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Effects of xylem cavitation and freezing injury on dieback of yellow birch (*Betula alleghaniensis*) in relation to a simulated winter thaw

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Summary Shoot dieback, shoot growth, stem xylem cavitation, stem and root freezing injury, and root pressure were measured in 2-year-old, cold-hardened, potted yellow birch (*Betula alleghaniensis* Britt.) seedlings that had been subjected to a simulated winter thaw for 0, 5, 10, 19 or 27 days followed by 10 weeks at –10 °C. Stem xylem cavitation was determined as percent loss of hydraulic conductivity. Stem freezing injury was measured as electrolyte leakage (EL). Root freezing injury was determined by EL and by triphenyl tetrazolium chloride (TTC) reduction. Thaw duration was significantly correlated with dieback, new shoot growth, stem xylem cavitation, stem and root freezing damage, and root pressure ($P < 0.05$). In particular, shoot dieback was positively correlated with stem xylem cavitation ($P < 0.001$), residual stem xylem cavitation ($P < 0.01$) and root freezing injury ($P < 0.010$), but only weakly correlated with stem freezing damage ($P < 0.05$). In roots, freezing damage was negatively correlated with root pressure ($P < 0.05$), which, in turn, was negatively correlated with residual stem xylem cavitation after root pressure development. In stems, there was no correlation between freezing damage and xylem cavitation. We conclude that long periods of winter thaw followed by freezing resulted in freezing injury to roots concomitant with a reduction in root pressures, leading to poor recovery from freezing-induced xylem embolism.

Keywords: dieback, new shoot growth, residual xylem embolism, thaw duration, stem xylem cavitation.

Introduction

Widespread decline of birch exhibited as branch dieback and mortality, was first recorded during the 1930s in central and southern New Brunswick, Canada. Since then, severe dieback of the species has been noted from the Maritime region (Pomerleau 1953) to eastern Ontario (Sinclair 1952, Walker et al. 1990). Birch decline has led to a 19% loss of the 368×10^6 m^3 growing stock of yellow birch (*Betula alleghaniensis* Britt.) in North America (Ward and Stephens 1997).

The primary cause of birch dieback is not fully understood. Pathologists (Hansborough and Stout 1947) and entomologists (Balch and Prebble 1940) have suggested that it is a physiological response to periods of unfavorable weather. Braathe (1957, 1995) and Auclair et al. (1996) postulated that winter freeze–thaw was the factor underlying birch dieback. Tolerance to winter injury is a well-known determinant of the geographic distribution of a plant species (Sakai and Larcher 1987). However, xylem cavitation may also play an important role in dieback because of the effects of irreversible cavitation on root-to-shoot water transport as the growing season commences. Xylem cavitation, defined as percent loss of hydraulic conductivity (PLC), is the process by which xylem vessels become embolized (air-filled), resulting in reduced water transport capability of the xylem. Two environmental conditions are primarily responsible for xylem cavitation: freeze–thaw cycles and water stress. In field-grown American beech (*Fagus grandifolia* Rhrh.), xylem embolism accumulates over winter and may remain through June, resulting in considerable dieback (Sperry 1993). Furthermore, injection-induced cavitation of water birch (*Betula occidentalis* Hook) was associated with considerable dieback (Sperry and Pockman 1993). In potted paper birch (*Betula papyrifera* Marsh.), correlations among branch dieback, relative xylem conductivity and thaw duration have been observed (Cox and Malcolm 1997). Intensive cavitation over winter has been reported in *Betula papyrifera* var. *cordifolia* (Regel) Fern. (Sperry 1993) and *Betula occidentalis* (Sperry et al. 1994).

For most diffuse-porous trees such as birch, winter-induced xylem cavitation is sometimes reversed by positive root pressure in spring. However, lack of complete refilling may occur if tree roots are injured. Birch root systems are generally shallow and thus susceptible to thaw–refreeze-induced injuries. Such injuries are particularly frequent when snow cover is temporarily lost as a result of thaws during winter. Re-freezing of potentially dehardened roots could lead to weak root pressure development during the following spring. The objectives of our study were to test four hypotheses: (1) thaw duration affects winter xylem cavitation in yellow birch; (2) thaw duration influences the extent of root injury during refreezing; (3) extent of root injury affects root pressure the following spring; and (4) residual stem xylem embolism contributes to twig dieback.

Materials and methods

Plant material

In the spring of 1996, yellow birch seed (collected between 46°17′ N and 63°8′ W) was sown in containers filled with a 3:1 (v/v) mix of fertilized peat and vermiculite at the Canadian Forest Service nursery in Fredericton (45°52′ N and 66°32′ W). Each container comprised 96 cavities, and the volume of each cavity was 105 cm^3. After germination, seedlings were grown for a month in a greenhouse, then outdoors under shade (50%). In early November the seedlings were removed from the containers, placed in plastic bags, sealed in cardboard boxes and stored in a freezer at –3 °C. In late May 1997, the seedlings were planted in 5-liter pots containing a 2:1:1 (v/v) mix of peat, soil and vermiculite and grown outdoors over the summer. The potted seedlings were set in the ground in early November 1997 and received a natural chilling treatment (0 to –30 °C) until the initiation of the thaw experiment in early February 1998. During the period, freezing air temperature varied from 0 to –30 °C, whereas soil temperature remained relatively stable around the freezing point.

Experimental design of thaw treatment

Five environmental-control growth chambers (ECGC) were used for the simulated thaw treatment that involved a randomized block design with 5 blocks (chambers) × 5 plots (duration of thaw) × 4 plants. Thaw treatments lasted for 0, 5, 10, 19, and 27 days, with thaw temperature varying between 2 and 17 °C in a diurnal fluctuation (Figure 1). To minimize water loss from stems during the treatment, all ECGCs were controlled at 90% relative humidity with a 9-h photoperiod. Buds were examined daily. It was observed that 27 days of thaw caused the buds to swell; however, bud burst did not occur until later.

Induction of stem xylem cavitation and freezing injury

After each thaw period, each pot was immediately placed inside an insulated polystyrene foam box, and moved to a freezer at –10 °C. The foam box insulated the pot, thereby allowing gradual re-freezing of the roots with a temperature drop of 1.08 and 0.44 °C h^{-1} for pre- and post-phase change, respectively. The control plants (Day 0 of thaw) were moved directly into the freezer from outside.

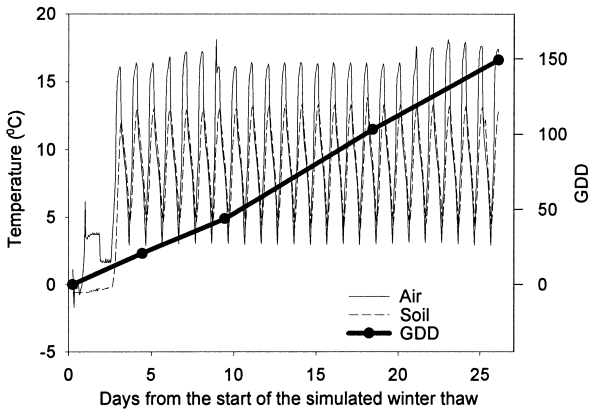

Figure 1. Mean hourly air and soil temperatures in potted yellow birch during simulated winter thaw. The five thaw regimes are presented as growing degree days above 4 °C, GDD.

In the freezer, xylem cavitation was regularly examined in additional seedlings that had been treated with 27 days of thaw. When freeze-induced xylem cavitation reached a relatively high level (> 80% PLC) after ten weeks at –10°C, five plants (one from each block) were sampled to evaluate xylem cavitation and freezing injury. Freezing injury to roots was measured after pots were thawed for 24 h at room temperature (21 °C). Both stem xylem cavitation and electrolyte leakage (EL) were measured in stem segments from the top, middle, and base of each main stem. Stem xylem cavitation was determined according to Sperry et al. (1988). Briefly, initial hydraulic conductivity was determined by mass flow rate (kg s^{-1}, with 10 mM HCl as the hydraulic solution) under low pressure (7 kPa) through 0.15-m stem segments after the frozen stem segments were thawed for 4 h under tap water. Maximum hydraulic conductivity of the same stem segments was measured following a series of high-pressure flushes (100–150 kPa). Xylem cavitation was calculated as percent loss of hydraulic conductivity (PLC). Stem electrolyte leakage (EL) was determined concurrently with cavitation, on adjacent segments. The segments were cut into 0.5- to 1-cm-long pieces and placed in test tubes containing 20 ml of deionized distilled water for 24 h before measuring electrical conductivity of the water (CDM 83 Conductivity Meter, Radiometer Copenhagen). The segments were then boiled for 30 min and allowed to cool at room temperature before electrical conductivity was remeasured. For each segment, electrolyte leakage was expressed as the ratio of electrical conductivity of the water before and after boiling (Ritchie 1991). Root electrolyte leakage was similarly measured in three samples (approximately 0.2 g in dry weight) per root system of five replicate plants of each treatment. Root triphenyl tetrazolium chloride (TTC) reduction was also determined in samples similar to those used for EL measurement after incubation for 18 h at 36 °C (Lassheikki et al. 1991). A unit of TTC reduction was defined as light absorption of extracted solution at 525 nm per gram dry weight.

To follow dieback, growth and residual xylem embolism, the remaining treated plants (75 in all) were warmed for 1 day from 2 to 20 °C and then grown in a greenhouse providing an air temperature of 18–22 °C, a 13-h photoperiod, and 85% relative humidity.

Measurement of root pressure

Once plants were moved to the greenhouse, five per thaw treatment (one per replicate block) were immediately fitted with bubble manometers (Sperry 1993). Root pressure was estimated as described by Fisher et al. (1997):

$$PV = nRT, \quad (1)$$

where P is pressure (Pa) of the air column in the graduated glass tube, n is the amount of air in the column (mol), R is mo-

lar gas constant (8.3143 m^3 J mol^{-1} K^{-1}), T is temperature (K) of the air column, and V is the volume of the air column (m^3), which can be expressed as:

$$V = \pi r^2 L, \quad (2)$$

where r and L represent the inner radius of the glass tube (m) and the length of the air column (m), respectively. Because T in Equation 1 was held constant during the measurement, it follows that:

$$P_1 L_1 = P_2 L_2, \quad (3)$$

where P_1 is the pressure of xylem sap, P_2 represents the current atmospheric pressure, and L_1 and L_2 represent the lengths of air column under the pressures of xylem sap and atmosphere, respectively. Heights of cut ends above soil were noted to subtract the gravitational pressure when calculating root pressure. In the greenhouse, the air column lengths of manometers were recorded daily until the pressure declined. This occurred shortly after bud burst and subsequent leaf spreading.

Dieback and residual cavitation

After 30 days in the greenhouse, percent length of dieback in shoots and lengths of new growth were scored in five replicate plants per thaw treatment. The plants were then harvested to evaluate residual stem xylem cavitation, defined here as the stem xylem cavitation remaining after root pressure development and leaf expansion.

Statistical analysis

Treatment means were compared by one-way ANOVA. Correlations among dieback, xylem cavitation, stem and root injury, and root pressure were examined. Before statistical analysis, all percent and proportional data were arc sine transformed, and the length data for new shoot growth was log transformed. Statistical analyses were performed with SAS software (1994; SAS Institute, Cary, NC).

Results

Stem freezing injury and xylem cavitation

Significant shoot injury (EL) was observed in the top segment of the main stem as thaw duration increased ($P < 0.05$, Figure 2). Shoot damage exceeded 20% (relative to that of the control treatment) in response to 100 growing degree days (GDD). No further damage occurred as the thaw progressed to the maximum 150 GDD. Shoot EL in the middle and base segments showed no differences in response to thaw treatments, with values from 25 to 28% (Figure 2).

Stem xylem cavitation increased significantly ($P < 0.05$) with thaw duration, both in the top and middle segments (Figure 3a). Following 100 GDD of thaw, xylem cavitation reached 98% PLC. There was no significant correlation between stem injury and xylem cavitation for the top, middle, and base stem segments (Table 1). Residual stem xylem embolism (RPLC) after root pressure development and xylem refilling was significantly correlated with root pressure ($P < 0.05$) in all segments of the main stem (Figure 3b, Table 1).

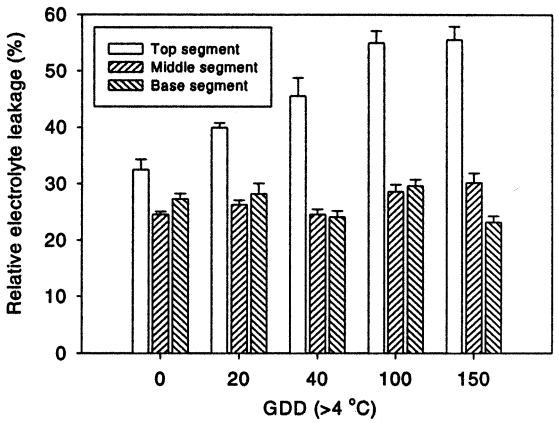

Figure 2. Response of stem electrolyte leakage in yellow birch to simulated winter thaws (represented as growing degree days above 4 °C, GDD) followed by 10 weeks of storage at –10 °C (Values are means ± 1 SE, $n = 5$). Treatment effects were only significant for the top stem segment ($P = 0.0158$, $F = 3.97$).

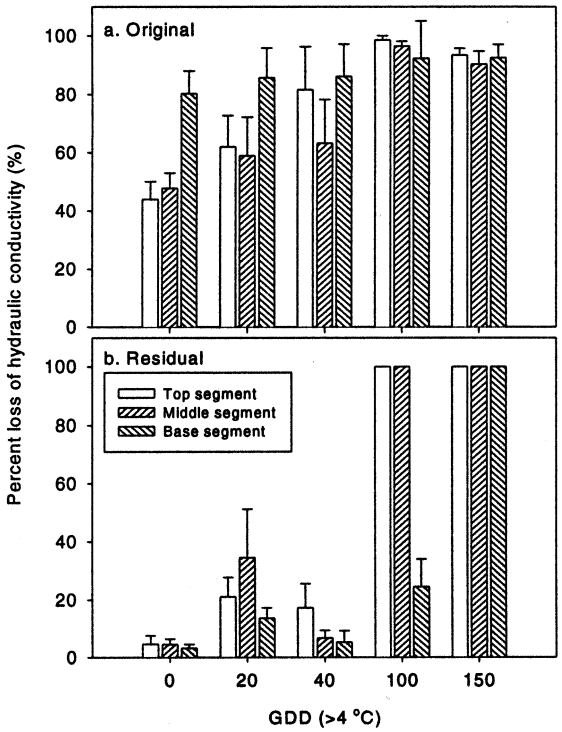

Figure 3. Comparison of stem xylem cavitation before and after root pressure development. (a) Original cavitation (PLC) was determined on stems harvested immediately after plants had been exposed to simulated winter thaws followed by 10 weeks at –10 °C. (b) Residual cavitation (RPLC) was measured on plants in the same treatments after they had been grown for 30 days in a greenhouse. Values are means ± 1 SE, $n = 5$.

Table 1. Correlation coefficients (r) and probability values (P, second line) between thaw duration, root and stem freezing injury, stem xylem cavitation and branch dieback (n = 25). The proportional and new shoot growth data were arc sine and log transformed, respectively. Values in boldface = $P < 0.05$.

	GDD[1]	DIEb	NEWs	PLCb	PLCm	PLCt	RPLCt	RPLCm	RPLCb	ELb	ELm	ELt	ELr	TTCr	PREr
GDD	1.0000	0.8380	−0.7936	0.1124	0.7415	0.6657	0.9059	0.9119	0.8117	−0.1374	0.3413	0.5726	0.8724	−0.7906	−0.6030
	0.0000	**0.0001**	**0.0001**	0.5929	**0.0001**	**0.0003**	**0.0001**	**0.0001**	**0.0001**	0.5127	0.0949	**0.0028**	**0.0001**	**0.0001**	**0.0014**
DIEb		1.0000	−0.7875	0.0502	0.6726	0.6298	0.7060	0.7281	0.5721	−0.2633	0.1668	0.4976	0.5962	−0.6833	−0.3968
		0.0000	**0.0001**	0.8118	**0.0002**	**0.0007**	**0.0001**	**0.0001**	**0.0028**	0.2035	0.4256	**0.0114**	**0.0017**	**0.0002**	**0.0496**
NEWs			1.0000	−0.3568	−0.6440	−0.576	−0.8078	−0.7845	−0.6764	0.2566	−0.4030	−0.5309	−0.7520	0.7989	0.4867
			0.0000	0.08000	**0.0005**	**0.0027**	**0.0001**	**0.0001**	**0.0002**	0.2157	**0.0458**	**0.0063**	**0.0001**	**0.0001**	**0.0136**
PLCb				1.0000	0.0182	−0.3196	0.0246	0.1211	0.1078	−0.2047	−0.0875	0.3405	0.2385	−0.1349	−0.2204
				0.0000	0.9313	0.1194	0.9071	0.5643	0.6079	0.3263	0.6774	0.0958	0.2510	0.5201	0.2897
PLCm					1.0000	0.5483	0.7427	0.7475	0.5509	−0.0681	0.1943	0.4076	0.5831	−0.6813	−0.4869
					0.0000	**0.0045**	**0.0001**	**0.0001**	**0.0043**	0.7463	0.3521	**0.0431**	**0.0022**	**0.0002**	**0.0136**
PLCt						1.0000	0.6812	0.5368	0.3129	0.0795	0.1177	0.3910	0.4886	−0.4946	−0.3013
						0.0000	**0.0002**	**0.0057**	0.1277	0.7057	0.5752	0.0533	**0.0132**	**0.0120**	0.1432
RPLCt							1.0000	0.9331	0.6495	0.0501	0.3290	0.5259	0.7700	−0.8455	−0.5271
							0.0000	**0.0001**	**0.0004**	0.8120	0.1083	**0.0069**	**0.0001**	**0.0001**	**0.0068**
RPLCm								1.0000	0.6962	0.0158	0.4180	0.5678	0.8242	−0.7866	−0.6039
								0.0000	**0.0001**	0.9402	**0.0376**	**0.0031**	**0.0001**	**0.0001**	**0.0014**
RPLCb									1.0000	−0.3001	0.3327	0.2646	0.7926	−0.5701	−0.4740
									0.0000	0.1450	0.1041	0.2012	**0.0001**	**0.0029**	**0.0167**
ELb										1.0000	0.2571	0.1398	−0.1604	0.0711	−0.0561
										0.0000	0.2147	0.5051	0.4438	0.7355	0.7899
ELm											1.0000	0.4354	0.4032	−0.1223	−0.2661
											0.0000	**0.0296**	**0.0456**	0.5604	0.1986
ELt												1.0000	0.5212	−0.5015	−0.5612
												0.0000	**0.0075**	**0.0107**	**0.0035**
ELr													1.0000	−0.6265	−0.5673
													0.0000	**0.0008**	**0.0031**
TTCr														1.0000	0.4758
														0.0000	**0.0162**
PREr															1.0000
															0.0000

[1] Abbreviations: GDD = growing degree days above 4 °C; DIEb = percent length of dieback in a single seedling; NEWs = length of new shoot growth; PLCb = percent loss of hydraulic conductivity in the base segment of main stem; PLCm = percent loss of hydraulic conductivity in the middle segment of main stem; PLCt = percent loss of hydraulic conductivity in the top segment of main stem; RPLCt = residual percent loss of hydraulic conductivity in the top segment of main stem; RPLCm = residual percent loss of hydraulic conductivity in the middle segment of main stem; RPLCb = residual percent loss of hydraulic conductivity in the base segment of main stem; ELb = electrolyte leakage in the base segment of main stem; ELm = electrolyte leakage in the middle segment of main stem; ELt = electrolyte leakage in the top segment of main stem; ELr = electrolyte leakage in root; TTCr = TTC reduction in roots; PREr = root pressure.

Root freezing injury, root pressure

Root EL increased significantly after a threshold of 40 GDD, rising to 32%, on average, following 150 GDD ($P < 0.05$, Figure 4a). Reduction of TTC was also significantly influenced by the thaw treatments ($P < 0.05$, Figure 4a), declining from 7.6 to 1.8 light-absorption units with increasing thaw duration. In roots, TTC reduction was significantly positively correlated with root pressure, whereas root EL was negatively correlated with root pressure ($P < 0.05$, Table 1).

Despite large variation in maximum recorded root pressures, a significant downward trend was observed with thaw duration ($P < 0.05$, Figure 4b). In particular, mean maximum daily root pressure reached about 16 kPa. This value did not drop significantly when thaw duration was less than 43 GDD (Figure 4b). However, root pressure decreased to about 5 kPa following 100 GDD of thaw. After 150 GDD of thaw, root pressure dropped to 1.0 kPa.

Dieback and new shoots

As thaw duration increased, percent length of dieback significantly increased, whereas the length of new shoots significantly decreased ($P < 0.01$, Figure 5). Dieback was correlated with root damage, measured either as electrolyte leakage or TTC reduction ($P < 0.01$, Table 1). Stem electrolyte leakage in the top segments also correlated with dieback ($P < 0.05$), as did root pressure ($P < 0.05$) and residual stem xylem embolism in the top ($P < 0.01$), middle, and base segments ($P < 0.01$) of the main stems. There were also correlations of new shoot length with residual stem xylem embolism ($P < 0.01$), root damage ($P < 0.05$), root pressure ($P < 0.05$) and stem

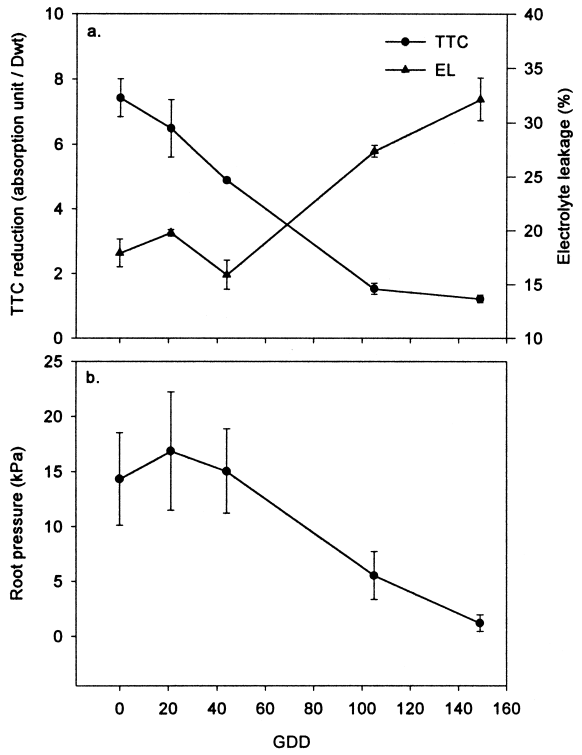

Figure 4. Responses of (a) root electrolyte leakage (EL), TTC reduction and (b) root pressure to simulated winter thaws (GDD > 4°C) and a subsequent 10 weeks at –10 °C. Values are means ± 1 SE, $n = 5$.

freezing damage of the top segment ($P < 0.01$, Table 1).

Discussion

Thaw–freezing treatment and tissue injury

The relationships of root electrolyte leakage (EL) and TTC reduction with thaw duration shown in Figure 4a may reflect root dehardening. During a thaw, significant depletion of soluble carbohydrates occurs as a result of respiration (van den Driessche 1979, Ögren 1996), resulting in less protection of cells and plasma membranes by sugars (Santarius 1982, Steponkus 1984), thus making root and shoot tissues more vulnerable to subsequent low temperatures. Electrolyte leakage is considered to be a more reliable indicator of the critical temperature when significant freezing injury occurs than the TTC method (DeHayes and Williams 1989, Strimbeck et al. 1995). In our experiment, freezing injury of roots was unlikely to be associated with rate of cooling following the thaw treatment, because the rates of soil temperature decline were 1.08 and 0.44 °C h^{-1} for pre-and post-phase change, respectively. These rates are much lower than those used in artificial freezing tests, i.e., 2–3 °C h^{-1} (Calmé et al. 1994, 1995).

Stem injury (measured as EL) was observed in the top segments of the stem after the thaw treatments and 10 weeks at –10 °C but not in the other parts of the main stem (Figure 2). Lack of injury in the stem as a whole may be a consequence of slow dehardening at mild air temperatures. The top stem segment comprises large amounts of parenchyma that have a high water content, and hence are more prone to freezing injury (Kozlowski and Kramer 1991).

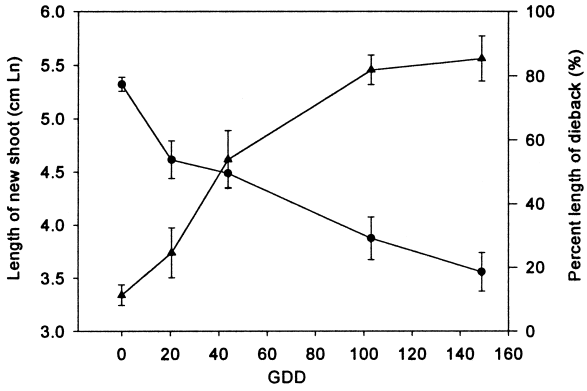

Figure 5. Length of new shoot (●) and the percent length of shoot dieback (▲) in response to simulated winter thaws (GDD > 4°C) followed by 10 weeks at –10 °C and 30 days in a greenhouse. Values are means ± 1 SE, $n = 5$.

Induction of stem xylem cavitation

The treatment-induced increase in stem cavitation may be attributed both to direct effects of freezing and to duration of thaw. When xylem sap is frozen, dissolved air forms bubbles in the ice that can nucleate cavitation as negative pressure develops in the vessels during thawing (Hammel 1967, Robson et al. 1988, Sperry and Sullivan 1992, Langan et al. 1997). During a previous experiment (Zhu and Cox, unpublished data), we noticed that substantial reductions in stem xylem cavitation can occur after roots thaw because of the development of root pressure and xylem refilling. In the current experiment, however, stem xylem cavitation increased with increasing thaw duration when examined 10 weeks after storage at –10 °C. A possible explanation is that ice formation in intercellular spaces and vessels may draw water from the surrounding parenchyma during freezing. This occurs because freezing of electrolyte solutions gives rise to large and transient potential differences between liquid and solid phase (Steponkus 1984). Thaw-induced dehardening may weaken the protection of plasma membrane from the water efflux during the freezing process of apoplastic water. Thus, the longer the duration of thaw, the more potential there is for dehydration of dehardened parenchyma tissues during subsequent refreezing. When frozen stems (–10 °C) were thawed in the laboratory, rehydration of xylem parenchyma caused rapid water loss from the xylem vessels and increased xylem tension, thereby facilitating cavitation. Water redistribution between xylem parenchyma and vessels caused by freeze–thaw cycles can cause parenchyma damage. However, deciduous hardwoods usually avoid this injury because their xylem parenchyma is tolerant to dehydration (George and Burke 1986). Similarly, we found no significant correlation between stem xylem cavitation and stem freezing injury (measured as EL) in the top, middle, and base segments (Table 1).

Root freezing injury, root pressure, and residual cavitation

Although root pressure was negatively correlated with root injury measured both as EL and TTC (Table 1), there was considerable variation in root pressure among the five replicate plants per thaw treatment, especially at GDD < 80 (Figure 4b). Variation was probably a result of varying root mass and phenotypic differences among individual plants. Formation of root pressure is the consequence of active water uptake, which is determined by the osmotic gradient between root xylem and root surface, an energy-consuming process (Kramer 1983). Because TTC reduction in roots can reflect the activity of some dehydrogenases in the respiratory electron transport system (Sakai and Larcher 1987), a low value of TTC reduction could indicate reduced energy availability for building an osmotic gradient (Palta and Li 1978, Cooke and Burden 1990). Increasing respiration during a simulated winter thaw can directly reduce the available respiratory substrate reserves (van den Driessche 1979, Ögren 1996), which are essential for generating sufficient root pressure in spring to refill the xylem. In addition, electrolyte leakage from the cell membrane as a result of freezing injury may directly disturb the osmotic potential gradient, thereby weakening root pressure.

Elimination of winter xylem cavitation in spring is determined by root pressure (Sperry et al. 1987). Because the mean height of the seedlings was 79.91 ± 2.26 cm (n = 33), a root pressure of 8 kPa is required to push water upward against gravity and fully refill the cavitated stem. However, root pressure was reduced to around 5 kPa in response to 100 GDD of thaw, and decreased to 1 kPa for seedlings exposed to 140 GDD of thaw (Figure 4b). We observed little embolism along the main stems when the mean maximum root pressure exceeded 16 kPa or when the thaw provided less than 50 GDD.

Dieback and new shoots

Severe root injury can cause dieback in shoots or even whole-tree death, however, dieback may not be entirely attributable to root freezing injury. The root system of yellow birch can tolerate temperatures as low as –33 °C in winter (Calmé et al. 1994), indicating that the temperature of –10 °C used in this experiment may not cause severe damage to roots even after the thaw treatments. Plants with more than 80% shoot length of dieback (Figure 5) had only 30% root EL, whereas control plants had 20% root EL but only 10% shoot dieback (Figure 4a). For root TTC reduction, control plants showed 7.6 units, whereas plants with 80% length of dieback had 1.8 units (Figure 4a). Regrowth of shoots was observed in plants with 30% root EL or 1.8 units of TTC reduction, but shoot regrowth decreased with GDD of thaw (Figure 5). Thus, the extent of dieback may depend directly on whether the remaining uncavitated vessels can provide enough water to maintain the living stem and branches. A 50% shoot dieback was recorded when the residual embolism was about 20% PLC (Figures 3b and 5). Birch may be very sensitive to residual embolism because it exhibited some shoot dieback at 0 GDD. This sensitivity may be consistent with the diffuse porous nature of the species, relying on old xylem for initial spring water supply. The relatively high dieback value under low xylem cavitation suggests that freezing injury of shoots or roots contributes to the dieback.

Conclusion

Duration of a simulated winter thaw can increase shoot dieback by accelerating the development of freeze–thaw-induced stem xylem cavitation and root and stem injury in potted yellow birch. During the process, root pressure was weakened by root damage and so it was unable to eliminate accumulated winter stem embolism caused by cavitation, resulting in residual stem xylem embolism. Thus, freezing damage to the root and shoot, together with residual stem xylem cavitation, can cause considerable shoot dieback.

Freeze–thaw-induced cavitation was generally attributed to the low solubility of air in frozen xylem sap (Hammel 1967, Robson et al. 1988). Freezing injury of xylem parenchyma was also considered to cause freeze–thaw-induced cavitation (Pockman and Sperry 1997). Our data indicate that duration of thaw plays an important role in the development of xylem embolism in winter. Based on predicted global warming, an important scenario in eastern Canada is that the winter will get warmer and the duration and frequency of winter thaws will increase. In response to these climatic changes the important roles of freezing damage and winter xylem cavitation in determining the frequency and intensity of spring shoot dieback and decline in birch deserve further study.

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References

Auclair, A.N.D., J.T. Lill and C. Revenga. 1996. The role of climate variability and global warming in the dieback of northern hardwoods. Water Air Soil Pollut. 91:163–186.

Balch, R.E. and J.S. Prebble. 1940. The bronze birch borer and its relation to the dying of birch in New Brunswick forests. For. Chron. 16:179–201.

Braathe, P. 1957. Is there a connection between the birch dieback and the March thaw of 1936? For. Chron. 33:358–363.

Braathe, P. 1995. Birch dieback caused by prolonged early spring thaws and subsequent frost. Norw. J. Agric. Sci., Suppl. No. 20, 50 p.

Calmé, S., H.A. Margolis, F.J. Bigras and C. Hébert. 1994. Frost tolerance and bud dormancy of container-growth yellow birch, red oak and sugar maple seedlings. Tree Physiol. 14:1313–1325.

Calmé, S., F.J. Bigras, H.A. Margolis and D. Mailly. 1995. The relationship between water content and frost tolerance in shoots of hardwood seedlings. Can. J. For. Res. 25:1738–1745.

Cooke, K.T. and R.S. Burden. 1990. Lipid modulation of plasma membrane-bound ATPases. Physiol. Plant. 78:153–159.

Cox, R.M and J.W. Malcolm. 1997. Effects of duration of a simulated winter thaw on dieback and xylem conductivity of *Betula papyrifera*. Tree Physiol. 17:389–396.

DeHayes, D.H. and M.W. Williams. 1989. Critical temperature: a quantitative method of assessing cold tolerance. USDA For. Serv. Gen. Tech. Rep. NE-134, 6 p.

Fisher, J.B., G.A. Angeles, F.W. Ewers and J. López-Portillo. 1997. Survey of root pressure in tropical vines and woody species. Int. J. Plant Sci. 158:44–50.

George, M.F. and M.J. Burke. 1986. Low temperature: physical aspects of freezing in woody plant xylem. *In* Forestry Science: Stress Physiology and Forest Productivity. Ed. Hennessey, T.C. Martinus Nijhoff Publishers, pp 133–150.

Hammel, H.T. 1967. Freezing of xylem sap without cavitation. Plant Physiol. 42:55–66.

Hansborough, J.R. and D.C. Stout. 1947. Virus-like symptoms accompanying birch dieback. Plant Dis. Rep. 31:327.

Kramer, P.J. 1983. Water relations of plants. Academic Press, pp 215–234.

Kozlowski, T.T. and P.J. Kramer. 1991. The physiological ecology of woody plants. Academic Press, Inc., San Diego, California, pp 189–206.

Langan, S.J., F.W. Ewers and S.D. Davis. 1997. Xylem dysfunction caused by water stress and freezing in two species of co-occuring chaparral shrubs. Plant Cell Environ. 20:425–437.

Lassheikki, M., P. Puttonen and P.K. Rasanen. 1991. Planting performance potential of *Pinus sylvestris* seedlings as evaluated by root growth capacity and triphenyl tetrazolium chloride reduction methods. Scand. J. For. Res. 6:91–104.

Ögren, E. 1996. Premature dehardening in *Vaccinium myrtillus* during a mild winter: a cause for winter dieback? Funct. Ecol. 10:723–732.

Palta, J.P. and P.H. Li. 1978. Cell membrane properties in relation to freezing injury. *In* Plant Cold Hardiness and Freezing Stress. Eds. P.H. Li and A. Sakai. Academic Press, New York, Vol 1, pp 93–115.

Pockman, W.T. and J.S. Sperry. 1997. Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. Oecologia 109:19–27.

Pomerleau, R. 1953. Development of dieback in trees and stands. *In* Report of Symposium on Birch Dieback, Part II. Canada Dept. of Agriculture, Forest Biology Division, Ottawa, pp 147–149.

Richie, G.A. 1991. Measuring cold hardiness. *In* Techniques and Approaches in Forest Tree Ecophysiology. Eds. J.P. Lassoie et al. CRC Press, pp 557–584.

Robson, D.J., W.J. McHardy and J.A. Petty. 1988. Freezing in conifer xylem. II. Pit aspiration and bubble formation. J. Exp. Bot. 39:1091–1098.

Sakai, A. and W. Larcher. 1987. Frost survival of plants. Responses and adaptation to freezing stress. Ecological Studies 62, Springer-Verlag, Berlin, pp. 174–266.

Santarius, K.A. 1982. The mechanism of cryoprotection of biomembrane systems by carbohydrates. *In* Plant Cold Hardiness and Freezing Stress. Vol. 2. Eds. PH. Li and A. Sakai. Academic Press, New York, pp 475–486.

Sinclair, G.A. 1952. Survey of the condition of birch in Ontario. Dept. of Agric. Sci. Serv. Serv. Bi-monthly Progr. Rep . 8 (2):2–3.

Sperry, J.S, N.M. Holbrook, M.H. Zimmermann and M.T. Tyree. 1987. Spring filling of xylem vessels in wild grapevine. Plant Physiol. 83:414–17.

Sperry, J.S., J.R. Konnelly and M.T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ. 11:35–40.

Sperry, J.S. and J.E.M. Sullivan. 1992. Xylem embolism in response to freeze–thaw cycles and water stress in ring-porous, diffuse-porous and conifer species. Plant Physiol. 100:605–613.

Sperry, J.S. 1993. Winter xylem embolism and spring recovery in *Betula cordifolia*, *Fagus grandifolia*, *Abies balsamea* and *Picea rubens*. *In* Water Transport in Plants under Climatic Stress. Eds. M. Borghetti, J. Grace and A. Raschi. Cambridge Univ. Press, Cambridge, pp 86–98.

Sperry, J.S. and W.T. Pockman. 1993. Limitation of transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. Plant Cell Environ. 16:279–287.

Sperry, J.S., K.L. Nichols, J.E.M. Sullivan and E. Eastach. 1994. Xylem embolism in ring-porous, diffuse-porous and coniferous trees of Northern Utah and Interior Alaska. Ecology 75:1736–52.

Steponkus, P.L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Annu. Rev. Plant Physiol. 35:543–584.

Strimbeck, G.R., P.G. Schaberg, D.G. DeHayes, J.B. Shane and G.J. Hawley. 1995. Midwinter dehardening of montane red spruce during a natural thaw. Can. J. For. Res. 25:2040–44.

van den Driessche, R. 1979. Respiration rate of cold-stored nursery stock. Can. J. For. Res. 9:18–15.

Walker, S.L., A.N. Auclair and H. Martin. 1990. History of crown dieback and deterioration symptoms of hardwoods in eastern Canada. Part I and II. Federal LRTAP Liaison Office, Atmospheric Environment Service, pp. 18–22.

Ward, J.S and G.R. Stephens. 1997. Survival and growth of yellow birch (*Betula alleghaniensis*) in southern New England. Can. J. For. Res. 27:156–169.