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Article

Effects of decreasing photoperiod on cold acclimation of asparagus seedlings

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Abstract

Cold acclimation is a vital process for surviving subzero temperatures in asparagus (*Asparagus officinalis* L.). A winter hardy cultivar, “Guelph Millennium” (GM), cold acclimates, senesces, and develops freezing tolerance earlier in the fall compared to “UC157” (UC), a cultivar adapted to warm climates. Decreasing photoperiod can induce timely senescence and freezing tolerance in both woody and herbaceous perennials, although its effects on asparagus are unclear. The objectives of this research were to study the effects of daylength and cold, and their interaction, on the induction of freezing tolerance in asparagus. Seedlings of GM and UC were subjected to four treatments: constant (15.5 h) or decreasing (15.5–14 h) photoperiods with either high (23 °C) or low (11 °C) temperatures. Decreasing daylengths did not impact the acquisition of freezing tolerance at either temperature. For UC, low temperature accompanied by decreasing photoperiod diminished root:shoot ratio and increased crown water percentage, which are both indicators of reduced dormancy. A decreasing photoperiod and high temperature increased crown fructan (total and high-molecular weight) and decreased glucose and proline concentrations in GM compared to UC. These results suggest that decreasing daylengths, similar to those occurring in late summer in Southern Ontario, may be a signal for crown metabolite partitioning. This effect may be a contributing factor to the timely senescence and cold acclimation seen in GM compared to UC.

Key words: *Asparagus officinalis*, percent water, partitioning, fructan, metabolites

Résumé

L’acclimatation au froid est un processus vital qui permet à l’asperge (*Asparagus officinalis* L.) de survivre aux températures inférieures à zéro. Le cultivar rustique "Guelph Millennium" (GM), s’endurcit au froid, flétrit et acquière une tolérance au gel plus tôt que la variété "UC157", adaptée aux climats chauds, quand arrive l’automne. La diminution de la photopériode entraîne la sénescence et la tolérance au gel des vivaces ligneuses et herbacées au moment opportun, mais ses effets sur l’asperge restent méconnus. Les chercheurs voulaient préciser les conséquences du froid et de la longueur du jour, ainsi que de leur interaction, sur l’induction de la tolérance au gel chez l’asperge. À cette fin, ils ont appliqué quatre traitements à des plantules GM et UC, soit une photopériode constante (15,5 h) ou décroissante (de 15,5 à 14 h) à haute (23 °C) ou à basse (11 °C) température. Le raccourcissement de la période diurne n’affecte pas l’acquisition de la tolérance au gel, peu importe la température. Une basse température et la diminution de la photopériode réduisent le rapport pousses:racines d’UC et augmentent la proportion d’eau dans la griffe, deux indices signalant une moins grande dormance. La plus courte photopériode et une température élevée augmentent la concentration de fructane (total et à haut poids moléculaire) dans la griffe et diminuent celles de glucose et de proline chez GM, comparativement à ce qui se produit chez UC. Ces résultats donnent à penser que le raccourcissement des jours, un peu comme cela arrive à la fin de l’été dans le sud de l’Ontario, pourrait correspondre au moment où les métabolites se répartissent dans la griffe, phénomène qui pourrait contribuer à la sénescence et à l’acclimatation au froid observées chez GM mais pas chez UC. [Traduit par la Rédaction]

Mots-clés : Asparagus officinalis, proportion d’eau, répartition, fructane, métabolites

Introduction

Asparagus (*Asparagus officinalis* L.) is a herbaceous perennial that is grown in a variety of diverse climates. To remain productive over the crop’s lifetime, which may be 15–20 years (Haynes 1987), cultivars must be well adapted to the environment in which they are grown. In southern Ontario, one of the coldest temperate climates where asparagus is cultivated, cultivars must be able to withstand early fall freezing events and cold winters.

Cold acclimation induces freezing tolerance in competent species primarily through the stabilization of membranes and protection of vital cellular processes such as

RNA metabolism (Melencion et al. 2017) and mitochondrial electron transport (Thebud and Santarius 1981; Steponkus 1984). A multigenic, quantitative trait, cold acclimation is associated with many processes including changes in membrane composition, cell compartmentalization, metabolism, antioxidant activity, osmotic adjustments, and production of primary and secondary metabolites (Kazemi-Shahandashti and Maali-Amiri 2018). Increased concentrations of crown proline, sucrose, and high-molecular weight (HMW) fructans, and dehydration of the crown have been correlated previously with increased freezing tolerance in asparagus seedling studies (Landry and Wolyn 2012; Kim and Wolyn 2015). Root:shoot ratio can also be used as a general indicator of carbon partitioning from the fern to crown in preparation for winter dormancy (Woolley et al. 1999; Guo et al. 2002).

In Southern Ontario, "Guelph Millennium" (GM), a locally bred cultivar with high longevity, cold acclimates and induces freezing tolerance earlier under field conditions in the fall compared to "UC157" (UC), an unadapted California-bred desert cultivar that typically dies after 3–4 years (Panjtandoust and Wolyn 2016). Interestingly, freezing tolerance in late fall does not differ between GM and UC, suggesting timely cold acclimation in the fall may be important for adaptation in Southern Ontario. In temperate climates, induction of dormancy may be achieved through photoperiodic control. Decreasing daylength is known to initiate endodormancy during late summer/early fall and be a key component of cold acclimation in woody perennials including poplar, and specific grape, dogwood, and silver birch cultivars (Li et al. 2003; Pearce 2004; Horvath 2009). Evidence of daylength perception resulting in increased freezing tolerance in herbaceous perennials is limited. In alfalfa, photoperiod is known to control the partitioning of photosynthates to the roots at the end of the growing period (Teixeira et al. 2008; Jing et al. 2020), and short daylengths can increase freezing tolerance in winter dormant cultivars (Bertrand et al. 2017).

Daylength may have an impact on cold acclimation in asparagus through carbon partitioning and dormancy induction. Both Sudjatmiko et al. (1997) and Woolley et al. (1999) observed increased carbon partitioning to the crowns of field-grown asparagus in late summer. A similar effect was found for asparagus seedlings grown in growth chambers as the photoperiod declined from 15.5 h to 14 h (Woolley et al. 2002). Other asparagus studies investigating the effects of a constant 8 h photoperiod in the field (Yamaguchi 2012) and controlled conditions (Kim and Wolyn 2015) indicated that photoperiod may not be used as a major signal in cold acclimation and dormancy. Mimicking the natural decline in daylength experienced in the summer to fall progression may be critical for observing a daylength effect in asparagus. For example, in Southern Ontario the photoperiod peaks at ~15.5 h in late June, and decreases to ~14 h in mid-August and ~11.5 h in early October.

The objective of this study was to determine the effects of decreasing photoperiod, similar to natural late summer daylengths in Southern Ontario, and its interaction with cold-acclimating temperatures, on the development of freezing tolerance in asparagus. Decreasing daylengths and cold temperatures were predicted to reduce growth, increase concentrations of cryoprotective compounds, and have a synergistic effect when applied together.

Table 1. Summary of treatments applied to 10-week-old asparagus seedlings.

Treatment No.	Temperature and photoperiod	Abbreviation	Duration
1	23 °C, 15.5 h	23C/15.5P	5 wk
2	11 °C, 15.5 h	11C/15.5P	5 wk
3	23 °C, 15.5 h→14 h	23C/14P	5 wk
4	11 °C, 15.5 h→14 h	11C/14P	5 wk

Materials and methods

Plant culture

Sixteen "cone-tainer" flats (SC10, Stuewe and Sons Inc., Corvallis, USA) each with 96 cells (164 mL volume) were filled with a peat-based soilless mix (Sunshine LC1, SunGro Horticulture Canada Ltd., Seba Beach, USA). Seeds for "UC157" (UC) were obtained from Walker Brothers (Pittsgrove, USA) and "Guelph Millennium" (GM) from Fox Seeds (Simcoe, Canada). After imbibing seeds in water at room temperature for 1 day, one seed was planted per cell. In each tray, 49 plants for each cultivar were established as split-plots. Replicate experiments were planted on 8 April 2019 and 7 April 2020.

Eight random trays were placed in each of two replicate greenhouse zones. Plants were grown at 23/18 °C (day/night), under natural light (daylength: 13 h 6 min to 15 h 25 min) supplemented with 16 h photoperiod from high-pressure sodium lighting (Model 67578, Sylvania, Mississauga, Canada) with a photosynthetic photon flux density (PPFD) of ~80 μmol m^{-2} s^{-1}. Plants were watered with deionized (DI) water as needed. A full-strength modified Hoagland's solution (Product ID: H353, PhytoTechnology Laboratories, Lenexa, USA), pH 6.5, was applied biweekly until saturation for fertilization.

Growth chamber treatments

Ten-week-old seedlings were subjected to four treatments (Table 1). Four randomly chosen flats were placed into each of two growth chambers at constant 23 °C and 60% relative humidity and two growth chambers at constant 11 °C and 60% relative humidity. All growth chambers initially had a 15.5 h photoperiod provided by alternating strips of fluorescent lights (Model 20906, Sylvania, Mississauga, Canada) and far-red light-emitting diode bulbs (Model 79292, Sylvania, Mississauga, Canada) with a total PPFD of 300 μmol m^{-2} s^{-1} measured 56 cm above the bottom of the flat. One 23 °C and one 11 °C growth chamber were chosen randomly for the decreasing photoperiod treatment (Table 1; Treatment No. 3 and 4), whereas the remaining 23 °C and 11 °C chambers maintained a constant photoperiod (Treatment No. 1 and 2). Daylengths were shortened by 15 min (minus 15 min alternating from morning and evening) every 5 days, for the 5-week duration of the experiment. Plants were watered as needed with DI water. At the end of each 5-week treatment, plants

were analyzed for metabolites, physiological parameters, and temperature at which 50% of the population dies (LT_{50}).

Sampling and LT_{50}

For each cultivar, crowns from seven randomly chosen seedlings in each flat were bulked for metabolite analyses as described below. The remaining 42 plants, per cultivar, in each flat were used for LT_{50} analysis. Ferns were removed 3 cm above the soil, and one replicate flat for each treatment was placed randomly into each of the four freezers. Temperature was maintained at 3 °C for 2 h then lowered to 0 °C for 24 h. Additional freezing treatments of −3 °C, −6 °C, −9 °C, −12 °C, −15 °C were achieved by decreasing the freezer temperature by 3 °C h^{-1} and maintaining for 24 h. A Hobo thermocouple (Pocasset, USA) inserted 2.5 cm below the soil surface was used to monitor soil temperature. After each freezing treatment (0 °C to −15 °C), seven random plants for each cultivar replicate were thawed at 4 °C for 24 h and two replicates per treatment were placed in each of the two greenhouse zones and watered with DI water as needed. After 4 weeks plants were rated as dead or alive to estimate LT_{50} values.

Tissue preparation, fresh/dry weight, and metabolite analysis

Sample preparation, water percentage determination, and analyses of proline, glucose, sucrose, and low-molecular weight (LMW) and HMW fructan concentrations were performed as described (Short and Wolyn, 2022).

Statistical analysis

Data analyses were conducted with SAS version 9.4 (SAS Institute, Cary, USA). A split-plot design was used, such that treatments served as whole-plots, and cultivars (GM, UC) were subplots. In 2019, both 23 °C chambers (Table 1; 23C/15.5P and 23C/14P) had fern die-back occurring approximately 2 weeks into the 5-week treatment from a thrips infestation. Plants were treated with beneficials, and ferns recovered. Therefore, 23 °C treatments were analyzed as independent completely randomized block designs where the least square means (LSM) of each cultivar were compared using a Tukey's test. The 11 °C chambers were combined across years, as the effects of year, treatment × year, and the interactions with cultivar were nonsignificant. The simple effects of treatment and variety were analyzed using the "slicediff" option with a Tukey's Honestly Significant Difference (HSD) adjustment ($P \leq 0.05$). Studentized residual plots were analyzed for homogeneity, and normality was tested using a Shapiro–Wilk test with PROC UNIVARIATE. Restricted maximum likelihood covariance parameter estimates were performed on parameters using PROC GLIMMIX. PROC CORR was used to generate correlation coefficients between analyzed variables, and LT_{50} was predicted using PROC PROBIT. No variation in LT_{50} was apparent for both 23 °C treatments (23C/15.5P and 23C/14P) in 2019, and therefore they were omitted from analysis.

Results

Statistical analysis

The fixed effect of cultivar was significant in the low-temperature treatment for LT_{50}, LMW fructan and HMW fructan concentrations, and crown water percentage. The fixed effects of treatment and cultivar × treatment were nonsignificant for all parameters.

Freezing tolerance

Decreasing photoperiod and low temperature had no effect on the acquisition of freezing tolerance in both cultivars (Fig. 1A). GM had approximately 1 °C lower LT_{50} values (increased freezing tolerance) compared to UC for the constant 15.5 h or decreasing 15.5–14 h photoperiod with 11 °C treatment. Cultivars did not differ under the high-temperature treatment in 2020 (Fig. 1B) and LT_{50} values appeared higher for 23 °C compared to 11 °C conditions (Figs. 1A and 1B).

Physiological parameters

Root:shoot ratio declined only for UC with decreasing, compared to constant photoperiod, for the 11 °C treatment; values for GM did not change and cultivars did not differ under constant or decreasing photoperiod (Fig. 2A). Cultivars did not differ for the 23 °C treatment (Fig. 2B), however, values appeared lower under 23 °C, compared to 11 °C conditions (Figs. 2A and 2B). Fern water percentage did not differ between cultivars for photoperiod treatments in either low or high temperatures (Figs. 2C and 2D).

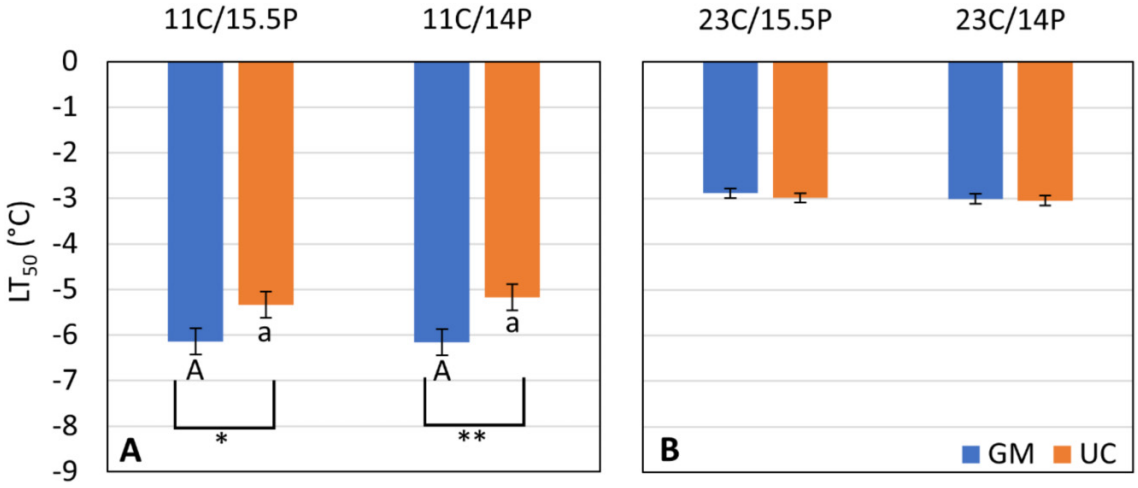
For UC, crown water percentage was 1.25% greater under decreasing, compared to constant photoperiod, for the 11 °C treatment; values for GM did not change (Fig. 2E). UC had greater crown water percentage than GM under decreasing photoperiod and low temperature. Crown water percentage appeared to be greater in 2019 compared to 2020 for both the constant and decreasing photoperiods and high-temperature treatments (Fig. 2F). GM had decreased water percentage compared to UC for both photoperiod treatments in 2020 (Fig. 2F).

Metabolites

Total fructan concentrations did not differ between cultivars or photoperiod treatments for plants grown at 11 °C (Fig. 3A). At 23 °C, fructan concentrations of GM were approximately 15% greater than those of UC in 2020 for the decreasing photoperiod treatment (Fig. 3B). Total fructan concentrations in the high-temperature treatments appeared lower in 2019 compared to those in 2020 (Fig. 3B).

LMW fructan concentrations were greater for UC than GM in the 11 °C treatment but the effects of variety within treatment, and treatments within variety were nonsignificant (Fig. 3C). Cultivars responded similarly to high temperature in 2019 and 2020 (Fig. 3D). Photoperiod treatments, in combination with low temperature, did not affect HMW fructan concentrations in both cultivars, however, values were greater for GM than UC (Fig. 3E). For the decreasing photoperiod and 23 °C treatment in 2020, the HMW fructan concentration for GM was greater than that for UC by approximately 40% (Fig. 3F). HMW fructan concentrations appeared

Fig. 1. LT_{50} of asparagus cultivars "Guelph Millennium" (GM) and "UC157" (UC). (A) Represents plants grown for 5 weeks at 11 °C under constant 15.5 h photoperiod (11C/15.5P) and decreasing 15.5–14 h photoperiod (11C/14P) treatments. Data were pooled over years (2019 and 2020). Mean ± SE, $n = 8$. (B) Represents plants grown 5 weeks at 23 °C under constant photoperiod (23C/15.5P) and decreasing photoperiod (23C/14P) treatments in 2020 only. Mean ± SE, $n = 4$. Uppercase and lowercase letters denote differences between treatments for GM and UC, respectively, and asterisks show the differences between cultivars within each treatment for Tukey's HSD test, $P \leq 0.05$. Significance is denoted by $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.



lower for the high-, compared to low-temperature treatment (Figs. 3E and 3F).

Proline, sucrose, and glucose concentrations did not differ between cultivars or photoperiod treatments in combination with low temperature (Figs. 4A, 4C, and 4E). Crown proline and glucose concentrations were greater in UC compared to GM by approximately 50% and 100%, respectively, in 2020 for plants grown with a decreasing photoperiod in the high-temperature treatment (Figs. 4B and 4F). Sucrose concentration was greater for UC compared to GM under constant photoperiod and high temperature in 2019 (Fig. 4D).

LT_{50} correlations

When analyzing high- and low-temperature data together, LT_{50} was correlated with all parameters except sucrose and glucose concentrations (Table 2A). For the 11 °C treatment HMW fructan and crown water percentage were weakly associated with freezing tolerance (Table 2B), whereas root:shoot ratio was associated with freezing tolerance in the 23 °C treatment in 2020 (Table 2C).

Discussion

Overall, reducing photoperiod from 15.5 to 14 h had no impact on the acquisition of freezing tolerance, although certain parameters previously correlated with cold tolerance and dormancy were altered under decreasing daylength. Low temperature induced greater freezing tolerance for GM compared to UC regardless of photoperiod. For UC, low temperature and decreasing photoperiod increased crown water percentage and reduced root:shoot ratio compared to a constant photoperiod, whereas GM maintained similar levels for both treatments. Decreasing photoperiods in combination with high temperature in 2020 induced a cultivar-specific response, whereby crown total and HMW fructan concentrations were increased, and proline and glucose concentrations were decreased in GM compared to UC.

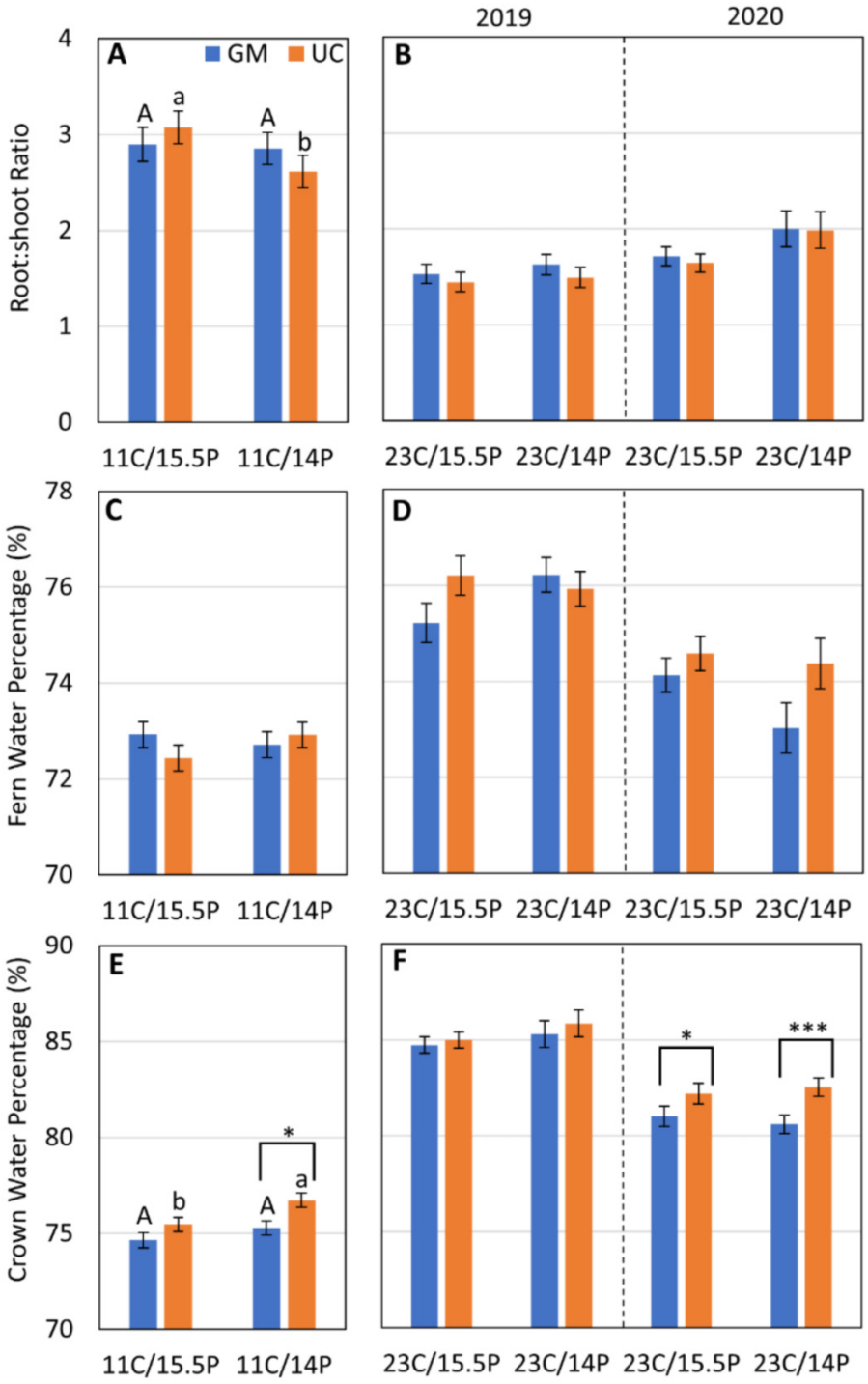
Effect of low temperature

Numerous cold acclimation studies have been reported for asparagus grown under controlled conditions. While the acclimation regimens vary, the temperature of 11 °C used here, was warmer compared to previous experiments of similar duration (Landry and Wolyn 2012; Kim and Wolyn 2015). In this study the magnitudes of freezing tolerance induction and cultivar differences were less than those observed by Kim and Wolyn (2015) where plants were acclimated at 7 °C. Induction of fern senescence in asparagus has been reported to occur at 2 °C–15 °C, with optimal temperatures ranging from 5 °C to 12 °C (Pressman et al. 1989; Krug 1996). Interestingly, GM had greater freezing tolerance compared to UC in both photoperiod treatments with low temperature, suggesting temperature is the main signal for inducing freezing tolerance and that GM cold acclimates more effectively than UC. Furthermore, many highly significant correlations between LT_{50} and metabolic parameters were present when data for high- and low-temperature treatments were combined (Table 2A). In contrast, few correlations with LT_{50} were found for temperature treatments considered separately (Table 2B and 2C), suggesting that the effects of cultivar and photoperiod on freezing tolerance were less than that for temperature.

Photoperiod effect at low temperature

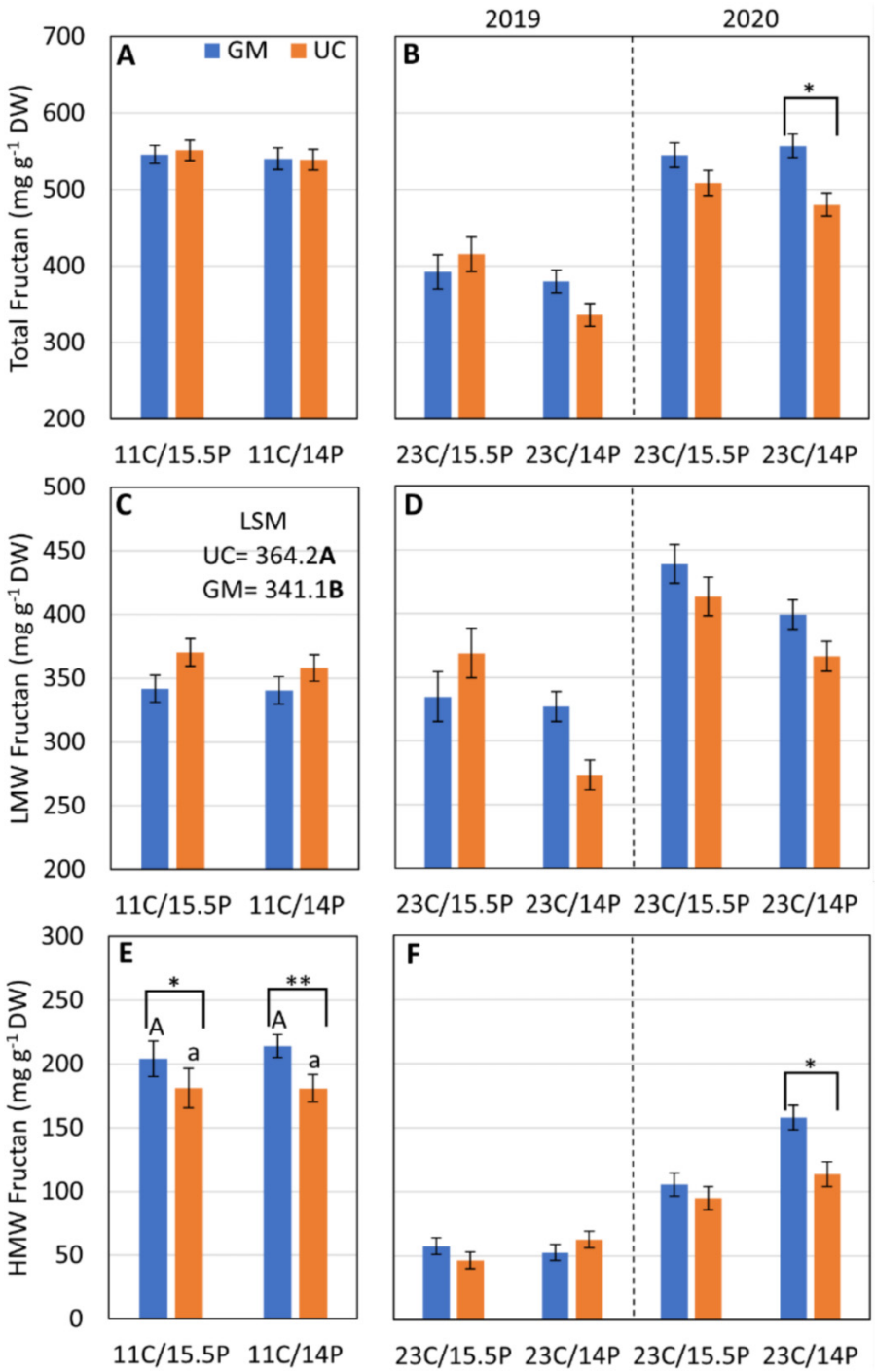
For UC under decreasing photoperiod with low temperature, root:shoot ratio decreased and crown water percentage increased compared to the constant photoperiod treatment. However, both treatments had similar freezing tolerances. UC, bred at the University of California (Riverside), may have

Fig. 2. Root:shoot ratio (A, B), fern water percentage (C, D), and crown water percentage (E, F) of asparagus cultivars Guelph Millennium (GM) and UC157 (UC). (A, C, and E) Represent plants grown for 5 weeks at 11 °C under constant 15.5 h photoperiod (11C/15.5P) and decreasing 15.5–14 h photoperiod (11C/14P) treatments. Data were pooled over years (2019 and 2020). Mean ± SE, $n = 8$. (B, D, and F) Represent plants grown for 5 weeks at 23 °C under constant photoperiod (23C/15.5P) and decreasing photoperiod (23C/14P) treatments in 2019 and 2020. Mean ± SE, $n = 4$. Uppercase and lowercase letters denote differences between treatments for GM and UC, respectively, and asterisks show the differences between cultivars within each treatment for Tukey's HSD test, $P \leq 0.05$. Significance is denoted by $^{*}P \leq 0.05$; $^{***}P \leq 0.001$.



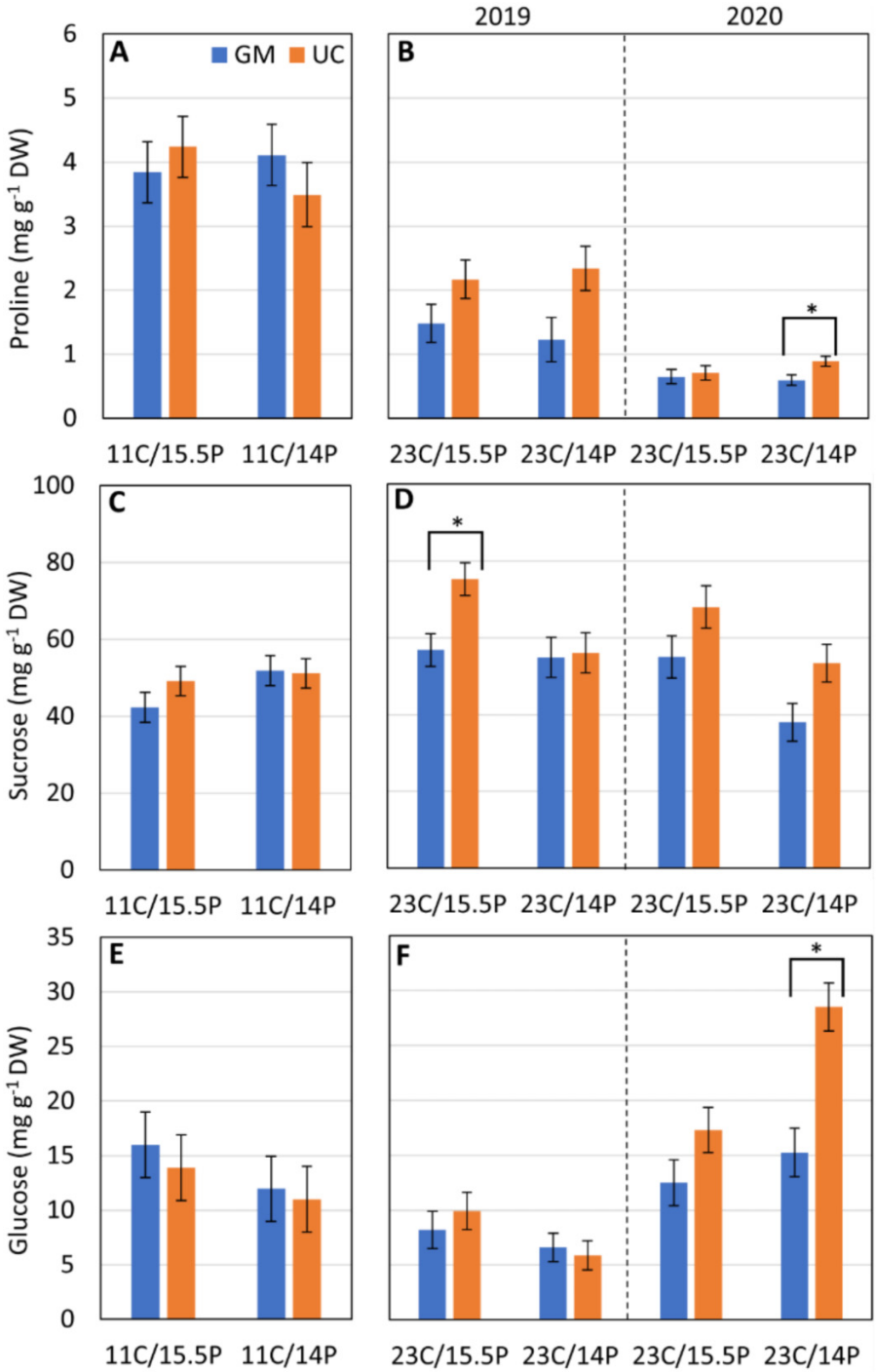
responded to the decreasing photoperiod as a signal to grow under low temperatures as a daylength of 14 h corresponds to late July in California when peak-high temperatures occur and persist into late August. In Southern Ontario, where GM was developed, a daylength of 14 h corresponds to mid-August when temperatures start to decline. For example, in Guelph, Ontario, average low temperatures are greatest in mid-July at ~15 °C and decrease to ~12 °C by mid-August

Fig. 3. Total crown fructan (A, B), low-molecular weight (LMW) fructan (C, D), and high-molecular weight (HMW) fructan (E, F) concentrations of asparagus cultivars Guelph Millennium (GM) and UC157 (UC). (A, C, and E) Represent plants grown for 5 weeks at 11 °C under constant 15.5 h photoperiod (11C/15.5P) and decreasing 15.5–14 h photoperiod (11C/14P) treatments. Data were pooled over years (2019 and 2020). Mean ± SE, $n = 8$. (B, D, and F) represent plants grown for 5 weeks at 23 °C under constant photoperiod (23 C/15.5P) and decreasing photoperiod (23 C/14P) treatments in 2019 and 2020. Mean ± SE, $n = 4$. Uppercase and lowercase letters denote differences between treatments for GM and UC, respectively, and asterisks show the differences between cultivars within each treatment for Tukey's HSD test, $P \leq 0.05$. Significance is denoted by $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.



(Weather Underground 2022). Interestingly, GM seemed insensitive to decreasing daylengths during cold acclimation as there were no significant differences between photoperiod treatments. Daylength insensitivity under low temperatures could be beneficial for growing this cultivar in different temperate regions, although additional temperature and photoperiod treatments would be needed to discount potential interactions.

Fig. 4. Crown proline (A, B), sucrose (C, D), and glucose (E, F) concentrations of asparagus cultivars "Guelph Millennium" (GM) and "UC157" (UC). (A, C, and E) Represent plants grown for 5 weeks at 11 °C under constant 15.5 h photoperiod (11C/15.5P) and decreasing 15.5–14 h photoperiod (11C/14P) treatments. Data were pooled over years (2019 and 2020). Mean ± SE, n = 8. (B, D, and F) represent plants grown for 5 weeks at 23 °C under constant photoperiod (23C/15.5P) and decreasing photoperiod (23C/14P) treatments in 2019 and 2020. Mean ± SE, $n = 4$. Uppercase and lowercase letters denote differences between treatments for GM and UC, respectively, and asterisks show the differences between cultivars within each treatment for Tukey's HSD test, $P \leq 0.05$. Significance is denoted by $^{*}P \leq 0.05$.



Photoperiod effect at high temperature

In this study, metabolic changes were observed under decreasing photoperiods combined with high temperatures in 2020, such that GM had higher total and HMW fructan, and decreased glucose and proline concentrations in the crown compared to UC. The cultivars, however, did not differ for freezing tolerance. Here and in other studies with asparagus seedlings, HMW fructan and proline crown concentra-

Table 2. Pearson correlation coefficients between LT_{50} and metabolic/physiological parameters analyzed for asparagus cultivars Guelph Millennium and UC157.

		Proline	Sucrose	Glucose	Fructan (total)	Fructan (LMW)[b]	Fructan (HMW)[c]	Root:shoot	Fern % H_2O[d]	Crown % H_2O
A	LT_{50}[a]	**−0.82*****	0.12	0.16	−0.34*	**0.59*****	**−0.75*****	**−0.78*****	**0.44****	**0.88*****
B	LT_{50}	−0.20	0.02	−0.19	−0.32	0.01	−0.36*	−0.24	−0.09	0.40*
C	LT_{50}	0.18	−0.18	0.07	−0.01	−0.01	0.01	**−0.70****	−0.11	−0.47

Note: (A) Represents correlations for plants grown at 23 °C and 11 °C with either a constant 15.5 h or decreasing 15.5–14 h photoperiod ($n = 48$). Data did not include 2019 high-temperature data. (B) Represents correlations using 11 °C treatments in 2019 and 2020 ($n = 32$). (C) Shows correlations for 23 °C treatments in 2020 ($n = 16$). Significant correlations are indicated by $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$; highly significant correlations ($^{**}P \leq 0.01$, $^{***}P \leq 0.001$) are highlighted in bold.
[a]LT_{50}, temperature at which 50% of the population dies.
[b]LMW, low-molecular weight.
[c]HMW, high-molecular weight.
[d]% H_2O, water percentage.

tions have been generally correlated with increased freezing tolerance when comparing warm- versus cold-acclimated plants (Landry and Wolyn 2012; Kim and Wolyn 2015). At high temperatures, decreases in photoperiod may be used by the plant to alter metabolism before senescence and freezing tolerance development. For example, Kim and Wolyn (2015) found that HMW fructan increased only for GM, not UC, with a warm/short day treatment compared to the respective untreated control, and freezing tolerances were similar. In addition, when the warm/short day treatment was subsequently subjected to an additional subfreezing treatment, GM gained freezing tolerance whereas UC did not. The increase in HMW fructan concentration in crowns could be a necessary step in cold acclimation, allowing efficient acquisition of freezing tolerance once cold temperatures are present, while not being directly responsible for enhanced freezing tolerance.

Decreasing daylengths may prime plants through metabolic adjustment to cold acclimate effectively once cold temperatures are present. The development of freezing tolerance in alfalfa is generally attributed to increased crown sucrose and raffinose, and decreased starch concentrations (Castonguay et al. 1995). In a study by Bertrand et al. (2017), alfalfa previously grown under 14 h daylengths, then cold acclimated, had elevated crown total soluble sugar and sucrose concentrations compared to plants grown under 16 h daylengths. In addition, raffinose family oligosaccharides were elevated in plants grown under 8, 10, and 12 h photoperiods followed by cold acclimation, compared to those grown under 14 and 16 h photoperiods in one of the two cultivars tested. Future studies should compare asparagus seedlings grown under both constant and decreasing photoperiods in high-temperature conditions, followed by a period of cold acclimation to determine if a priming effect exists.

Fern die-back and regrowth occurring for both photoperiod treatments grown under the high-temperature treatment in 2019 most likely caused general decreases in total, LMW, and HMW fructan concentrations and increased crown water percentage compared to 2020 observations. Similar metabolic changes were found after defoliation of asparagus in mid-August for both rhizome and storage roots in a field study (Nolet and Wolyn 2020). Damage and regrowth of ferns in this study may have masked any metabolic changes between cultivars caused by decreasing daylengths in 2019.

Summary

GM had a greater freezing tolerance compared to UC under cold acclimation irrespective of photoperiod treatment. A photoperiod effect with low temperature was observed where crown water percentage was elevated and root:shoot ratio was reduced in UC. Decreasing photoperiods for the warm treatment did not appear to impact freezing tolerance and caused crown metabolite changes between cultivars in 2020, which may be evidence of priming for the acquisition of freezing tolerance. These findings contribute to our understanding of the role photoperiod has in the cold acclimation process of asparagus, and could be used in the breeding of new cultivars suited for temperate regions.

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