Day/Night Temperatures Influence Growth and Photosynthesis During Containerized Production of Selected Species of *Helleborus* (Hellebores)[1]

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Abstract

Containerized seedlings of *Helleborus foetidus* L. (stinking hellebore), *H. niger* L. (Christmas rose), and *H. ×hybridus* L. (Lenten rose) were grown under long-day conditions in controlled-environment chambers for 95 days with 9-hr days of 14, 18, 22, 26, or 30C (57, 64, 72, 79, or 86F) in factorial combination with 15-hr nights of 10, 14, 18, 22, or 26C (50, 57, 64, 72, or 79F). Long-day conditions were provided by a 3-hr night interruption. Growth of each species responded differently to day and night temperatures. Calculated maximum root, top, and total dry weight, and leaf area of *H. foetidus* occurred with days/nights of 20/15, 18/13, 19/14, and 18/15C (68/59, 65/55, 66/57, and 65/59F), respectively. While night temperature (NT) had no effect on root:top ratio [RTR (root dry weight ÷ top dry weight)], RTR was greatest (0.65) with days of 22C (72F). *Helleborus niger* had calculated maximum root dry weight and total dry weight with days of 14C (57F) and nights of 16 and 13C (60 and 55F), respectively. Top growth of *H. niger* decreased linearly as NTs increased for days of 14 or 22C (57 or 72F). Day temperatures (DTs) had no effect on RTR, whereas RTR responded quadratically as NT increased with a calculated maximum RTR at nights of 19C (66F). Leaf area was maximized at days/nights of 14/10C (57/50F). At days of 22 or 26C (72 or 79F), top growth of *H. ×hybridus* responded quadratically as NT increased with maxima occurring at nights of 18 or 17C (64 or 63F). Root dry weight responded quadratically at days of 14, 22, or 26C (57, 72, or 79F) and calculated maxima occurred with nights of 18C (64F). At days of 22 or 26C (72 or 79F), there were quadratic responses in total dry weight with calculated maximum growth of *H. ×hybridus* at nights of 18 or 17C (64 or 63F), respectively. For days of 14, 22, or 30C (57, 72, or 86F), there were quadratic responses in RTR with greatest RTR calculated at nights of 15, 18, or 16C (59, 64, or 60F), respectively. There were quadratic responses at days of 22 or 26C (72 or 79F) for leaf area with calculated maxima at nights of 18 or 17C (64 or 63F), respectively. As DTs increased from 14 to 30C (57 to 86F) net CO_2 assimilation (P_N) of *H. ×hybridus* also increased linearly whereas increased NTs had no effect on P_N. In contrast, stomatal conductance was not impacted by DT or NT.

Index words: *Helleborus foetidus*, *Helleborus niger*, *Helleborus ×hybridus*, perennials, heat tolerance, optimal temperature, thermoperiod.

Species used in this study: *Helleborus foetidus* L. (stinking hellebore); *Helleborus niger* L. (Christmas rose); *Helleborus ×hybridus* L. (Lenten rose).

Significance to the Nursery Industry

Quantitative data are presented concerning the influence of day temperatures (DTs) of 14, 18, 22, 26, or 30C (57, 64, 72, 79, or 86F) in factorial combination with night temperatures (NTs) of 10, 14, 18, 22, or 26C (50, 57, 64, 72, or 79F) on growth during containerized production of *Helleborus foetidus* (stinking hellebore), *H. niger* (Christmas rose), and *H. ×hybridus* (Lenten rose). Data are also provided regarding net CO_2 assimilation (P_N) and stomatal conductance (g_s) of *H. ×hybridus*. For each species, DT and NT affected root, top, and total dry weights, root:top ratio (root dry weight ÷ top dry weight), and leaf area differently. In general, to maximize growth, *H. foetidus* and *H. ×hybridus* should be grown under long-day conditions at days/nights of 18/14C (64/57F) whereas *H. niger* is best grown at days/nights of 14/10C (57/50F). The need for such temperatures, particularly during summer months in the southeastern United States, may require various temperature control methods such as reflective shade, fans, and cool cells to reduce temperatures within growing structures.

Introduction

The genus *Helleborus* L. (hellebores) includes approximately 17 species native primarily to Europe and western Asia and is a member of the buttercup family (Ranunculaceae) (4). Although various species have been cultivated in European gardens for centuries, interest and cultivation of these plants in North America has until recent times been limited. However, more North American gardeners began to take notice of these plants in the early 1980s and interest accelerated in 2005 when the Perennial Plant Association named *Helleborus ×hybridus* L. (Lenten rose) the Perennial Plant of the Year (17). This recognition increased the popularity of the genus *Helleborus* which includes many species with outstanding garden merit.

A rainbow of colors aptly describes the extremely attractive, winter to early spring, single or double flowers species of *Helleborus* exhibit in the wild and in shade gardens in various

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regions of the world (4, 20). In addition to outstanding shade garden attributes, *H. niger* (Christmas rose) is a popular cut flower in Europe (4) and Fanelli and Dole (7) reported that flowers of *H. ×hybridus* have a vase life of approximately 17 days, suggesting it may also have merit as a cut flower.

In a nursery setting, containerized production of all species of *Helleborus* can be very challenging. Plants are most often propagated by seed but can also in some cases be propagated by division or micropropagation (tissue culture) (4). Regardless of the manner of propagation, containerized culture is utilized to grow plants to a saleable size. Hellebores are sold as 1-year-old seedlings, 2-year-old liners, and larger 3- to 5-year-old plants in 6.3-liter (6-qt) containers that are ready to flower. The later, being most common when selection of phenotype, including flower color and color patterns (e.g., picotee margins of sepals), is desired.

Currently, nursery professionals commonly use fans, plastic covers, and shade cloth to regulate substrate moisture levels, light intensity (irradiance), and temperatures in growing structures during production of *Helleborus* sp. Also, when grown in containers, species often exhibit mineral nutrient deficiencies, root rot, and foliar disorders caused by various viral and fungal pathogens, and slow growth rates taking 2 to 4 years from seed, depending on the species, to reach flowering size (4). The inordinate length of time in production results in increased costs to consumers while decreasing profits to growers. The long production time also causes growers to be constantly vigilant to avoid cultural problems.

Species of *Helleborus* appear to be sensitive to high temperatures (Richard and Judith Tyler, Pine Knot Farms Perennials, Clarksville, VA, personal communication). Although some research has been reported on mineral nutrition, potting substrates, substrate pH, and irrigation for such species as *H. ×hybridus* and *H. foetidus* (stinking hellebore) (11, 12, 14, 23), it appears no research has been reported to date on the influence of temperature on this genus. Additionally, since members of the genus are native to varying geographic and climatic environments, it is likely individual species may require different temperature regimes during container production to maximize growth.

Elevated summer temperatures can limit plant survival and growth in warm-temperate climates such as those experienced in the southeastern United States (1). Heat stress imposed by supraoptimal temperatures can be the predominant ecological factor defining the distribution and adaptability of cultivated species (2). Biological activity for the majority of plant species occurs within a temperature range 0 to 50C (32 to 122F) and within this temperature range optimum growth for most horticultural crops occurs from 10 to 30C (50 to 86F) (19). Determining the optimal temperatures for growth of *Helleborus* sp. would aid nursery professionals in reducing losses of plants during summers in the Southeast and other regions of the United States experiencing hot summers.

Temperature directly affects many chemical reactions, in particular rates of P_N (3). Optimal temperatures for photosynthesis of various species growing in different habitats are often contrasting (22), and such temperatures for P_N often correlate with optimal temperatures for plant growth (2). Temperature also has an influence on respiration. Respiration is utilization of the products of photosynthesis, i.e., sugars and starches, for biomass accumulation. During heat stress, respiration exceeds the rate of photosynthesis up to two to three times, causing a reduction of carbohydrates that can be used for growth (19). Respiration also proceeds during periods of darkness. When night temperatures are higher than day temperatures, respiration often causes plants to be stunted phenotypically. Studying growth under varying thermoperiods would enable development of production schedules to optimize growth of *Helleborus* sp. Therefore, the following research was conducted to study the influence of day/night temperatures on growth and photosynthesis during containerized production of selected species of *Helleborus*. The three species selected represented a range of phenotypes (growth forms), geographic or cultivated origins, and hypothesized physiological responses (Table 1).

Table 1. Descriptive information of *Helleborus* sp. included in this study.[z]

Scientific name	Common name	Growth form in situ	Nativity	Habitat	Comments
H. foetidus (FOE)[y]	stinking hellebore	Herbaceous perennial, caulescent	Widespread in western Europe, from Portugal east to Great Britian and Germany	Oak and mixed oak-pine woodlands on limestone-derived soils; grows from sea level to 2135 m (7000 ft)	Small root system compared to top growth; often short-lived; purportedly, easiest of the three species listed to grow in a container; hypothesized to have good tolerance to high temperatures based on native habitat
H. niger (NIG)	Christmas rose	Herbaceous perennial, acaulescent	Localized alpine plant in central and eastern Europe from Croatia, Austria, and Italy north to Switzerland and Germany	Mixed coniferous and deciduous forests, meadows, rocky pastures, and along road cuts; grows at elevations from 305 m (1000 ft) to 1829 m (6000 ft)	Valued in breeding programs for large outward-facing flowers; hypothesized to have lowest tolerance to high temperatures
H. ×hybridus (HYB)	Lenten rose	Herbaceous perennial, acaulescent	Widespread in southwestern and eastern Europe	Mixed woodlands and the edges of meadows; grows from near sea level to 1829 m (6000 ft)	Natural hybrid of species in the section *Helleborastrum*; hypothesized to have the greatest tolerance to high temperatures

[z]Information derived from Burrell and Tyler (4), Rice and Strangman (20), and Woodard (25).
[y]Abbreviations for species mentioned in text.

Materials and Methods

On June 5, 2007, seedlings of *H. foetidus* (FOE), *H. niger* (NIG), and *H. ×hybridus* (HYB), obtained from Pine Knot Farms Perennials, Clarksville, VA, were transplanted into square 1-liter (1.1-qt) black plastic containers filled with a substrate of pine bark:sand (4:1, by vol) amended with 1.8 $kg \cdot m^{-3}$ (3 $lb \cdot yd^{-3}$) dolomitic lime. At potting, four additional plants of each species were harvested to determine initial root, top, and total dry weights (root dry weight + top dry weight), root:top ratio (RTR), and leaf area. These plants were dried at 70C (158F) until dry weights remained unchanged (72 hr). After potting, the plants were acclimated in a controlled-environment greenhouse at the Horticulture Field Laboratory, Raleigh, under natural photoperiod and irradiance with days/nights of 24/18C (75/64F).

All seedlings were transferred to the Southeastern Plant Environment Laboratory (NC State Univ. Phytotron) on June 12, 2007, and temperature treatments were initiated the following day using four controlled-environment A-chambers and one B-chamber (24). Seedlings were arranged as a 3 × 5 × 5 factorial in a completely random design using four single-plant replications per temperature treatment per species. The two main factors were five day temperatures (DTs) [14, 18, 22, 26, or 30C (57, 64, 72, 79, or 86F)] and five night temperatures (NTs) [10, 14, 18, 22, or 26C (50, 57, 64, 72, or 79F)] provided as 9/15-hr thermoperiods. Temperatures were maintained within 0.25C (0.45F) of the set point. Plants were moved between chambers at 0730 and 1630 HR daily to maintain appropriate day/night temperatures. Plants exposed to constant day and night temperatures were also moved daily to different areas of a chamber to simulate transient mechanical perturbations and to avoid possible gradient effects within chambers.

During the 9-hr portion of a thermoperiod, chamber irradiance was provided by a combination of cool-white fluorescent lamps and incandescent bulbs resulting in a photosynthetic photon flux (*PPF*) of 642 $\mu mol \cdot m^{-1} \cdot s^{-1}$ (24). Incandescent bulbs providing a *PPF* of 44 $\mu mol \cdot m^{-1} \cdot s^{-1}$ were used as a dark interruption between 2300 and 0200 HR daily to provide long-day conditions. Plants were fertigated every other day with the standard Phytotron nutrient solution providing N, P, and K at 106, 10, and 111 $mg \cdot liter^{-1}$ (ppm), respectively (24).

On September 1, 2007, 82 days after treatment initiation (DAI), P_N ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) and g_s ($mol\ H_2O \cdot m^{-2} \cdot s^{-1}$) were measured on individual, recently matured leaves of HYB using a LI-6400 gas analyzer (LI-COR, Lincoln, NE). Photosynthetically active radiation, air and leaf temperatures, and relative humidity inside a 0.25-liter leaf chamber were measured concurrently with gas exchange for 30 sec. P_N rates and g_s were calculated using the LI-COR 6400 measurements. Measurements were recorded on each of three plants grown at days of 14, 18, 22, 26, or 30C (57, 64, 72, 79, or 86F) in combination with nights of 10, 18, or 26C (50, 64, or 79F).

The experiment was terminated September 14, 2007, 95 DAI. Plants were separated into roots and tops by severing the crown (root collar) of each plant at the substrate surface. Roots were washed to remove substrate and leaf area was measured using a LI-COR 3100 leaf area meter (LI-COR). Leaf area of the caulescent, FOE, also included the green, photosynetically active stems. Roots and tops were then dried at 70C (158F) until dry weights remained unchanged (72 hr) and weighed. These data were used to calculate total plant dry weight and RTR. Data were subjected to analysis of variance (ANOVA) procedures and regression analyses in SAS (version 9.1.3; SAS Inst., Cary, NC). The maximum of the polynomial was calculated as the zero point in a first-order derivative of the independent variable.

Results and Discussion

ANOVA revealed a significant species × DT × NT interaction for all variables. Therefore, data were reanalyzed by species. For each species D and NTs affected root, top, and total dry weights, RTR, and leaf area differently.

Helleborus foetidus. The DT × NT interaction was nonsignificant for all measured variables (Table 2). As such, DT and NT main effects are presented.

Total, top, and root dry weights and leaf area responded quadratically as DT and NT increased (Fig. 1). This is the prototypical effect to temperature with dry matter production increasing as temperature increases to a point at which growth starts to decrease (16). Total plant dry weight (Fig. 1A) was highest at NTs of 10 to 18C (50 to 64F), the measured peak occurring at 14C (58F) and declining 58% from 18 to 26C (64 to 79F). Presumably, increased dark respiration (not measured in this study) resulted in greater loss of respiratory carbohydrates from high NT, which reduced dry weight accumulation (6, 9). Responses of top and root dry weights and leaf area to NT were similar to that of total plant dry weight (Fig. 1B–D). All measured growth responses of which data are presented showed a clearly defined optimum NT of 14C (57F).

Optimum DT for total plant dry weight (Fig. 1A), top dry weight (Fig. 1B), and leaf area (Fig. 1D) was 18C (65F), but for root dry weight (Fig. 1C) optimum DT was 22C (72F). Root growth was apparently less sensitive to high DT than the other measured variables. In contrast, other studies have reported optimum root growth was realized at lower DT than

Table 2. ANOVA of root, top, and total dry weight, root:top ratio (RTR), and leaf area of three *Helleborus* sp. grown under contrasting day/night temperatures.

Species	Root dry wt.	Top dry wt.	Total dry wt.[z]	RTR[y]	Leaf area
H. foetidus					
DT[x]	***	***	***	*	***
NT	***	***	***	NS	***
DT × NT	NS	NS	NS	NS	NS
H. niger					
DT	***	***	***	NS	***
NT	***	***	***	*	***
DT × NT	NS	*	NS	NS	*
H. ×hybridus					
DT	***	***	***	***	***
NT	***	***	***	NS	***
DT × NT	*	*	**	**	*

[z]Total dry weight = root dry weight + top dry weight.

[y]RTR = root dry weight ÷ top dry weight.

[x]DT, NT, and DT × NT represent day temperature (DT) and night temperature (NT) main effects, and the DT × NT interaction, respectively.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

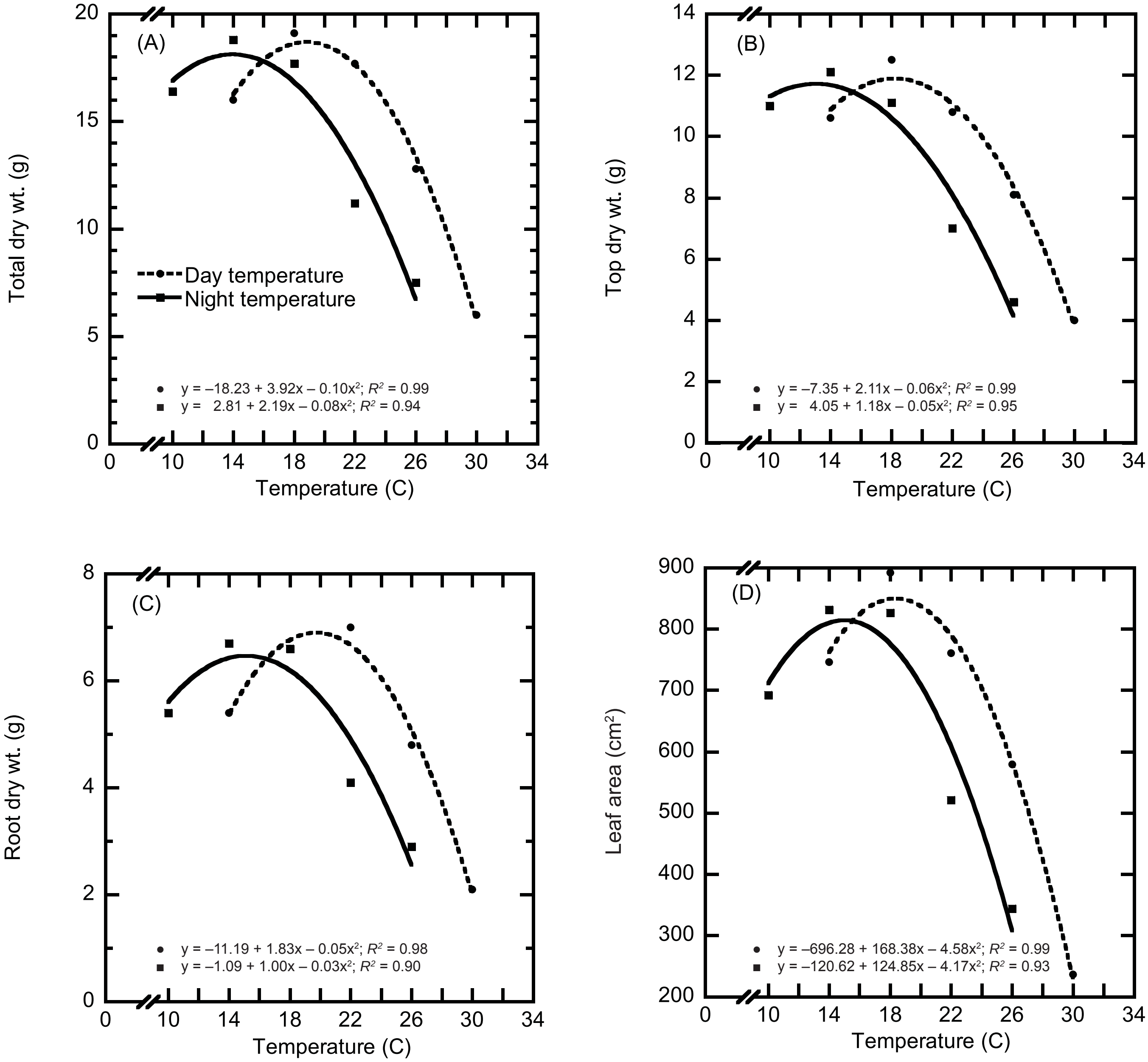

Fig. 1. Effects of day temperature (averaged over all night temperatures) and night temperature (averaged over all day temperatures) on (A) total plant dry weight, (B) top dry weight, (C) root dry weight, and (D) leaf area of seedlings of *H. foetidus*. Each symbol is the mean of 20 observations and the legend in (A) applies to all figures. There was a nonsignificant day × night temperature interaction.

top growth (10, 13, 15, 21). Maximum calculated total, top, and root dry weight, and leaf area occurred at days/nights of 19/14C (66/57F), 18/13C (65/55F), 20/15C (68/59F), and 18/15C (65/59F), respectively. While NT had no effect on RTR, RTR was greatest (0.65) with days of 22C (72F) (data not presented). Surprisingly, root growth was maximized at higher DT conjointly with RTR than the other growth parameters. This could have occurred by the high heat capacity of water in the container. However, substrate temperatures recorded for 22C days utilizing a Watchdog 425 temperature data logger (Spectrum Technologies, Contoocook, NH) revealed that on days the plants were fertigated maximum day substrate temperature was 24C (75F) and on days without fertigation maximum day substrate temperature was on average 6C (11F) higher than ambient air temperature. This is in contrast to widespread belief that roots of *Helleborus* sp. prefer cool substrate temperatures.

Helleborus niger. The DT × NT interaction was not significant for root and total plant dry weights, and RTR (Table 2). Thus, main effects are discussed. Root and total dry weights decreased linearly as DT increased indicating optimal DT for root and total dry weight was greatest at DTs ≤ 14C (58F) (Table 3). DT had no effect on RTR, whereas RTR responded quadratically as NT increased with a calculated maximum RTR at nights of 19C (66F). Even at nights of 22C (72F), RTR (0.56) indicates root growth was less temperature-sensitive than top growth (Table 3). Root and total dry weights also responded quadratically as NT increased with actual peaks occurring at 14C (57F) and total dry weight decreasing rap-

Table 3. **Influence of day temperature averaged over all night temperatures and night temperature averaged over all day temperatures on root and total plant dry weight, and root:top ratio (RTR) of *Helleborus niger*.[z,y]**

Day temp. (C)	Root dry wt. (g)	Total dry wt. (g)	RTR
14	1.7	5.4	0.50
18	1.5	4.6	0.49
22	1.4	4.6	0.47
26	1.1	3.6	0.47
30	0.7	2.1	0.47
Linear[x]	***	***	NS
Quadratic	NS	NS	NS
Night temp. (C)			
10	1.3	4.6	0.37
14	1.7	5.3	0.49
18	1.5	4.3	0.55
22	1.2	3.5	0.56
26	0.7	2.5	0.43
Linear	**	***	NS
Quadratic	***	*	***

[z]Total dry weight = root dry weight + top dry weight. RTR = root dry weight ÷ top dry weight.

[y]Data are means of 20 observations. There was a nonsignificant day × night temperature interaction. Initial root dry weight, total plant dry weight, and RTR = 0.2 g (0.007 oz), 0.6 g (0.021 oz), and 0.41, respectively.

[x]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 4. **Influence of day and night temperatures on top dry weight of *Helleborus niger*.[z]**

Night temp. (C)	Day temp. (C) 14	18	22 (g)	26	30	Linear[y]	Quadratic
10	5.4	3.2	3.4	3.2	1.8	**	NS
14	4.1	3.6	5.7	2.7	1.8	*	*
18	3.8	3.5	3.1	1.9	2.0	*	NS
22	2.7	3.3	1.8	2.8	1.0	*	NS
26	2.3	2.2	1.9	1.8	0.6	**	NS
Linear[x]	**[w]	NS	**	NS	***		
Quadratic	NS	NS	NS	NS	*		

[z]Data are means of four observations. There was a significant day × night temperature interaction. Initial top dry weight = 0.4 g (0.014 oz).

[y]Regression response of day temperature within each night temperature.

[x]Regression response of night temperature within each day temperature.

[w]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

idly with higher NTs while root dry weight only decreased 29% from 14 to 22C (56 to 72F) days. At DTs and NTs of 14C (56F), there was no evidence that alternating temperatures enhanced root or total plant growth. Similarly, Malek et al. (15), found no optimal thermoperiodicity for total dry weight of seedlings of *Rhododendron calendulaceum* (Michx.) Torr. (flame azalea). In the present investigation, maximum root and total dry weights were calculated to occur with NTs of 16 and 13C (60 and 55F), respectively. However, unexpectedly the differential between D/NTs that optimized growth of NIG was quite small.

There was a significant DT × NT interaction for top dry weight and leaf area of NIG (Table 2). As such, treatment comparisons were made within each DT and NT. For days of 14 or 22C (56 or 72F), top growth decreased linearly as NT increased (Table 4). At days of 30C (86F) there was a quadratic response to top dry weight with calculated maximum top dry weight at nights of 14C (56F). For all NTs except 14C (56F), top growth decreased linearly as DT increased. For nights of 14C (56F), total dry weight responded quadratically with increasing DT with calculated maximum top dry weight at days of 19C (67F). In general, greatest top growth was achieved with days/nights of 14/10C (57/50F) (Table 4) whereas, for root and total dry weight, maximum growth was at days/nights of 14/14C (57/57F) (Table 3).

The response of top dry weight (Table 4) to various temperature regimes was closely related to leaf area (Table 5). These responses agree with research of Dale (5) who reported that during the vegetative phase, dry matter production was highly correlated with the capacity of the leaves to intercept light. For days of 14 or 22C (57 or 72F), leaf area decreased linearly as NT increased (Table 5) indicating leaf area was optimized at NTs ≤ 10C (50F). As temperatures increase above optimum, leaf area typically decreases resulting in less carbohydrate production which when coupled with potentially higher respiration rates, results in less dry weight accumulation (6, 9). At days of 18C (64F) there was a quadratic response with calculated maximum leaf area at nights of 15C (59F). At nights of 10, 18, or 26C (50, 64, or 79F), leaf area decreased linearly as DT increased. For nights of 14C (57F) there was a quadratic response with a calculated maximum leaf area at days of 20C (68F). This reflects the response of top dry weight at nights of 14C (57F) (Table 4). Overall, leaf area was high at days/nights of 14/10C (57/50F) but was maximized at 22/14C (72/57F). In general, leaf area responded similarly as root, top, and total dry weights where growth was greatest at lower DTs and NTs (Tables 3 and 4). Thus, while NIG may contribute enhanced flowering characteristics to selective breeding programs, it should not be expected to contribute heat tolerance.

Table 5. **Influence of day and night temperatures on leaf area of *Helleborus niger*.[z]**

Night temp. (C)	Day temp. (C) 14	18	22 (cm^2)	26	30	Linear[y]	Quadratic
10	279.2	193.0	186.1	130.5	63.7	***	NS
14	199.8	205.6	327.5	147.1	64.7	*	**
18	213.1	205.9	143.2	112.6	85.6	**	NS
22	144.5	176.2	93.1	148.8	117.2	NS	NS
26	108.6	95.0	139.7	69.2	12.4	*	NS
Linear[x]	**[w]	*	*	NS	NS		
Quadratic	NS	*	NS	NS	NS		

[z]Data are means of four observations. There was a significant day × night temperature interaction. Initial leaf area = 30.6 cm^2 (4.7 in^2).

[y]Regression response of day temperature within each night temperature.

[x]Regression response of night temperature within each day temperature.

[w]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 6. Influence of day and night temperatures on top dry weight of *Helleborus ×hybridus*.[z]

Night temp. (C)	14	18	22	26	30	Linear[y]	Quadratic
	Day temp. (C) (g)						
10	5.0	5.4	3.8	3.6	2.6	**	NS
14	4.6	8.0	4.9	5.6	1.9	NS	*
18	8.1	5.2	5.5	4.6	2.6	***	NS
22	7.3	6.3	5.8	5.0	3.2	**	NS
26	7.2	4.1	3.2	2.1	1.1	***	NS
Linear[x]	*[w]	NS	NS	NS	NS		
Quadratic	NS	NS	**	**	NS		

[z]Data are means of four observations. There was a significant day × night temperature interaction. Initial top dry weight = 0.6 g (0.021 oz).

[y]Regression response of day temperature within each night temperature.

[x]Regression response of night temperature within each day temperature.

[w]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 7. Influence of day and night temperatures on root dry weight of *Helleborus ×hybridus*.[z]

Night temp. (C)	14	18	22	26	30	Linear[y]	Quadratic
	Day temp. (C) (g)						
10	2.9	3.1	2.2	2.1	2.0	**	NS
14	3.1	6.4	4.5	3.3	1.6	NS	**
18	4.2	3.6	5.0	3.5	2.0	NS	NS
22	3.9	3.9	4.1	3.5	2.2	*	NS
26	2.3	2.4	2.1	1.8	1.6	NS	NS
Linear[x]	NS[w]	NS	NS	NS	NS		
Quadratic	**	NS	***	***	NS		

[z]Data are means of four observations. There was a significant day × night temperature interaction. Initial root dry weight = 0.5 g (0.018 oz).

[y]Regression response of day temperature within each night temperature.

[x]Regression response of night temperature within each day temperature.

[w]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Helleborus ×hybridus. The DT × NT interaction was significant for all measured variables (Table 2). As such, treatment comparisons were made within each DT and NT. At days of 14C (57F), top growth increased linearly as NT increased (Table 6). Elevated NTs usually coincide with excessive dark respiration, which is unfavorable for growth (8). However, Malek et al. (15), reported that at days of 18C (64F) total, top, leaf, and stem dry weights of seedlings of *R. calendulaceum* were maximized with 26C (79F) nights. In the present investigation, at days of 22 or 26C (72 or 79F), top growth responded quadratically as NT increased with maximum calculated top growth occurring with NTs of 18 or 17C (64 or 63F), respectively. In contrast, top growth of HYB decreased linearly at each NT except 14C (57F) as DT increased. At nights of 14C (57F) there was a quadratic response to top dry weight with calculated maximum top dry weight at days of 20C (68F).

Table 8. Influence of day and night temperatures on total dry weight of *Helleborus ×hybridus*.[z,y]

Night temp. (C)	14	18	22	26	30	Linear[x]	Quadratic
	Day temp. (C) (g)						
10	7.9	8.5	6.0	5.6	4.6	**	NS
14	7.7	14.4	9.4	8.9	3.5	*	**
18	12.3	8.8	10.4	8.1	4.6	***	NS
22	11.2	10.2	9.9	8.5	5.4	**	NS
26	9.4	6.5	5.3	3.9	2.7	***	NS
Linear[w]	NS[v]	NS	NS	NS	NS		
Quadratic	NS	NS	***	***	NS		

[z]Total dry weight = root dry weight + top dry weight.

[y]Data are means of four observations. There was a significant day × night temperature interaction. Initial total dry weight = 1.1 g (0.039 oz).

[x]Regression response of day temperature within each night temperature.

[w]Regression response of night temperature within each day temperature.

[v]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

At days of 14, 22, or 26C (57, 72, or 79F) root dry weight responded quadratically and calculated maximum root dry weight occurred with nights of 18C (64F) (Table 7). Root dry weight decreased linearly with increasing DT for 10 or 22C (50 or 72F) nights. At nights of 14C (57F) there was a quadratic response to root dry weight and maximum root dry weight was calculated at days of 20C (68F). As observed with the other acaulescent species in this study, NIG, roots of HYB were able to tolerate high NT while high DT decreased root growth by 33 to 75% (Table 7). Hence, long durations of exposure to elevated DTs could decrease root growth and lower plant quality.

There were no clear trends of total dry weight to NTs at DTs of 14, 18, or 30C (57, 64, or 86F). At days of 22 or 26C (72 or 79F), there were quadratic responses in total dry weight with calculated maxima at nights of 18 or 17C (64 or 63F), respectively (Table 8). In contrast, at all NTs except 14C (57F), total dry weight decreased linearly as DT increased. At nights of 14C (57F) there was a quadratic response to total dry weight with calculated maximum total dry weight at days of 20C (68F) which was the same as root dry weight (Tables 7 and 8).

Responses of dry matter partitioning between roots and tops of HYB to DT and NT were different. RTR increased linearly at days of 26C (79F) over all NTs, while for days of 14, 22, or 30C (57, 72, or 86F), there was quadratic responses in RTR with greatest RTR calculated at nights of 15, 18, or 16C (59, 64, or 60F), respectively (Table 9). At nights of 10, 18, or 26C (50, 64, or 79F), RTR increased linearly as DT increased. Apparently, less carbohydrates were used for top growth than root growth, and root growth occurred at the expense of top growth resulting in increasing RTRs. This supports an earlier observation that root growth decreased less with increasing NT compared to top growth.

Leaf area increased linearly at days of 14C (57F), while at days of 22 or 26C (72 or 79F) there were quadratic responses in leaf area with calculated maximum leaf area at nights of 18 or 17C (64 or 63F), respectively (Table 10). For all NTs except 14C (57F), leaf area decreased linearly as DT increased. For nights of 14C (57F) there was a quadratic response to leaf area with calculated maximum leaf area at days of 20C (68F)

Table 9. Influence of day and night temperatures on root:top ratio (RTR) of ***Helleborus ×hybridus***.[z,y]

Night temp. (C)	Day temp. (C) 14	18	22	26	30	Linear[x]	Quadratic
10	0.57	0.61	0.60	0.63	0.86	*	NS
14	0.71	0.86	0.94	0.60	0.83	NS	NS
18	0.57	0.69	0.89	0.74	0.84	*	NS
22	0.56	0.63	0.70	0.70	0.78	NS	NS
26	0.32	0.62	0.64	0.91	1.61	***	NS
Linear[w]	*[v]	NS	NS	*	*		
Quadratic	*	NS	**	NS	*		

[z]RTR = root dry weight ÷ top dry weight.

[y]Data are means of four observations. There was a significant day × night temperature interaction. Initial RTR = 0.96.

[x]Regression response of day temperature within each night temperature.

[w]Regression response of night temperature within each day temperature.

[v]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 10. Influence of day and night temperatures on leaf area of ***Helleborus ×hybridus***.[z]

Night temp. (C)	Day temp. (C) 14	18	22	26	30	Linear[y]	Quadratic
	(cm^2)						
10	362.5	405.9	265.3	230.5	165.2	***	NS
14	343.7	579.4	378.7	413.2	123.4	*	**
18	632.0	438.4	391.8	390.7	179.2	***	NS
22	606.6	503.5	487.8	388.3	192.7	**	NS
26	565.4	342.6	229.8	140.9	81.5	***	NS
Linear[x]	*[w]	NS	NS	NS	NS		
Quadratic	NS	NS	**	***	NS		

[z]Data are means of four observations. There was a significant day × night temperature interaction. Initial leaf area = 59.6 cm^2 (9.2 in^2).

[y]Regression response of day temperature within each night temperature.

[x]Regression response of night temperature within each day temperature.

[w]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

the same as root dry weight (Table 7). Reductions in leaf area with high DT along with reductions in growth and increased RTR may indicate a decrease in carbohydrate production via P_N instead of increased respiration. However, P_N increased as DT increased indicating loss of carbohydrates for growth was due to increased respiration and not decreased P_N (Table 11). While NT did not impact P_N, DT had an effect. Neither DT nor NT impacted g_s (Table 11). There was a linear increase in P_N as DT increased to 30C (86F) (Table 11), whereas top dry weight was greatest at days of 18C (64F) (Table 6). Malek et al. (15) reported that for seedlings of *R. calendulaceum*, P_N was highest at days of 30C (86F), whereas, maximum total dry weight occurred at lower day temperatures. However, dry weight increase does not always coincide with an increase in photosynthesis. More often leaf expansion is a more relevant indicator of growth than photosynthetic rates (15, 18). Results herein support these reports. At days/nights of 18/14C (64/57F) greatest total dry weight was realized (Table 8), these temperatures of which corresponded to a high leaf area (Table 10). However, higher values for leaf area were observed at days/nights of 14/18C (57/64F) and 14/22C (57/72F) with lower total dry weight. Results suggest that while leaf area may be optimized with warmer nights than days, a different temperature regime would be necessary to optimize all growth variables.

In general, growth of HYB was greatest with a cool 14C (57F) DT for each NT except 14C (57F). Interestingly, with 14C (57F) nights, HYB was able to tolerate slightly higher DTs of 18C (64F) as evidenced by high top dry weight (Table 6), root dry weight (Table 7), and leaf area (Table 10). Thus, it appears HYB and FOE require a greater differential between day and night temperatures than NIG.

In summary, determination of an optimal day/night temperature regime for a particular species is complex as temperature optima vary depending upon a particular growth parameter. Results of this study demonstrate slight variations in heat tolerance among three cultivated species of hellebores. Growth of FOE, NIG, and HYB was enhanced by low to moderate DTs and NTs although growth of HYB was impacted mostly by DT. Thus, better management of DT will benefit growth of HYB. FOE and HYB were the most tolerant to elevated day/night temperatures [18/14C (64/57F)] while NIG preferred cooler day/night temperatures [14/10C (57/50C)]. Poor growth of NIG at high DT and NT supports observations of nursery professionals who have experienced problems growing NIG in the southeastern United States (Richard and Judith Tyler, Pine Knot Farm Perennials, Clarksville, VA, personal communication). Thus, containerized culture of particular species of *Helleborus*, such as NIG, during summer in the southeastern United States may require reduced temperatures within growing structures. We therefore suggest that NIG and also FOE and HYB be grown in a structure such that high DT stress can be monitored and reduced if necessary. This should shorten the time to produce a salable plant. Ideally, FOE and HYB should be grown at days/nights of 18/14C (64/57F) while NIG is best grown at days/nights of 14/10C (57/50F). Finally, if ideal temperature

Table 11. Net CO_2 assimilation (P_N) and stomatal conductance (g_s) of ***Helleborus ×hybridus*** grown under contrasting day/night temperatures.[z]

Day temp. (C)	P_N (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	g_s (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)
14	4.85[z]	0.17
18	4.45	0.20
22	6.75	0.20
26	7.74	0.16
30	8.33	0.41
Linear	*	NS
Quadratic	NS	NS
Night temp. (C)		
10	4.47	0.11
18	8.44	0.39
26	6.37	0.18
Linear	NS	NS

[z]Data are means of 20 observations. There was a nonsignificant day × night temperature interaction.

NS, * Nonsignificant or significant at $P \leq 0.05$, respectively.

regimes are not feasible we suggest the same 4C (7F) differential between DT and NT while, lowering temperatures as far as possible.

Data herein support in part initial hypotheses about heat tolerance of FOE, NIG, and HYB (Table 1). Overall, FOE had the greatest tolerance to high DT, followed by HYB, and the least exhibited by NIG. However, with NT, HYB had the greatest heat tolerance, followed by FOE, and the least exhibited by NIG.

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