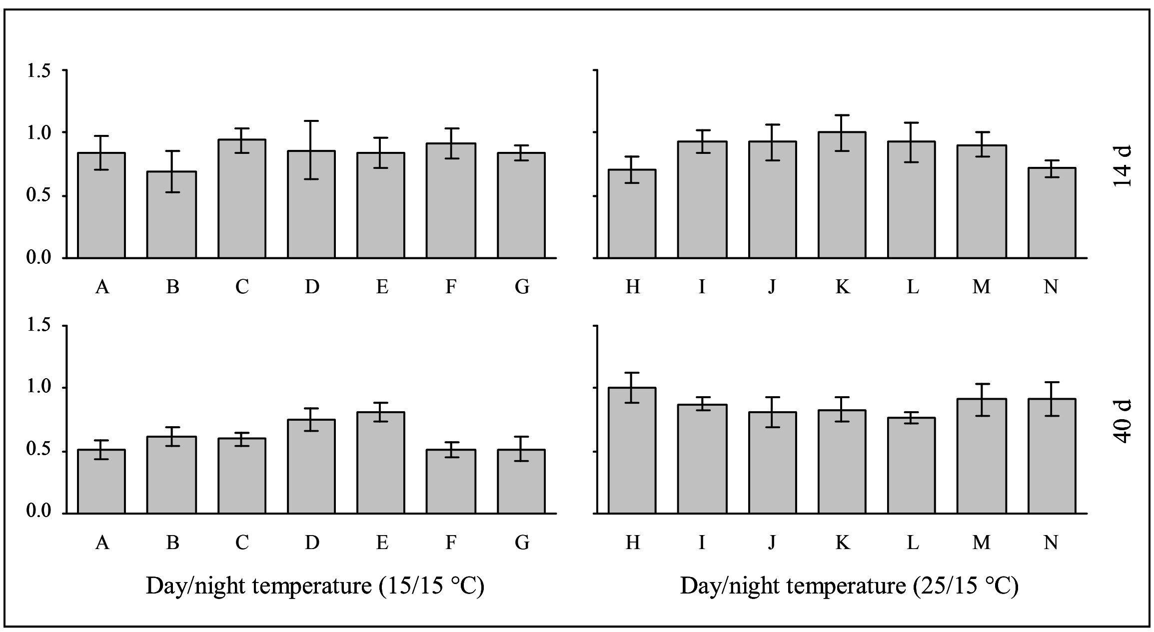
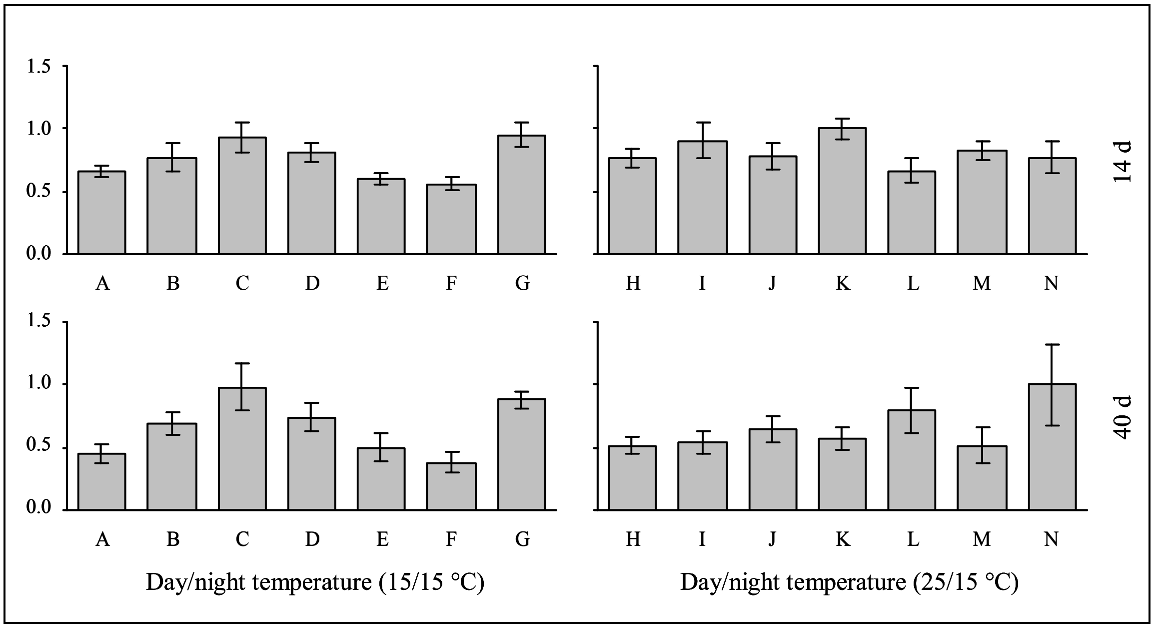


Fig. 3.1. Influence of temperature variation during the 12-h light period on total plant DW of *D.* × *cultorum* and *R. fulgida* after 14 and 40 d (Expt. 1). Letter labels refer to Table 1. Bars are treatment means (n=5) and vertical lines are ± 1 SE. Units are scaled to maximum mean observed DW by taxon and harvest with maximum = 1. Temperature was constant within both day and night periods in regimes labeled G and N.

Delphinium × cultorum



Rudbeckia fulgida

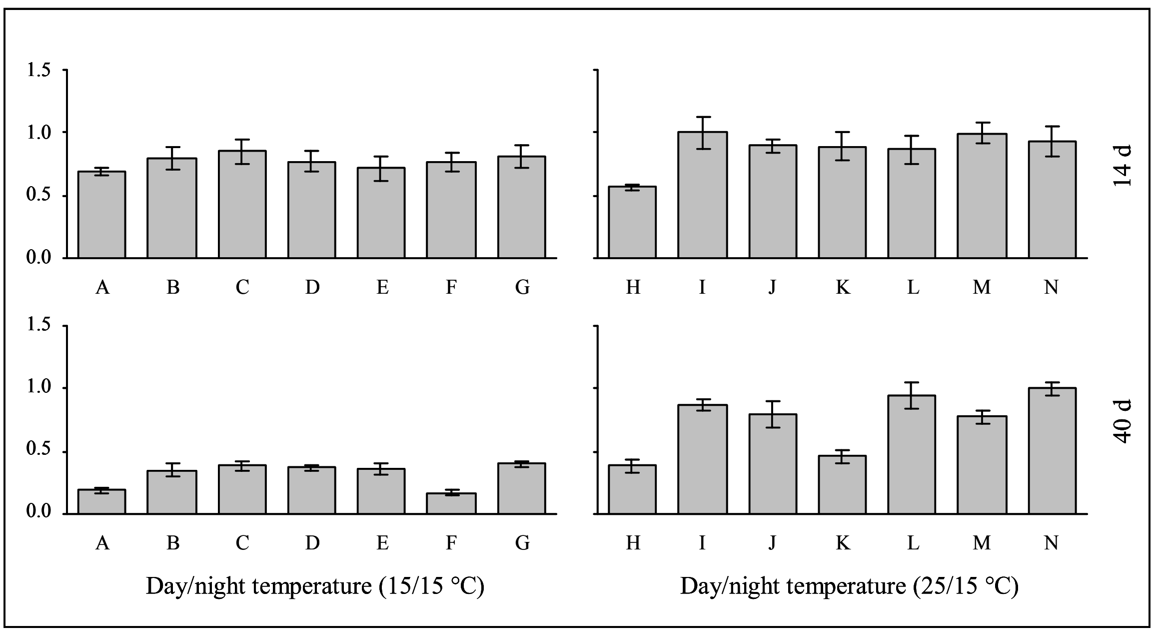


Fig. 3.2. Influence of temperature variation during the 12-h dark period on total plant DW of *D.* × *cultorum* and *R. fulgida* after 14 and 40 d (Expt. 2). Letter labels refer to Table 2. Bars are treatment means (n=6) and vertical lines are ± 1 SE. Units are scaled to maximum mean observed DW by taxon and harvest with maximum = 1. Temperature was constant within both day and night periods in regimes labeled G and N.