**Fall Defoliation Affects Acquisition of Freezing Tolerance and Spring Regrowth in Asparagus**

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Fall Defoliation Affects Acquisition of Freezing Tolerance and Spring Regrowth in Asparagus

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Asparagus (Asparagus officinalis L.) acquires freezing tolerance during a period of fall acclimation when both photoperiod and temperature decrease. The above-ground vegetative growth may be important for sensing changing environmental conditions and translocating compounds to the below-ground crown. Defoliation experiments, repeated over two years, were conducted by removing fern in mid-August, -September and–October, and evaluating crown metabolites and LT$_{50}$, the temperature at which 50% of plants die, at monthly intervals to mid-November. Spring emergence and vigor were also assessed in separate experiments. In one year, only mid-August defoliation affected LT$_{50}$ values, decreasing freezing tolerance, which was associated with diminished rhizome proline concentration and storage root low- and high-molecular weight fructan concentrations. All defoliation treatments in the second year decreased LT$_{50}$ values, or increased freezing tolerance, possibly resulting from an interaction between defoliation and drought which increased rhizome sucrose concentrations. Defoliation decreased spring vigor in both experiments; the response was proportional to the earliness of the treatment and associated with rhizome and storage root fructan levels. Crowns of plants defoliated in mid-August had increasing proline concentrations during the fall, similar to control plants, suggesting the below-ground organs may have sensed soil temperature to cold acclimate. Autumn defoliation to control disease, harvest seed or implement other cultural practices can reduce vigor and likely attenuate long-term performance of a plantation.

Keywords: freezing tolerance, metabolites, proline, fructan, LT$_{50}$, regrowth
INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is an herbaceous perennial vegetable crop where emerging stems or spears are harvested for several weeks in the spring, followed by the growth of a vegetative structure, commonly called ‘fern.’ During summer, photosynthesis produces carbohydrates to replenish storage roots for sustaining harvest during the subsequent season. In temperate regions, fern senesces in autumn and the underground crown, consisting of a rhizome and storage roots, acquires freezing tolerance and becomes dormant to survive winter.

Cultivars differ for adaptation to the cold winter climate of southern Ontario, Canada, and for the best hybrid, Guelph Millennium, fern senesces in mid-October and the crown acquires freezing tolerance in a timely manner, earlier than other genetic lines (Panjtandoust and Wolyn 2016a). Physiological traits and cryoprotective compounds correlated with freezing tolerance, or LT$_{50}$, the temperature at which 50% of plants die, included high proline, low-molecular weight fructan (LF) and sucrose concentrations, and low water percentage in the rhizome, and high proline and sucrose concentrations and low water percentage and LF concentration in the storage roots. Rhizome traits were thought to be more important for winter survival than those of storage root as they had higher correlations with LT$_{50}$ values (Panjtandout and Wolyn 2016a, 2016b).

The induction of senescence, dormancy and freezing tolerance occurs during autumn as both photoperiod and temperature decrease. In woody plants, in the absence of shortening photoperiod, cold temperature alone can cause trees to undergo cold acclimation (Weiser 1970). Light, however, may be important for low temperature perception through changes in photosynthetic processes leading to the production of reactive oxygen species (Winfield et al.
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2010) which serve as signaling molecules. Twice as many cold-responsive gene were
upregulated in light compared to darkness in Arabidopsis (Soitamo et al. 2008) supporting an
interaction between light and cold in freezing tolerance. Although the sensing of photoperiod for
the acquisition of freezing tolerance in asparagus is uncertain, the interaction of light and cold
temperatures, sensed in the fern, could be critical. Decreasing photoperiod affected asparagus
carbohydrate partitioning, altering distribution from the fern to the crown as daylength decreased
to 14 h (Woolley et al. 2002). The role of decreasing soil temperature in the autumn and its
perception directly in the rhizome or storage roots to induce freezing tolerance is also unknown.

Autumn fern removal before complete senescence is practiced in hybrid seed production
in southern Ontario where unusual early snowfalls in October could compromise the harvest.
Defoliation has also been suggested as a means to control foliar disease inoculum levels for the
subsequent growing season (Kelly and Bai 1997) or allow the implementation of cultural
practices, such as application of plastic to fields for white spear production, before soils become
saturated and susceptible to structural damage from heavy machinery (Feller and Müller 2018).
Late-September fern removal in asparagus decreased yield the following year, while that in
early-October did not (Kelly and Bai 1997), however, the effect on fall acclimation and the
acquisition of freezing tolerance was not addressed. In another study, late-September fern
removal did not affect storage root carbohydrate concentration in November, and no impact on
yield the following season was predicted (Feller and Müller 2018).

Alfalfa harvested in late-summer or early-autumn in northern climates had increased
winter damage and reduced yields in the following year (McKenzie and McLean 1980,
Haagenson et al. 2003). Roots of alfalfa plants defoliated in the autumn had decreased dry matter
percentage, and starch and protein concentrations (Silkett et al. 1937, Haagenson et al. 2003). In addition, reduced transcript levels of cold responsive genes have been reported after defoliation (Dhont et al. 2006).

The objective of this research was to understand the role of the fern in the acquisition of freezing tolerance in asparagus by defoliating plants during late-summer and autumn, and measuring LT$_{50}$ levels, and concentrations of cryoprotective metabolites. Emergence and regrowth vigor the following season were also studied to determine associations with freezing tolerance and defoliation.

MATERIALS AND METHODS

Experiment 1: Fall Acclimation and Metabolite Profile after Autumn Defoliation

Experimental Design

Ten treatment combinations (Table 1) were planted in a randomized complete block design with four replicates. The experiment was repeated independently in 2015 and 2016. In 2015, plots were sampled on 17 Aug., 15 Sep., 14 Oct., 9 Nov. and 16 Nov. In 2016, plots were sampled on 15 Aug., 13 Sep., 17 Oct., 7 Nov. and 14 Nov. Half of the replicates were harvested on each of the November dates in each year to accommodate the large number of treatments, but collectively were classified as mid-November sampling.

Plant Establishment

Seed for cultivar Guelph Millennium was obtained from Fox Seeds (Simcoe, ON) and
planted on 19 Mar. 2014 and 25 Mar. 2015 into 288-cell plug trays containing Sunshine mix #5 (Sun Gro Horticulture Canada, Seba Beach, AB). The seedlings were transplanted on 2 May 2014 and 19 May 2015 into 50-cell plug trays filled with Sunshine mix #4 (Sun Gro Horticulture Canada, Seba Beach, AB) and fertilized weekly with 20N-3.5P-16.6K (1.25 g/L; Plant Products Limited; Brampton ON). Plants were grown in a greenhouse with 25°C/20°C (day/night) temperatures and natural light supplemented with a 12 h photoperiod provided by 250-watt high pressure sodium lamps with a photosynthetic photon flux density (PPFD) of 80 μmol m⁻² s⁻¹.

Seedlings were transplanted on 25 May 2014 and 18 June 2015 at the Simcoe Research Station, Ontario, Canada (lat. 42°51' N, long. 80°16' W, elevation 240.5 m) into a Norfolk sandy loam (coarse sand and sandy loam). Before transplanting seedlings to the field, phosphorus was added to the bottom of 15cm deep trenches at a rate of 50 kg ha⁻¹. Each plot was 6 m long and contained 35 plants spaced 30 cm apart. Rows were spaced 125 cm on centers. Guard rows were placed on both sides of each treatment plot. Seedlings were planted at the bottom of trenches and covered with 5 cm of soil. Weeds were mowed between rows and removed manually within rows. Trenches were gradually filled during the first growing season as plants were cultivated.

**Plot Sampling**

In 2015 and 2016, plots of one-year-old asparagus plants, planted in 2014 and 2015, respectively, were defoliated at monthly intervals (Table 1) by removing the fern at ground level. The experiment was monitored weekly for regrowth; spears were removed three times in 2015 and no emergence occurred in 2016. On each sampling date, crowns were dug mechanically,
cleaned of excess soil, and stored in burlap bags at 6°C until processing the following day. From the 35 crowns in each plot, five were chosen randomly for metabolite analysis, and five to determine water percentage. The remaining 25 crowns were used for $LT_{50}$ estimation.

$LT_{50}$ Estimation

Storage roots were trimmed to approximately 20 cm in length and crowns were potted in 3.785 litre pots with presoaked bark mix (70% aged pine bark fines, 25% peat moss, 5% compost; ASB Greenworld Ltd. Mount Elgin, ON). The 25 crowns from each treatment replicate were randomly distributed into four freezers. Each freezer contained 25 pots with six or seven from each of the four field replicates. Pots were incubated in each freezer at 4°C for 12 h, after which five random pots for each treatment were removed as controls. The temperature was lowered at a rate of 2°C h$^{-1}$ and maintained sequentially at 0°C, -4°C, -8°C, -12°C and -16°C, each for 12 h, after which four pots for each treatment were removed from each freezer. Pots were stored at 4°C for 4 wk to break dormancy, then transferred to the greenhouse and grown at 20°C/15°C (day/night) with a 14 h photoperiod supplemented by high pressure sodium lamps as described above. Regrowth was monitored for 4 wk and the numbers of living and dead plants were recorded to estimate $LT_{50}$ values.

Water Percentage

Ten to 15 storage roots sections approximately 7 cm in length, proximal to the rhizome, were sampled randomly throughout the crown; soil was removed with a dry cloth. The rhizome
was separated from storage roots and epidermal tissue removed. Approximately 15 g fresh weight (FW) for each of storage roots and rhizome were weighed separately to determine fresh weight, then dried at 50°C for 2 weeks. Dry weight (DW) was recorded and water percentage was calculated according to Tanino et al. (1990).

Metabolite Analysis

Storage roots and rhizomes were sampled as described for water percentage, frozen in liquid nitrogen, and stored at -80°C. Samples were lyophilized (FreeZone 4.5 L Freeze Dry System, Model 77510, LABCONCO; Kansas City, MO) and ground separately using a bench-top grinder (Blender Model 7011S, Waring, New Hartford, CT). Storage root powder was passed through a 60-mesh sieve to remove epidermal tissue. All samples were then stored at -80°C.

Chlorophyll concentration was estimated for cladophylls, and proline, nitrogen and low- and high-molecular weight fructan concentrations were measured for storage roots and rhizomes as described by Landry and Wolyn (2011). Sucrose was measured using a commercially available kit (K-SUCGL; Megazyme International Ireland, Bray, Ireland). Tissue samples (500 mg DW) were extracted using 50 mL distilled H₂O at 70°C for 30 min followed by filtering through a glass fibre filter paper (Whatman GF/A). From each extraction, 200 μL aliquots were added to each of four, 20 mL borosilicate test tubes. Two-hundred microliters of sodium acetate buffer were added to each of two duplicate test tubes for D-glucose determination, while 200 μL of supplied β-fructosidase were added to the remaining two duplicate test tubes for sucrose and D-glucose determination. All tubes were incubated at 50°C for 20 min after which 3 mL of
supplied glucose oxidase/peroxide reagent were added, and the tubes were incubated for 20 min at 50°C. Absorbance was measured at 510 nm with a spectrophotometer (DU-64; Beckman, Fullerton, CA). Sucrose concentration (g 100 g⁻¹) was calculated as: average absorbance of samples for sucrose and D-glucose determination – average absorbance of samples for D-glucose determination (both read against reagent blanks) x absorbance conversion factor (100/absorbance of 100 µg of D-glucose standard) x 0.0095 (K-SUCGL Assay Procedure; Megazyme International Ireland, Bray, Ireland).

**Experiment 2: Spring Vigor of Asparagus after Autumn Defoliation**

Guelph Millennium seedlings were grown and transplanted in 2014 and 2015 using the same method and dates as Experiment 1, except 20 plants were used per plot in a randomized complete block design with four treatments. In 2015, fern defoliation treatments occurred on 16 Aug., 14 Sep., 13 Oct., and 8 Nov. for plots planted in 2014. In 2016, fern defoliation treatments occurred on 14 Aug., 12 Sep., 16 Oct., and 7 Nov. for plots planted in 2015. Undefoliated rows on each side of a treatment row were controls. The Julian date on which the first emerged spear was 2.5 cm tall for each plant was recorded in 2016 and 2017 for the experiments defoliated in 2015 and 2016, respectively.

In the summer following fall defoliation, total plant yield for each treatment plot and undefoliated controls was estimated through a large-medium fern index (LMFI) which is highly correlated (r=0.91) with total harvested yield (Wolyn 1993). Diameters of all stalks were measured 2.5 cm above the ground and LMFI was calculated as: 3 x (number of large stalks with
a diameter > 15 mm) + 2 x (number of medium stalks with a diameter of 10 to 15 mm). LMFI was measured on 20 June 2016 and 4 July 2017. Undefoliated rows did not differ from those defoliated in mid-November and only the later are presented as controls.

**Statistical Analysis**

A generalized linear mixed model was used to analyze data with PROC GLIMMIX in SAS software (SAS Version 9.3; SAS Institute, Cary, NC). Treatment was a fixed effect and year, block(year) and year x treatment were random effects. Significance of random effects was determined by a likelihood ratio test. LMFI of undefoliated rows on each side of treatment plots were included as covariates in the yield analysis. Data were tested for a normal distribution with the Shapiro Wilks test, equality of variance with the Levene’s test, and random distribution of residuals using residual plots. Tukey's honest significant difference test was used for separation of least square means ($P \leq 0.05$). The PROC PROBIT function of SAS was used to determine $LT_{50}$ of freezing treatments and $E_{50}$, the day 50% of plants in a plot had emerged.

**RESULTS**

**Statistical Analysis**

The year and year x treatment effects were significant for all parameters. Therefore, data were
analyzed separately by year.

**Temperature and Precipitation**

Both years of the experiment were warmer than the 30-year average (data not shown). Fall temperatures were greater in 2016 than in 2015; consistent daytime temperatures of 15°C occurred in late-September 2015, while similar temperatures were observed in early-October 2016. Soil temperatures followed similar trends to those of air temperatures, where values for 2016 were higher than those of 2015. The frequency and intensity of rainfall during the growing season was lower in 2016 compared to 2015; cumulative rainfall for 2016 was 60% of that for 2015 (data not shown).

**LT<sub>50</sub>**

LT<sub>50</sub> values of undefoliated controls decreased from mid-August to mid-November in 2015 (Fig. 1a). Levels also decreased from mid-October to mid-November for plants defoliated in mid-August and mid-September, however, those for the mid-August treatment were greater than LT<sub>50</sub> values for the undefoliated control. October defoliation decreased LT<sub>50</sub> compared to the control when sampled in mid-November. Overall, defoliation in mid-August had the greatest effect, increasing LT<sub>50</sub> and decreasing freezing tolerance.

In 2016, LT<sub>50</sub> of the undefoliated controls did not change from mid-August to mid-October, but levels decreased from mid-October to mid-November for the control and plants
defoliated in mid-August and mid-September (Fig. 1b). On the November sampling dates, controls had greater LT$_{50}$ values, decreased freezing tolerance, compared to all defoliation treatments, a pattern opposite to that observed in 2015.

**Water Percentage**

Water percentage of the rhizome decreased over the sampling period for all treatments in both 2015 and 2016 (Figs. 2a and b). Values did not differ among treatments on each sampling date in 2015; a similar trend was observed in 2016 except water percentage for the mid-August defoliation treatment, sampled in mid-September, did differ from that of the control.

Storage root water percentage of undefoliated controls decreased between mid-August and mid-November in both years (Figs. 2c and d). Defoliation in mid-August prevented dehydration of storage roots; values did not change from mid-September to mid-November and were greater than those of other treatments on each sampling date. Removal of fern in mid-September and mid-October had little or no effect; values for plants in subsequent months generally did not differ from those of the undefoliated controls. Overall, only defoliation in mid-August affected water percentage or dehydration and the effect was limited to the storage roots.

**Sucrose**

In 2015, rhizome sucrose concentrations generally increased throughout the sampling period for the different treatments (Fig. 3a). On each sampling date, defoliation treatments did
not differ from the control. In 2016, rhizome sucrose concentrations of undefoliated control plants did not change from mid-August to mid-October, but increased from mid-October to mid-November, as did those for plants defoliated in mid-August and mid-September (Fig. 3b). For the October and November sampling dates, values for the defoliation treatments were greater than those of the undefoliated controls.

Sucrose concentrations increased in the storage roots of undefoliated control plants during the sampling period in both years (Fig. 3c and d). All treatments did not differ on each sampling date in both years except that for plants defoliated in mid-August and sampled in mid-November, where the value was greater than those for all other treatments.

**Proline**

Rhizome proline concentrations increased in undefoliated control plants throughout the sampling periods in both 2015 and 2016 (Figs. 4a and b). Although proline concentrations of plants defoliated in mid-August increased from mid-September to mid-November, values were lower than those of the undefoliated control on most sampling dates. Defoliation of the fern in mid-September delayed rhizome proline accumulation, values were lower than, and similar to, those of the controls in mid-October and mid-November, respectively. Mid-October defoliation had no effect on proline levels when plants where assessed in mid-November.

Storage root proline concentrations increased throughout the fall sampling period in undefoliated control plants in both years (Figs. 4c and d) although levels varied one- and three-
fold in 2015 and 2016, respectively. Levels for the controls also increased substantially between mid-October and mid-November in 2016, but no change was observed during the same period in 2015. Defoliation had no effect on proline concentrations in 2015, however, plants from all defoliation treatments had lower proline concentrations than the undefoliated control at the mid-November sampling date in 2016.

**Nitrogen Percentage**

In 2015 and 2016, rhizome total nitrogen percentage of undefoliated plants increased from mid-August to mid-October (Figs. 5a and b). Values for plants defoliated in mid-August and mid-September did not increase throughout the subsequent sampling periods. Nitrogen percentage was generally lower on most sampling dates for defoliation treatments compared to the control and the magnitude of the decrease appeared associated with earliness of defoliation.

Storage root nitrogen percentage did not change in undefoliated control plants throughout the sampling periods in both years (Figs. 5c and d). Removal of the fern in mid-August caused an increase in storage root nitrogen percentage compared to the control at most subsequent sampling dates in both years. Values for plants defoliated in mid-September and mid-October did not differ from those of the undefoliated controls at subsequent sampling dates.

**Low-Molecular-Weight Fructan**

Rhizome LF concentrations for undefoliated controls increased or did not change from
mid-August to mid-September in 2015 and 2016, respectively (Figs. 6a and b). In both years, values decreased from mid-September to mid-November. For plants defoliated mid-August, LF concentrations were lower than those of the controls at most subsequent sampling dates in both years; values also did not change from mid-September to mid-November. Defoliation in mid-September resulted in different responses for the two years. In 2015, LF concentrations were similar to, and greater than, those of the undefoliated control in mid-October and mid-November, respectively; values were similar to those of the undefoliated control on both dates in 2016. Plants defoliated in mid-October had greater rhizome LF concentrations compared to the control in mid-November of 2016.

Storage root LF concentrations of control plants did not change from mid-August to mid-November in 2015 but decreased in 2016 (Figs. 6c and d). LF concentrations for plants defoliated in mid-August were lower than those of the control at most subsequent sampling dates in both years; values also decreased from mid-September to mid-October in both years but not subsequently. For plants defoliated in mid-September, LF concentrations did not differ from those of the control at subsequent sampling dates. Mid-October defoliation did not affect LF concentrations in mid-November for both years.

High-Molecular-Weight Fructan

In 2015, rhizome HF concentrations of control plants increased from mid-August to mid-September then decreased to mid-November (Fig. 7a). In 2016, values for control plants decreased from mid-August to mid-September but did not change thereafter (Fig. 7b). Plants
defoliated in mid-August had lower HF concentrations than the controls at most subsequent dates in both years. Plants defoliated in mid-September and mid-October did not differ for HF concentrations compared to the control on any sampling date.

Storage root HF concentrations in control plants increased from mid-August to mid-October in 2015 but remained constant in 2016 (Figs. 7c and d). Early removal of fern generally decreased storage root HF concentrations; mid-August and mid-September treatments had the lowest and intermediate values, respectively, compared to the controls on most subsequent sampling dates in both years. In mid-November, values for plants defoliated in mid-October did not differ from those of the control.

**Chlorophyll**

Cladophyll chlorophyll concentrations decreased throughout the sampling period in both years (data not shown). In 2016, ferns were fully senesced by the mid-November sampling date, with no cladophylls remaining on stems for sampling.

**Spring Emergence and Yield Estimates**

Defoliation in mid-August delayed spring emergence compared to the other defoliation treatments for both years of the experiment (Figs. 8a and b). The control and plants defoliated in mid-September and mid-October of 2015 had similar emergence in the spring of 2016 (Fig. 8a). Plants defoliated in mid-October 2016 and the control, emerged the earliest in 2017 and those
defoliated mid-September had an intermediate emergence date (Fig. 8b).

Timing of defoliation in the fall affected LMFI yield estimates the following spring for both years of the experiment (Figs. 8c and d). Similar trends were seen, where defoliation in mid-August had the greatest effect on yield reduction, defoliation in mid-September and mid-October had intermediate effects, and the control generally produced the highest yields. Yield estimates for plots evaluated in 2016 appeared greater than those for 2017.

**DISCUSSION**

Autumn defoliation of asparagus impacted the acquisition of freezing tolerance, crown metabolite levels, spring emergence and yield. Defoliation had different effects on LT$_{50}$ in the two years of the experiment; in 2015 defoliation increased values, or decreased freezing tolerance, while an opposite effect was observed in 2016. In both years, rhizome proline concentration, and storage root LF and HF concentrations were reduced consistently compared to controls on subsequent sampling dates after mid-August defoliation. Increased sucrose concentration after defoliation was associated with improved freezing tolerance in 2016. Some treatments also delayed emergence and reduced yield in the subsequent season.

The discrepancy between years for response to defoliation, decreasing and increasing freezing tolerance in 2015 and 2016, respectively, may be related to varying growing conditions. The climate of 2016 likely stressed plants with drought, while the 2015 had adequate rainfall. Since both freezing and drought tolerance share common physiological processes (Shinozaki and
Yamaguchi-Shinozaki 1996), defoliation, drought stress, and cold acclimation may have interacted to produce the results observed in 2016. Notably, levels of proline, a compound associated with both freezing and drought tolerance (Verslues et al. 2006) appeared greater in 2016 than 2015.

In 2015, mid-August defoliation increased LT$_{50}$ values and freezing tolerance decreased. The effect was associated with decreased concentrations of many cryoprotective compounds, including proline, LF and HF, and increased water percentage which are consistent with previous studies in asparagus (Landry and Wolyn 2011, Kim and Wolyn 2015, Panjtandoust and Wolyn 2016a). Although mid-September defoliation did not affect LT$_{50}$ values in 2015, levels for many of these compounds decreased compared to controls on specific sampling dates and were intermediate between values for controls and the mid-August defoliation treatment, suggesting concentrations for some cryoprotective agents may have been above a critical threshold in controls to impart freezing tolerance on the plants. Autumn defoliation also decreased freezing tolerance in alfalfa (Haagenson et al. 2003); protein and starch concentrations were reduced, however, those of cryoprotective root sugars increased. Interpretation of these results are confounded by the normal conversion of starch to sugars. In a separate study, fall harvest of alfalfa did reduce sugars, as well as amino acids and soluble proteins (Dhont et al. 2004).

Temporal patterns and concentrations of cryoprotective metabolites during the sampling period were generally similar between years for defoliation treatments, with decreased levels of compounds often proportional to the earliness of defoliation. The increased freezing tolerance from defoliation in 2016 was inconsistent with most metabolic profiles except sucrose.
concentration in the rhizome, where levels for defoliation treatments were greater than those of the control only in 2016. Since LT_{50} levels were most correlated with rhizome traits in past asparagus studies (Panjtandoust and Wolyn 2016a, 2016b) the increased defoliation-induced sucrose concentrations may be responsible for the unexpected enhancement of freezing tolerance.

Water percentage of both rhizomes and storage roots decreased in control plants acclimated during the fall, consistent with previous results in asparagus (Panjtandoust and Wolyn 2016a) and other crops (Fowler and Carles 1979, Clifton-Brown and Lewandowski 2000). For plants defoliated in mid-August, rhizome water percentage decreased from mid-September to mid-November, however, values for storage roots did not change. Failure of the storage roots to dehydrate is likely related to the lack of translocated carbohydrates and decreasing fructan concentrations from metabolism prior to dormancy. In bromegrass, the amount of water in cells (mg per million cells) did not change with cold acclimation, rather water percentage decreased from dry matter accumulation. (Tanino et al. 1990). Decreasing water percentage for rhizome tissue in defoliated asparagus could be explained by metabolite translocation from storage roots. Both proline and sucrose concentrations increased in rhizomes of defoliated plants during the autumn and could have contributed to decreasing water percentage. Interestingly, both HF and LF concentrations did not change in rhizomes to affect the trait.

Rhizome proline concentrations increased during the sampling period in both control plants and those defoliated in mid-August and mid-September. Levels for defoliated plants, however, were lower than those of the control, and those for the mid-September treatment were
intermediate, between values for controls and the mid-August treatment. The presence of fern appears necessary to optimize proline concentration, however, increasing values during the autumn in the absence of fern suggests the crown alone is sensing decreasing soil temperatures and acclimating to some level independent of above-ground conditions.

For plants defoliated in mid-August, rhizome nitrogen percentages on subsequent sampling dates were lower than values for the control. The differences resulted from the percentage remaining constant in the treatment and increasing in the control. In storage roots, nitrogen percentage was greater for the mid-August defoliation treatment on subsequent dates compared to the control, however, levels for each treatment did not change during the sampling period. Decreased nitrogen percentage observed in the rhizome was expected since the removed fern cannot translocate this element to the crown. Increased percentage in storage roots may be a result of decreased fructan levels, altering the root dry weight and indirectly increasing levels. Although fructan concentrations also decreased in rhizomes from defoliation, values were lower than those in the storage roots and probably had a minimal influence on dry weight. Decreasing root nitrogen percentage has also been observed with defoliation in alfalfa (Dhont et al. 2006).

Mid-August and mid-September defoliation in both years and the mid-October treatment in 2016 had a negative effect on yield estimates in the following season. These observations are supported by decreases in fructans, the most abundant storage carbohydrate in the crown (Wilson et al. 2001), and most notably losses of HF in the storage roots. Mid-August defoliation also delayed spear emergence the following spring in both years, while the mid-September treatment had an effect only in 2016. In Michigan, spring yields of asparagus following autumn
defoliation in late-September were lower than those of the control, but a mid-October treatment had no effect (Kelly and Bai 1997). Differences with results reported here could be explained by studies conducted on mature plants in Michigan, established for 5 yr, compared to defoliating plants in the second growing season in this experiment. In alfalfa, a third fall defoliation in early-September, late-September or mid-October also reduced the yield of the first harvest the following year compared to that which was harvested twice (Dhont et al. 2004).

Overall, growing season affected the acquisition of freezing tolerance in response to defoliation during fall acclimation in asparagus. In 2015, mid-August defoliation decreased freezing tolerance and treatments in mid-September and -October had no effect; LT\textsubscript{50} levels were generally consistent with concentrations of several cryoprotective metabolites. With warm and dry conditions in 2016, defoliation in mid-August, -September and -October increased freezing tolerance, however, only changes in rhizome sucrose concentration supported observations. All defoliation treatments generally decreased yield the subsequent season which was associated mostly with diminished HF in the storage roots. Since mid-September and mid-October defoliation treatments did not affect freezing tolerance in 2015 when climate likely did not stress plants, the perception of signals to induce fall acclimation may have occurred late in the summer such that fern was not required thereafter. The ability of crowns defoliated in mid-August to increase proline concentration in subsequent months, suggests the plant could also be acclimating partially through the sensing of soil temperatures in the absence of fern. Further research to study the interaction of cold acclimation and drought stress is warranted.

Although the effects of defoliation to decrease freezing tolerance were minimal, loss of yield the following season was observed and correlated with the timing of fern removal.
Defoliation before complete senescence in order to harvest seed, minimize disease inoculum for the subsequent seasons, or implement certain cultural practices will likely reduce crown vigor that, with repeated practice, could compromise the profitability and longevity of a plantation.

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REFERENCES


concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 45: 523-539.


Table 1. Fern defoliation and crown sampling treatments of two-year-old asparagus plants in Simcoe, Ontario.

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<td>7</td>
<td>Mid-August</td>
<td>Mid-November&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>Mid-September</td>
<td>Mid-October</td>
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<tr>
<td>9</td>
<td>Mid-September</td>
<td>Mid-November&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>Mid-October</td>
<td>Mid-November&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup>Two replicates were harvested in each of first and second weeks of November and data averaged over the two November sampling dates.
LIST OF FIGURES

**Fig. 1.** Lethal temperatures at which 50% of plants die (LT$_{50}$) when asparagus was defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a) and 2016 (b). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.

**Fig. 2.** Water percentages of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.

**Fig. 3.** Sucrose concentrations of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.
Fig. 4. Proline concentrations of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.

Fig. 5. Nitrogen percentages of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.

Fig. 6. Low-molecular-weight fructan (LF) concentrations of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.
Fig. 7. High-molecular-weight fructan (HF) concentrations of asparagus rhizomes (a, b) and storage roots (c, d) when plants were defoliated in mid-August, -September and -October and sampled in all subsequent months until mid-November, and undefoliated controls sampled monthly from mid-August to mid-November, in 2015 (a, c) and 2016 (b, d). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.

Fig. 8. The Julian Day 50% of plants in a plot emerged ($E_{50}$) (a, b) and Large-Medium Fern Index (LMFI) of asparagus plots (c, d) measured June 2016 (a, c) and June 2017 (b, d) after defoliation in mid-August, -September, or -October of previous year. LMFI was calculated as: 3 x (number of large stalks with a diameter > 15mm) + 2 x (number of medium stalks with a diameter > 10mm, but < 15 mm). Means ± standard errors (n=4) are presented. Letters represent significant differences as determined by Tukey’s Honest Significant Difference test (P≤0.05) within each sub-figure.
Figure 1.
Figure 2.
Figure 3.
Asparagus Fall Defoliation Affects Freezing Tolerance

Nolet and Wolyn

Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.