Differing Vernalization Responses of *Veronica spicata* ‘Red Fox’ and *Laurentia axillaris*

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ABSTRACT. Many polycarpic herbaceous perennials are known to have a cold-requirement for flowering. To determine the range and relative effectiveness of vernalization temperatures for flower induction, clonally propagated plants of veronica (*Veronica spicata* L.) ‘Red Fox’ and laurentia [*Laurentia axillaris* (Lindl.) E. Wimm.] were exposed to temperatures from –2.5 to 20 °C at 2.5 °C increments for 0, 2, 4, 6, or 8 weeks (veronica ‘Red Fox’) and 0, 2.5, 5, 7.5, 10, 12.5, or 15 weeks (laurentia). After treatments, growth and flowering were monitored in a glass greenhouse set at 20 °C with an average daily light integral of $\approx 5 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. Both veronica ‘Red Fox’ and laurentia exhibited obligate vernalization requirements for flowering, but the temperature–response curves were distinctly different. A minimum of 4 weeks at –2.5 and 0 °C, 6 weeks at 2.5 °C, and 8 weeks at 5 and 7.5 °C was required for complete (100%) flowering of veronica ‘Red Fox’, while a minimum of 5 weeks at 5 to 10 °C, 7.5 weeks at 12.5 °C, and 10 weeks at 2.5 °C were required for complete flowering of laurentia. For veronica ‘Red Fox’, node number under each flower and flower timing were relatively fixed following up to 8 weeks at each temperature, although these values generally decreased at each temperature with extended exposure for laurentia. Based on percent flowering and percentage of lateral nodes flowering, vernalization of veronica ‘Red Fox’ was most effective at 0 and –2.5 °C, while based on percent flowering and flower number, vernalization of laurentia was most effective at 5 to 10 °C.

Many herbaceous winter annual, biennial, and perennial plants require vernalization, exposure to low temperatures, for flower induction (Chouard, 1960; Thomas and Vince-Prue, 1984). While vernalization responses of numerous plant species have been studied, the bulk of our knowledge comes from extensive research on a relatively few plant species. In particular, numerous studies have evaluated the vernalization responses of winter annuals and biennials such as winter wheat (*Triticum aestivum* L.), ‘Petkus’ winter rye (*Secale cereale* L.), carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), sugar beet (*Beta vulgaris* L.), onion (*Allium cepa* L.), arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] var. Stockholm, *Hyoscyamus niger* (L.), and *Lunaria annua* (L.) (Chouard, 1960; Lang, 1952, 1965; Thomas and Vince-Prue, 1984).

For most winter annuals, it has been shown that the vernalization requirement is nonobligate or facultative, because they will eventually flower even without exposure to low temperatures. Reported effective temperatures for vernalization of winter cereals generally have ranged from below zero to ≈ 15 °C, with an optimum between 5 and 10 °C, depending on the species, cultivar, and method of assessment (Atherton et al., 1990; Baloch et al., 2003; Lang, 1965; Rawson et al., 1998). Potter and Gawith (1999) reviewed numerous published studies on vernalization of winter wheat and concluded that the

minimum effective temperature was –1.3 °C, the optimum was 3.8 to 6.0 °C, and the maximum was 15.7 °C.

Many herbaceous perennials are known to have a cold-requirement for flowering, and knowledge of the specific temperatures and durations for vernalization is of special concern during horticultural production, because growers can be required to bring perennials into flower for marketing on specific dates. Limited empirical data have been available to help growers, and it is not known which herbaceous perennials have vernalization requirements similar to winter cereals and biennials and which do not. We have conducted extensive trials to better understand vernalization requirements of numerous herbaceous perennial selections. During this process, we identified two species, *Veronica spicata* ‘Red Fox’ and *Laurentia axillaris* (syn. *Isotoma axillaris* Lindl.), with obligate or near-obligate vernalization requirements yet with substantially differing temperature requirements.

Veronica (Scrophulariaceae) is an extremely cold-hardy (to –40 °C) ornamental species native from Europe to Northern Asia (Armitage, 1989; Griffiths, 1994). The species has characteristic blue flowers, and many clonal hybrids are available, including the pink-flowered ‘Red Fox.’ Outdoors, veronica ‘Red Fox’ typically flowers for several weeks in early summer then remains vegetative until the following year. Enfield (2002) determined that veronica ‘Red Fox’ had an obligate vernalization requirement, and plants held in the greenhouse at constant 20 °C for up to a year rarely produced flowers. Growers in southern locations have expressed specific concerns about incomplete flowering after attempted vernalization of veronica ‘Red Fox.’

Laurentia (Campanulaceae) is native to Queensland, Victoria, and New South Wales in eastern Australia (Bentham and

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Mueller, 1844), generally frost-free regions. In Australia, *laurentia* plants naturally flower in late winter and early spring for several months and sporadically thereafter until autumn (Blomberry, 1977). Our preliminary trials indicated that *laurentia* selections started from seed had near-obligate vernalization and long-day requirements for flowering (Fausey et al., 2003). Plants not exposed to low temperatures eventually produced a few flowers but only after several months. *Laurentia* is sometimes grown from seed as a garden annual in northern climates, and anecdotal reports suggest that first-year seedlings flower late in the season if at all.

The objectives of this study were to characterize flower induction of *veronica* 'Red Fox' and *laurentia* as a function of vernalization temperature and duration to determine the most effective temperatures and how they differ between these two species. Our approach was to use single clones of each species of the same physiological age grown under strictly controlled photoperiod and temperature conditions to eliminate confounding effects of pre- and post-vernalization growth environment on subsequent growth and developmental responses.

Materials and Methods

PLANT MATERIAL. *Veronica* 'Red Fox' is a clonal selection propagated from cuttings. The clone used in these experiments was originally received from Yoder Green Leaf (Lancaster, PA). *Laurentia* seedlings were provided by Bluebird Nursery (Clarkson, NE). A single *laurentia* seedling was selected from this population based on superior foliage and flower characteristics and cloned for all subsequent experiments. This clone was later named and made commercially available as 'Beth's Blue' from Four Star Greenhouses (Carleton, MI).

To create stock plants, rooted cuttings were transplanted into 13-cm-square pots (1.1 L) filled with a commercial peat-perlite media (Sure-Mix; Michigan Grower Products, Galesburg, MI) and grown in a glass greenhouse at $\approx 20^\circ\text{C}$ in East Lansing, MI (lat. 43°N) until established. Six weeks before the beginning of each experiment, stock plants were transferred to a 22°C growth chamber with a 13-h photoperiod provided by 12 cool-white fluorescent lamps (215 W, VHO F96T12; Philips Lighting Co., Somerset, NJ) supplemented with 12 incandescent lamps (60 W, 60 A-130 V; Philips Lighting Co.) at $150\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0600 to 1900 HR. The proportion of light based on wattage output was 78% cool-white fluorescent and 22% incandescent. Stock plants were watered as necessary using acidified well water containing nutrients (see below) in the greenhouse and growth chamber.

EXPERIMENT 1. *Veronica* 'Red Fox' cuttings with three to four nodes were taken at 2-week intervals for 8 weeks beginning on 22 May 2003. *Laurentia* cuttings with three to four nodes were taken at 2.5-week intervals for 15 weeks beginning on 13 May 2003. Cuttings were dipped into $1000\ \text{mg}\cdot\text{L}^{-1}$ indole-3-butyric acid and $500\ \text{mg}\cdot\text{L}^{-1}$ naphthalene acetic acid rooting hormone (Dip 'N Grow, Clackamas, OR) and placed in 72-cell plug trays (50-mL cell volume per plant) filled with the above media. Cuttings were rooted for 2 weeks under a 9-h photoperiod in a glass propagation house with air and media temperatures of 23°C and 26°C , respectively. Once rooted, plants were grown for an additional 3 weeks in a 20°C growth chamber with a 13-h photoperiod receiving $150\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($\approx 7\ \text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) before transfer to temperature treatments.

Five weeks from the start of rooting, plants were transferred to controlled environmental chambers set at -2.5 to 20°C , at 2.5°C increments. *Veronica* 'Red Fox' and *laurentia* plants were all of the same physiological age and averaged 5–6 and 12–14 nodes, respectively, at the start of temperature treatments. Because propagation was staggered, as described in the previous section, plants were exposed at each temperature for 0, 2, 4, 6, or 8 weeks (*veronica* 'Red Fox') and 0, 2.5, 5, 7.5, 10, 12.5, or 15 weeks (*laurentia*). Controlled environmental chambers varied by $<0.5^\circ\text{C}$ from set point. During treatment at 5 to 20°C , plants were illuminated with $100\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for $11\ \text{h}\cdot\text{d}^{-1}$ ($\approx 4\ \text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) provided by a combination of fluorescent and incandescent lamps as previously described. During treatment at -2.5 to 2.5°C , light levels were reduced to $20\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ($\approx 0.8\ \text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$), because of the lower output from fluorescent light sources at low temperatures and the fact that growth and photosynthesis would be much slower at these low temperatures.

Propagation was staggered such that *veronica* 'Red Fox' and *laurentia* plants were moved from temperature treatments and transplanted to the greenhouse on 6 Aug. 2003 and 9 Sept. 2003, respectively. A complete second run for *veronica* 'Red Fox' was initiated on 22 Aug. 2003 and conducted with exactly the same protocol, such that plants were removed from the temperature treatments and transplanted on 25 Nov. 2003. A second complete second run for *laurentia* was initiated 13 Dec. 2002. For this run, all cuttings were taken on a same day, so that treatments were transferred to the greenhouse at 2.5-week intervals beginning on 17 Jan. 2003.

EXPERIMENT 2. To further determine the minimum vernalization requirement for flowering of *veronica* 'Red Fox', cuttings were collected on 1 Mar. 2004, propagated and treated as described, and transferred to -2.5 , 0 , or 2.5°C on 5 Apr. 2004 and held for 0, 12, 16, 20, 24, 28, or 32 d. After the cold treatments, plants were transplanted and grown in a glass greenhouse. This experiment was repeated starting on 31 Mar. 2004, and plants entered the greenhouse starting 5 May 2004.

PLANT CULTURE. Following vernalization treatment, plants were transplanted to 13-cm-square pots (1.1 L) filled with the above media. All plants were irrigated as needed with reverse osmosis water and a water soluble fertilizer providing $125\ \text{mg}\cdot\text{L}^{-1}$ N, $12\ \text{mg}\cdot\text{L}^{-1}$ P, $100\ \text{mg}\cdot\text{L}^{-1}$ K, $65\ \text{mg}\cdot\text{L}^{-1}$ Ca, and $12\ \text{mg}\cdot\text{L}^{-1}$ Mg (12.6% nitrate nitrogen, 2% ammoniacal N) plus $1.0\ \text{mg}\cdot\text{L}^{-1}$ Fe, $0.5\ \text{mg}\cdot\text{L}^{-1}$ Mn, $0.5\ \text{mg}\cdot\text{L}^{-1}$ Zn, $1.0\ \text{mg}\cdot\text{L}^{-1}$ Cu, $0.3\ \text{mg}\cdot\text{L}^{-1}$ B, and $0.1\ \text{mg}\cdot\text{L}^{-1}$ Mo (MSU RO Special, Greencare Fertilizers, Chicago).

ENVIRONMENTAL CONTROL. Following treatment, plants were grown in a glass greenhouse with a 20°C setpoint and 16-h photoperiod provided by 400-W high-pressure sodium (HPS) lamps from 0600 to 0800 HR and from 1700 to 2200 HR. Instantaneous light in each treatment was measured at plant height with LI-COR line quantum sensors (LI-COR, Lincoln, NE) connected to a CR10 datalogger (Campbell Scientific, Logan, UT). Greenhouse air temperatures were controlled by a climate-control computer (model CD750; Priva North America, Vineland Station, ON) and were monitored on each bench with 36-gauge (0.127-mm diameter) type-E thermocouples connected to a CR10 datalogger. Temperature and light measurements were collected every 10 s, and the hourly average was recorded. Average daily temperature (ADT) and integrated photosynthetic photon flux *PPF* [daily light integral (DLI)] were calculated for each run of each experiment for each species.

DATA COLLECTION AND ANALYSIS. Node number before treatment, at transplant, and following transplant below the first visible flower bud were collected for all plants. For veronica 'Red Fox', the date of first visible inflorescence, the date of first open flower along the inflorescence, and the number of reproductive and vegetative lateral shoots were collected for all flowering plants at first open flower. For laurentia, the date of first macroscopic visible flower bud (≈ 1 mm in length) and first open flower were collected for all flowering plants. Flower bud number at first open flower was also recorded for laurentia. Experiments were ended 15 weeks after transfer to the greenhouse, and all plants without a macroscopic flower bud were considered non-reproductive.

Each experiment was a completely randomized design. Data were analyzed using PROC GLM general linear model procedures in SAS (version 8.0; SAS Institute, Cary, NC). Regression analysis was performed using Sigma Plot 8.0 (Systat Software, Inc., San Jose, CA). Linear regression lines are presented only when the correlation was statistically significant ($P < 0.05$).

Results

TEMPERATURE AND LIGHT LEVELS. Average daily temperatures ranged from ≈ 20 to 24 °C, while DLI ranged from ≈ 5 to 10 mol·m⁻²·d⁻¹ from transplant to first flower during the different experimental runs (Table 1). Temperature and light changed with season of the experiment and whitewash application. For the second run of laurentia, weekly DLI varied by <2 mol·m⁻²·d⁻¹ from weeks 5 to 20.

SURVIVAL DURING VERNALIZATION. Newly rooted plants were actively growing when moved from 20 °C post-rooting conditions to vernalization treatments, and plant survival at the low temperatures differed between species. Veronica 'Red Fox' plants survived all treatment durations at -2.5 to 20 °C, while laurentia plants did not survive 2.5 or more weeks at -2.5 °C and had variable survival after 7.5 or more weeks at 0 and 2.5 °C in both runs (Table 2). Laurentia plants survived all treatment durations at 5 to 20 °C. Data for visibly injured plants were removed from further analysis.

VERNALIZATION EFFECTS ON FLOWERING. Veronica 'Red Fox' exhibited obligate vernalization requirements for flowering, because no plants formed a visible flower bud within 15 weeks if vernalized for 0 or 2 weeks at any temperature. A minimum of 4 weeks at -2.5 and 0 °C, 6 weeks at 2.5 °C, and 8 weeks at

5 and 7.5 °C was required for complete (100%) flowering (Fig. 1A). After 8 weeks, only marginal flowering (30% to 50%) occurred after vernalization at 10 °C, and no plants flowered when held at or above 12.5 °C. In Expt. 2, the

Table 2. Percent survival percentage of laurentia plants after transfer from 20 °C to -2.5 to 5 °C for 2.5–15 weeks and subsequently grown in a greenhouse with a 20 °C setpoint (average of Runs 1 and 2).^z

Duration (weeks)	Treatment temp. (°C)			
	-2.5	0	2.5	5
2.5	0	100	100	100
5	0	100	100	100
7.5	0	63	25	100
10	0	50	100	100
12.5	0	0	63	100
15	0	0	38	100

^zAll plants survived when treated at 7.5 to 20 °C.

Fig. 1. Flowering percentage of veronica 'Red Fox' plants (A) and percent of lateral shoots that flowered (B) following treatment at -2.5 to 20 °C for 4, 6, and 8 weeks. No plants held for 2 weeks or control plants flowered. Percent flowering plants is average of Runs 1 and 2. For flowering laterals, each symbol represents the mean of 16 plants when 100% flowering was achieved, and error bars represent SE.

Table 1. Mean air temperatures and daily light integrals (DLI) for each experiment and replication.^z

Expt. and run no.	Temp (°C)	DLI (mol·m ⁻² ·d ⁻¹)
Expt. 1—veronica 'Red Fox'		
Run 1	24.0	9.8
Run 2	20.3	4.7
Expt. 1—laurentia		
Run 1	20.9	5.7
Run 2	21.4	10.6
Expt. 2—veronica 'Red Fox'		
Run 1	24.0	10.0
Run 2	20.2	4.5

^zFor veronica 'Red Fox' and the first run of laurentia, averages were calculated for the first 8 weeks from the time of transplant, by which time the majority of plants had flowered. For Run 2 of laurentia, averages were calculated for the first 18 weeks following transplant.

vernalization requirement for 90% to 100% flowering of veronica 'Red Fox' plants was a minimum of 20 d at -2.5°C , 28 d at 0°C , and 32 d at 2.5°C (Fig. 2A).

All reproductive veronica 'Red Fox' plants formed a single inflorescence spike, with vegetative or floral buds forming in the leaf axil of each node below the spike. Each plant formed an average of 18 total lateral nodes, and this was not significantly affected by treatment. Flower induction of nodes below the spike occurred in a basipetal fashion, and induced buds developed into secondary inflorescences. The percent of nodes induced to flower was strongly influenced by vernalization temperature and duration (Figs. 1B and 2B). About 60% of lateral buds flowered following 8 weeks of vernalization at -2.5 or 0°C and decreased with increasing temperature (Fig. 1B). For every 2.5°C increase in vernalization temperature above -2.5°C , the percentage of flowering nodes decreased. In Expt. 2, the induction of secondary inflorescences down the stem was consistently higher after treatment at -2.5°C than at 0 or 2.5°C , but never exceeded 50% (Fig. 2B).

Laurentia also exhibited obligate vernalization requirements for flowering, because no control plants formed a visible flower bud within 15 weeks. Complete flowering occurred after a minimum of 5 weeks at 5 to 10°C , 7.5 weeks at 12.5°C , and

10 weeks at 2.5°C (Fig. 3A). Treatment for 12.5 and 15 weeks did not change percent flowering (data not shown). Shorter durations at each temperature resulted in lower flowering percentages. Treatment for 2.5 weeks at 5 , 7.5 , and 10°C induced flowering (Fig. 3A), but the number of flower buds at first flower was significantly less than when vernalized for 5 or more weeks (Fig. 3B). Complete flowering was not achieved after treatment at 0°C , even after 10 weeks, and longer durations resulted in total plant death. An average of >60 flower buds per plant were present at first open flower when plants had been treated at 7.5 or 10°C for 5 or more weeks or at 12.5°C for 10 or more weeks (Fig. 3B). The number of flower buds did not significantly change as treatment duration increased from 10 to 15 weeks (data not shown). The average number of flower buds that formed on plants following treatment at 5°C was consistently lower than those treated at 7.5 or 10°C (Fig. 3B). Those plants that survived treatment at 2.5 and 0°C formed relatively few flowers if at all.

GROWTH DURING VERNALIZATION. For both species, nodes formed during treatment increased with time (Fig. 4A and C). For veronica 'Red Fox', the rate of node formation exhibited a curvilinear response in relation to increasing temperature with

Fig. 2. Flowering percentage of veronica 'Red Fox' plants (A) and percent of lateral shoots that flowered (B) treated at -2.5 , 0 , or 2.5°C for 12–32 d. Percent flowering plants is average of Runs 1 and 2. Each symbol represents the mean of 16 plants when 100% flowering was achieved, and error bars represent SE.

Fig. 3. Flowering percentage of laurentia plants (A) and number of flower buds at first open flower following treatment at -2.5 to 20°C for 2.5–10 weeks. No control plants flowered. Percent flowering plants is average of Runs 1 and 2. Each symbol represents the mean of 16 plants when 100% flowering was achieved, and error bars represent SE.

Fig. 4. Number of nodes formed during temperature treatment (A, C) and the rate of node formation during treatment (B, D) for veronica 'Red Fox' (A, B) and laurentia (C, D) held at -2.5 to 20 °C for 2–8 or 2.5–15 weeks, respectively. Error bars represent SE.

essentially no growth at -2.5 and 0 °C (0.01 node per day) and a maximum of 0.14 node per day at 20 °C (Fig. 4B). In contrast, the rate of node formation in laurentia increased linearly from ≈ 0 node per day at 0 °C to 0.2 node per day at 20 °C (Fig. 4D). Between 5 and 10 °C, laurentia node formation rate was about twice that of veronica (Fig. 4B and D).

VERNALIZATION EFFECTS ON NODE DEVELOPMENT AND TIME TO FLOWER. Vernalization temperature but not duration of treatment affected the number of nodes formed below the inflorescence following vernalization for veronica 'Red Fox' ($P < 0.0001$) (Fig. 5A). Node number after transplant increased nonlinearly following vernalization treatment above -2.5 °C but did not correlate with time to flower (data not shown). Flowering of veronica 'Red Fox' plants occurred in 38–41 d (Run 1) and 53–58 d (Run 2) for all treatments that resulted in complete flowering.

In contrast, node formation after transplant in laurentia generally decreased with increasing duration at each vernalization temperature (Fig. 5B). There was a decrease of 2–4 nodes as treatment duration increased from 5 to 10 weeks. With the exception of plants that flowered after 2.5 weeks and a few that were injured at low temperatures, time to flower in laurentia was highly correlated with node formation following vernalization ($r^2 = 0.93$) (Fig. 6). The fewest nodes below an inflorescence were observed following 15 weeks at 7.5 and 10 °C and were associated with the fewest days from transplant to first open flower (Figs. 5B and 6). Flowering of plants that survived 7.5 and 10 weeks of vernalization at 0 °C was significantly delayed, although plants formed a similar number of nodes as other treatments (Fig. 6). Plants that flowered after treatment for only 2.5 weeks at 5, 7.5, or 10 °C took >80 d until the first flower opened (data not shown).

Discussion

Optimum temperatures for vernalization of a species depend on the duration of thermoinduction treatments as well as the method of assessment (Lang, 1965). The greatest flowering response of veronica 'Red Fox' was observed following all vernalization durations at -2.5 °C based on the percentage of primary and lateral nodes flowering. To our knowledge, no other species examined to date has exhibited an optimum vernalization temperature below 2 °C (Atherton et al., 1990; Chouard, 1960; Lang, 1965). Conversely, 5 to 10 °C was most effective in vernalizing laurentia plants following 5 or more weeks of cold based on percent flowering, and vernalization at 12.5 °C was equally effective after 10 or more weeks. Based on number of flower buds at first open flower, vernalization at 7.5 and 10 °C were somewhat superior to that at 5 °C. Optimum vernalization temperatures of 3 to 7 °C have been described for carrot, beet, 'Petkus'

winter rye, and *A. thaliana* var. Stockholm, while slightly higher optimum temperatures of 5 to 10 °C have been reported for *H. niger* and onion (Atherton et al., 1990; Bernier et al., 1981; Brewster, 1987; Chouard, 1960; Lang, 1965).

Lang (1965) cited occurrences of vernalization in winter rye, *H. niger*, and onion at 15 to 17 °C, and Lange (1992) found that *Lilium longiflorum* Thunb. could be vernalized at or below 15 °C. Maximal temperatures for vernalization of veronica 'Red Fox' and laurentia were 7.5 and 12.5 °C, respectively. It is possible that with longer durations at 10 °C, vernalization of veronica 'Red Fox' would have eventually saturated.

Laurentia exhibited an obligate vernalization requirement in this study, but there is evidence that laurentia can flower via a vernalization-independent, autonomous pathway. We have observed flowering of nonvernalized plants when grown for extended durations (≥ 150 d) under higher greenhouse and light conditions than encountered in this study (B. Fausey and A.C. Cameron, unpublished data). In addition, anecdotal reports from gardeners indicate that laurentia will flower in late summer if started from seed in early spring, although the environmental history of these plants is unclear. Attempts to flower inadequately vernalized laurentia plants (0 and 2.5 weeks at 7.5 °C) in controlled environment chambers set at 20 °C with 16-h photoperiods and high light quantity ($9\text{--}26 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) only marginally improved flowering (0% to 15%) after 12 weeks of growth (B. Fausey and A.C. Cameron, unpublished data). Although growth at any temperature may result in flowering when given enough time, vernalization at 5 to 10 °C clearly promoted the most rapid, uniform flowering of this laurentia clone. Recently, new laurentia cultivars (e.g., Avant Garde) have been released that flower much earlier without vernalization in garden trials (A.C. Cameron, personal observations).

Fig. 5. New nodes formed under the inflorescence after transplanting in the greenhouse as a function of treatment temperature for veronica 'Red Fox' (A) and laurentia (B) held at -2.5 to 10 °C for 2–8 or 2.5–10 weeks, respectively. Only data for flowering plants are presented, and error bars represent SE.

The effectiveness of vernalization treatments has been determined empirically by several methods, and the proportion of plants flowering in a given population following vernalization treatment readily represents the quantitative nature of thermoinduction (Lange, 1992; Thomas and Vince-Prue, 1984). Other methods to evaluate induction include evaluation of floral morphology, node and flower development, and time required to reach a particular stage of development. Lang (1965) generalized that, for most species, the number of nodes formed below the inflorescence after transfer to warmer inductive conditions decreased with increasing effectiveness of a vernalization treatment until an optimal level was reached.

Node number, time to flower, and flowering characteristics of veronica 'Red Fox' and laurentia responded differently to vernalization treatments, and treatment effectiveness was determined by differing criterion. In veronica 'Red Fox', node number (Fig. 5A) and flower timing were relatively fixed at each temperature with increasing duration (data not shown). Under conditions of higher ADT and DLI in Run 1 (Table 1), average time to first flower was almost 2 weeks faster than in Run 2 across all treatments, but node formation and other patterns of flowering were very similar between the two runs.

In contrast to veronica 'Red Fox', node number and time to flower of laurentia generally decreased at each temperature

Fig. 6. Correlation between new nodes formed and days to flower after transplant for laurentia plants stored at 0 to 12.5 °C for 5–15 weeks. Treatments with <100% survival are presented with (+) inside symbol and were not included in regression analysis.

with extended exposure (Figs. 5B and 6). Flower initiation first occurred following a minimum of 2.5 weeks, although time to visible bud was greatly delayed (≈ 70 d) and flower bud number was greatly reduced (Fig. 3B). Plants vernalized for 5 weeks at 5 to 10 °C formed ≈ 9 nodes (Fig. 5B), and flower buds ≈ 1 mm in length were noted 22 d after transplant. In contrast, plants vernalized for an additional 10 weeks formed 4–6 nodes, and flower buds were visible 8 d after transplant. Laurentia will flower under both long and short day photoperiods once the cold requirement for flowering is satisfied (B. Fausey and A.C. Cameron, unpublished data). Although plants were not dissected to determine whether flower primordia were present following vernalization treatments, initiation and development likely occurred following a minimum of 5 weeks of vernalization at 5 to 10 °C with an 11-h photoperiod.

Veronica 'Red Fox' and laurentia plants were actively growing and not acclimated to low temperatures before vernalization treatments. Veronica 'Red Fox' showed no signs of injury after transfer from 20 to -2.5 °C, while laurentia plants failed to survive prolonged exposure to temperatures < 2.5 °C. Veronica 'Red Fox' is an alpine plant that survives at least down to -40 °C, while hardiness of laurentia is reported to be about -10 °C (Griffiths, 1994). Engle (1994) found that 20 of 24 herbaceous perennial species benefited from exposure to 0 or 5 °C for several weeks before low-temperature storage at -2.5 °C, and it is well known that specific genes induce cold tolerance in arabidopsis.

The fact that the vernalization requirements differ for these two herbaceous perennials will impact production techniques for growers who wish to sell plants in flower on specific dates. Growers who rely on natural low temperatures in mild climates or those who are using artificial refrigeration for vernalization will need to take this information into account. For instance, although exposure to 10 °C for 8 weeks should be completely effective for vernalization of laurentia, it would result in unacceptable flowering of veronica 'Red Fox.' When possible, most perennial growers have targeted 5 °C for vernalization, and, based on the results from this research, this still appears to be a suitable compromise for these two species. Still, growers need to

be aware that some perennials such as *laurentia* can vernalize even at 12.5 °C, while others such as *veronica* 'Red Fox' will not. Further, vernalization of *veronica* 'Red Fox' is more effective at 0 or -2.5 °C than at 5 °C, so the duration of exposure at 5 °C will need to be increased for satisfactory flowering.

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