

# Phenological cues drive an apparent trade-off between freezing tolerance and growth in the family Salicaceae

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**Abstract.** With increasing concern about the ecological consequences of global climate change, there has been renewed interest in understanding the processes that determine species range limits. We tested a long-hypothesized trade-off between freezing tolerance and growth rate that is often used to explain species range limits. We grew 24 willow and poplar species (family Salicaceae) collected from across North America in a greenhouse common garden under two climate treatments. Maximum entropy models were used to describe species distributions and to estimate species-specific climate parameters. A range of traits related to freezing tolerance, including senescence, budburst, and susceptibility to different temperature minima during and after acclimation were measured. As predicted, species from colder climates exhibited higher freezing tolerance and slower growth rates than species from warmer climates under certain environmental conditions. However, the average relative growth rate (millimeters per meter per day) of northern species markedly increased when a subset of species was grown under a long summer day length (20.5 h), indicating that genetically based day-length cues are required for growth regulation in these species. We conclude that the observed relationship between freezing tolerance and growth rate is not driven by differences in species' intrinsic growth capacity but by differences in the environmental cues that trigger growth. We propose that the coordinated evolution of freezing tolerance and growth phenology could be important in circumscribing willow and poplar range limits and may have important implications for species' current and future distributions.

**Key words:** cold acclimation; freezing tolerance; geographic distributions; growth rate; *Populus* spp.; range limits; *Salix* spp.

## INTRODUCTION

Understanding the mechanisms that determine species range limits is a central pursuit in ecology that is of paramount concern given the likely impact of climate change on species distributions (Thuiller et al. 2008, McMahon et al. 2011). Some of the earliest research on plant distributions addressed how freezing temperatures could impact species northern range limits (Merriam 1894, Hutchinson 1918). Later studies documented large correlations between freezing tolerance and latitudinal distributions across species (Sakai and Weiser 1973, Larcher and Bauer 1981, Koehler et al. 2012). A common explanation for these correlations is that the cost associated with freezing tolerance reduces species' growth capacity, causing a trade-off between freezing tolerance and growth rate (MacArthur 1972, Woodward and Pigott 1975, Loehle 1998). The general idea is that species northern boundaries are determined by their susceptibility to low temperatures and their southern

boundaries are limited by their inability to compete successfully with faster-growing species. Despite the appeal of this explanation for species range limits, relatively little evidence exists for the proposed trade-off in the literature (but see Woodward and Pigott [1975], Loehle [1998] and Koehler et al. [2012]), and little is known about the mechanism(s) that might maintain it across species.

Typically, the freezing tolerance–growth trade-off is explained in terms of differences in the structure and carbon allocation of freezing tolerant and nonfreezing tolerant species (MacArthur 1972, Woodward and Pigott 1975, Loehle 1998). For example, physiological changes during cold acclimation, e.g., structural modifications such as cell wall thickening, and compositional changes such as increased cellular lipid, sugar, and protein concentrations (Larcher et al. 1973, Graham and Patterson 1982), often require reallocation of carbon and nutrients, which could limit the growth rate of freezing tolerant species. Another possibility is that constraints on xylem anatomy could prevent freezing tolerant species from achieving high growth rates. Smaller xylem conduits confer higher freezing tolerance because they limit the size of emboli, therefore minimizing the loss of vascular function following freeze–thaw events (Sperry and Sullivan 1992, Hacke

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and Sperry 2001). While smaller conduits are advantageous in colder climates, they may result in slower growth rates because they restrict hydraulic conductivity, which could in turn limit carbon assimilation (Sperry 2000, Brodribb et al. 2007). Despite the potential of these two mechanisms to explain the proposed trade-off, they provide a fairly simplistic view of plant function and do not consider how traits vary seasonally or under different climatic conditions, or whether there are functional and ecological differences between lineages. These issues are central to assessing whether the proposed trade-off could explain species distributions globally.

Freezing tolerance is a complex trait that varies both seasonally and across tissue types (Parker 1963, Sakai and Larcher 1987). As a result, many traits could limit species distributions including their rate of cold acclimation and timing of senescence, cold-acclimated freezing tolerance, timing of spring de-acclimation and budburst, and flowering phenology (Sakai and Larcher 1987, Woodward 1990, Chuine 2010). Understanding the mechanistic basis of this trade-off requires knowledge about what aspects of freezing tolerance influence species' northern range limits and their respective carbon costs. However, research on this trade-off has often focused on species' cold-acclimated freezing tolerance and overlooked the potential for more broadly defined cold tolerance strategies (MacArthur 1972, Loehle 1998).

Both freezing tolerance and growth rate are dependent on growth conditions (Oleksyn et al. 1992, Cavender-Bares et al. 2005, Cavender-Bares 2007, Tanino et al. 2010), and their plasticity could affect whether the proposed trade-off influences species range limits. For example, if the trade-off is driven by constraints on xylem anatomy, and if species' vessel diameters are plastic, species could have wider vessels at their southern range limits and therefore achieve higher growth rates while maintaining the ability to have narrow vessels in colder climates. Under this scenario, freezing tolerance would only be costly to growth rate when it is expressed and would not limit species' southern migration.

Last, the biogeographic and evolutionary history of plant lineages could influence the expression of this trade-off across species. For example, subtropical lineages may have a steeper trade-off than circumpolar lineages if it is more difficult to evolve freezing tolerance during a northward migration than it is to maintain it following a southward migration (assuming a northern hemisphere orientation). Different phylogenetic lineages may also employ freezing tolerance strategies that vary in their metabolic costs. Either of these scenarios could create lineage-specific variation that could impact how this trade-off is manifested across species.

To test the validity of this hypothesized trade-off and explore whether it could be important in explaining species range limits, we examined the relationship

TABLE 1. Willow (*Salix*) and poplar (*Populus*) species and their collection locations.

Species	Species code	Site
<i>S. alaxensis</i> (Andersson) Coville	ALA	AK <sup>1</sup>
<i>S. arbusculoides</i> Andersson	ARB	AK <sup>1</sup>
<i>S. candida</i> Flueggé ex Willd.	CAN	MN <sup>2,3</sup>
<i>S. caroliniana</i> Michx.	CAR	FL <sup>4</sup>
<i>S. eriocephala</i> Michx.	ERI	MN <sup>2,3</sup>
<i>S. exigua</i> Nutt.	EXI	OR <sup>5</sup> , NV <sup>6</sup> , AZ <sup>7</sup>
<i>S. fuscescens</i> Andersson	FUS	AK <sup>1</sup>
<i>S. gooddingii</i> C.R. Ball	GOO	NV <sup>6</sup>
<i>S. hookeriana</i> Barratt ex Hook.	HOO	OR <sup>5</sup>
<i>S. interior</i> Rowlee	INT	MN <sup>2,3</sup> , AK <sup>1</sup>
<i>S. lasiandra</i> Benth.	LAS	OR <sup>5</sup>
<i>S. lucida</i> Muhl.	LUC	MN <sup>2,3</sup>
<i>S. nigra</i> Marsh	NIG	MO <sup>8</sup>
<i>S. pedicellaris</i> Pursh	PED	MN <sup>2,3</sup>
<i>S. petiolaris</i> Sm.	PET	MN <sup>2,3</sup>
<i>S. pseudomyrsinites</i> Andersson	PMY	AK <sup>1</sup>
<i>S. pseudomonticola</i> C.R. Ball	PSM	AK <sup>1</sup>
<i>S. pulchra</i> Cham.	PUL	AK <sup>1</sup>
<i>S. pyrifolia</i> Andersson	PYR	MN <sup>2,3</sup>
<i>S. sitchensis</i> Sanson ex Bong.	SIT	OR <sup>5</sup>
<i>P. balsamifera</i> L.	BAL	AK <sup>1</sup>
<i>P. deltoides</i> Bartram ex Marsh.	DEL	MN <sup>2,3</sup>
<i>P. fremontii</i> Watson	FRE	NV <sup>6</sup>
<i>P. trichocarpa</i> Torr. & A. Gray ex Hook.	TRI	OR <sup>5</sup>

Notes: Specimens of each species were deposited in the UMN herbarium and more detailed collection information is in Appendix A. Collection sites are marked as follows: (1) Bonanza Creek LTER, and along the Tanana River, Fairbanks, Alaska; (2) Cedar Creek LTER, East Bethel, Minnesota; (3) Boot Lake and Savage Fen Scientific and Natural Area, Minnesota; (4) San Felasco and O'Leno State Parks, Florida; (5) Willamette and Suislaw River Valleys, Oregon; (6) U.S. Bureau of Land Management Land, Nevada; (7) Apache National Forest, Arizona; (8) Kansas City, Missouri.

between freezing tolerance and growth rate in a group of 24 species in the family Salicaceae. Species in this family span North America from the Arctic Circle to Mexico (Argus 2007) and often have high local diversity (Savage and Cavender-Bares 2012). By focusing on one phylogenetic lineage, we hold constant many aspects of ancestry and genetic background. Considering the potential importance of seasonality and plasticity to this trade-off, we comprehensively describe species' freezing tolerance based on suites of traits related to cold acclimation, cold-acclimated freezing tolerance, and phenology and examine how species' growth rate changes under a variety of climatic conditions.

#### MATERIALS AND METHODS

We selected 20 willow (*Salix*; see Plate 1) and four poplar (*Populus*) species from across North America (Table 1), including two species (*S. interior* and *S. exigua*) with wide latitudinal distributions (Appendix A: Table A1). We modeled species distributions based on herbaria records (Appendix B: Table B1) and Worldclim climate data (2.5 arc-minutes) (Hijmans et al. 2001) using the program Maxent, version 3.3.1 (Philips 2004). Maxent estimates the probability that a species will

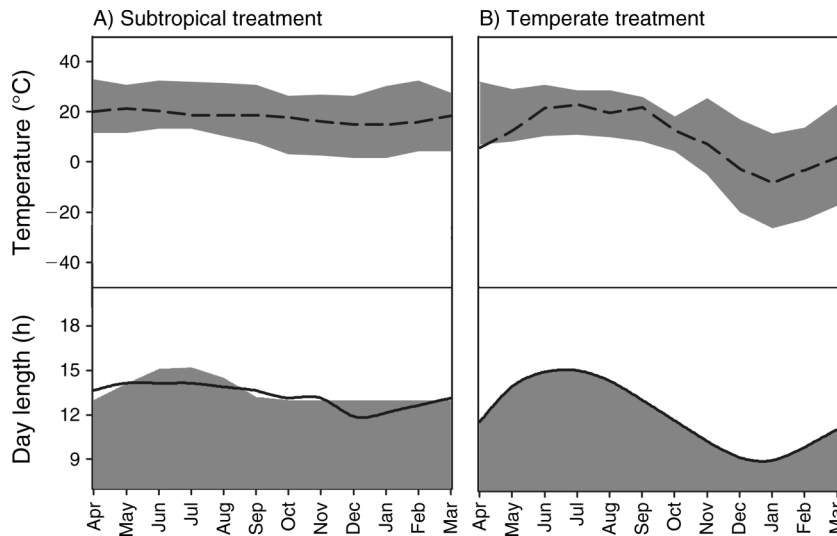


FIG. 1. Willow (*Salix*) and poplar (*Populus*) species from across North America were grown in (A) a subtropical treatment with a monthly temperature and day length similar to Morelia, Mexico, and (B) a temperate treatment with a monthly temperature and day length similar to Franklinville, New York, USA. The maximum and minimum temperatures and the average day length in the greenhouse are marked with gray shading, and the average monthly temperatures and day lengths at the target sites are noted with dashed lines and solid lines, respectively.

occur at a site based on a maximum entropy model constrained by occurrence data. For quality assurance, we did not include incomplete herbaria records or records that fell outside of published ranges (Argus 2007). We found between 73 and 1200 collections per species and ran each model with a 25% random test set. Our models described the occurrence data with a high specificity, and only two species had a test AUC (area under the curve)  $< 0.9$  (*S. alaxensis*, 0.89; *P. balsamifera*, 0.88). We described species distributions based on the most probable, average annual minimum temperature in their modeled climatic niche ( $T_{\min}$ ), which was determined by calculating the zenith of the average minimum temperature probability curve. Other climatic variables are reported in Appendix B.

We collected cuttings from six to 10 unique genotypes per species in the middle of their latitudinal range in the spring and summer of 2007. We grew plants in a mixture of 50:30:20 sand, compost, and peat in 6.25-L treepots and fertilized them with Osmocote (19-6-12 slow release fertilizer; Scotts Miracle-Gro, Marysville, Ohio, USA). The cuttings were allowed to root under 15.5 h light and an average temperature of 25°C. In the autumn, plants were divided into a temperate and a subtropical treatment each of which was replicated into two greenhouse rooms (four total). The subtropical treatment simulated the temperature and photoperiod of Morelia, Mexico (19.77° N, 101.19° W), which is near the southernmost range limit of any of the species in this study (Fig. 1A). The temperate climate treatment simulated the temperatures and photoperiod of Franklinville, New York, USA (42.34° N, -78.46° W), where the greenhouse was located (Fig. 1B). During the first

winter we kept the plants in the temperate treatment inside the greenhouse ( $T_{\min} \geq 2^{\circ}\text{C}$ ), but during the second winter (when freezing tolerance and phenology was monitored) we put them outside. We used clones of some of the genotypes for each treatment whenever possible, allowing us to control for genotypic differences within species. During the final summer, we added a long-photoperiod treatment conducted on 4–6 individuals of 10 species that spanned the same range of latitudes as the full species' set. It had a temperature regime identical to the temperate treatment (Fig. 1A) and a 20.5-h summer photoperiod (similar to Fairbanks, Alaska).

To test for the proposed trade-off, we measured a suite of traits related to cold and freezing tolerance through the autumn and winter in the temperate treatment and in the autumn in the subtropical treatment. First, we compared species' stem and leaf freezing tolerance at  $-10^{\circ}\text{C}$  and  $-6^{\circ}\text{C}$ , respectively, in the autumn. All but three of the species had leaves at this point during the experiment (Appendix C: Table C1). These freezing temperatures were selected because they cause freezing damage but do not kill the majority of samples. For the leaf experiments, we cut 6-cm stem segments, keeping at least two fully expanded leaves per sample, and for the stem experiments, we used 5-cm stem segments without leaves. In both cases, samples were cut under water and one end was put in a water-filled rose tube. We assessed freezing injury by measuring the maximum quantum efficiency of dark-adapted leaves and stems using a pulse-modulated fluorometer (Hansatech Instruments, Norfolk, UK) before and after freezing. We also assessed cambial mortality by visually examining the stems.

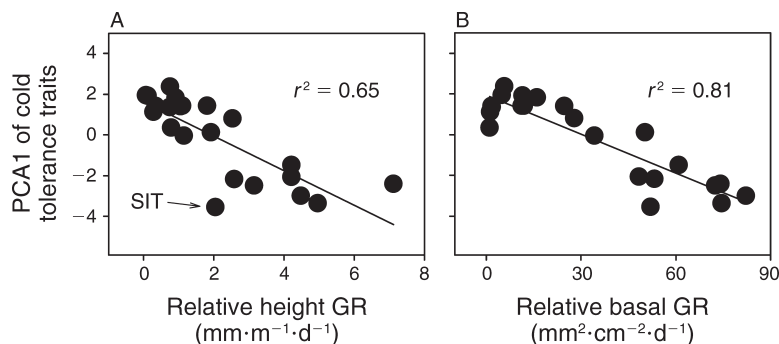


FIG. 2. The relationship between freezing tolerance in the temperate treatment and growth in the subtropical treatment in terms of (A) relative height and (B) relative basal growth rates (GR). The same genotypes were used in both treatments. Freezing tolerance is described by the primary axis of a principal components analysis (PCA) of all measured cold tolerance traits. Each point represents a species' average. *Salix sitchensis* (SIT) is marked because it is shrubby and demonstrated more lateral than vertical growth.

Second, we measured cold-acclimated freezing tolerance in February. We froze 1-cm stem segments at  $-55^{\circ}\text{C}$  (record  $T_{\min}$  in North America is  $-62^{\circ}\text{C}$ ) and assessed stem injury using electrolyte leakage (Burr et al. 1990, Friedman et al. 2008). This technique is based on the assumption that the amount of electrolyte leakage from cells after freezing is proportional to cell mortality. We estimated the index of injury based on Flint et al. (1967). Concurrently, we monitored extracellular freezing in the pith of 5-cm stem segments of a subset of five species using thermocouples. We considered an exotherm to occur when there was a temperature spike  $>1^{\circ}\text{C}$  (Cavender-Bares et al. 2005). A detailed description of the freezing experiments and the injury assessments are described in Appendix C.

Third, we made cross sections of one-year-old branches from five to six individuals per species to compare their vessel diameters. Samples were collected from the temperate climate treatment in the winter. We measured vessels in a quarter of each cross section (typically  $>100$  vessels) using ImageJ, version 1.44 (National Institutes of Health, Bethesda, Maryland, USA).

Last, we calculated the proportion of each species that senesced each week of the autumn and the maximum growing degree-days (GDD) required for budburst in the spring. GDD was calculated as  $\sum (T_{\text{high}} - T_{\text{low}})$  where  $T_{\text{high}}$  and  $T_{\text{low}}$  are the maximum and minimum temperature each day (after January) that had an average temperature  $>1^{\circ}\text{C}$ , the threshold temperature (Lennartsson and Ogren 2004). *Salix pseudomonticola* and *S. pulchra* were excluded from this analysis because of their small sample size.

To compare species' growth rates and to determine how growth rates vary with climatic conditions, we measured plant height and basal area in all the climate treatments in May and September 2008 and 2009 (the beginning and end of the growing season). We measured height growth because of its potential importance in

light acquisition, and basal growth because it correlates with total biomass (J. A. Savage, *unpublished data*). We calculated plant relative growth rate as  $[\ln(g_2) - \ln(g_1)] / (t_2 - t_1)$  where  $g_1$  and  $g_2$  are the growth measurements at times  $t_1$  and  $t_2$ . We also monitored dieback and mortality in the summer 2008. We did not measure the growth of *S. arbusculoides* and *S. petiolaris* in the subtropical treatment because of their high mortality.

To test for a trade-off, we used the primary axis of a principal components analysis (PCA) as an integrated measure of freezing tolerance (including autumn freezing tolerance, senescence, cold-acclimated freezing tolerance, budburst, and vessel diameter) and completed a regression analysis of the PCA1 loadings on species' growth rates. We used growth rates from the subtropical treatment, where there was no low temperature stress and maximum growth rate could be expressed. By comparing growth rate in the tropical treatment and freezing tolerance in the cold treatment, we examined the proposed trade-off on a geographic scale that could be relevant to species distributions. For all comparative analyses, plants with the same genotype in the different treatments were matched. We used JMP 8.0 (SAS Institute, Cary, North Carolina, USA) and PC-ORD 5.0 (MJM Software Design, Gleneden Beach, Oregon, USA) for the statistical analyses.

## RESULTS

Species' height and basal growth rate in the subtropical treatment were inversely correlated with their freezing tolerance in the temperate treatment (least squares regression (LSR),  $F = 67.4$ ,  $df = 1, 22$ ,  $P < 0.0001$ ; Fig. 2A, B) in a manner consistent with the proposed trade-off. In this analysis, species' freezing tolerance was described by the primary axis of a PCA conducted on all traits associated with cold tolerance, an axis that explained 61.9% of the trait variation. Each trait was also inversely correlated with growth rate, individually (Appendix D: Fig. D1). Freezing tolerance

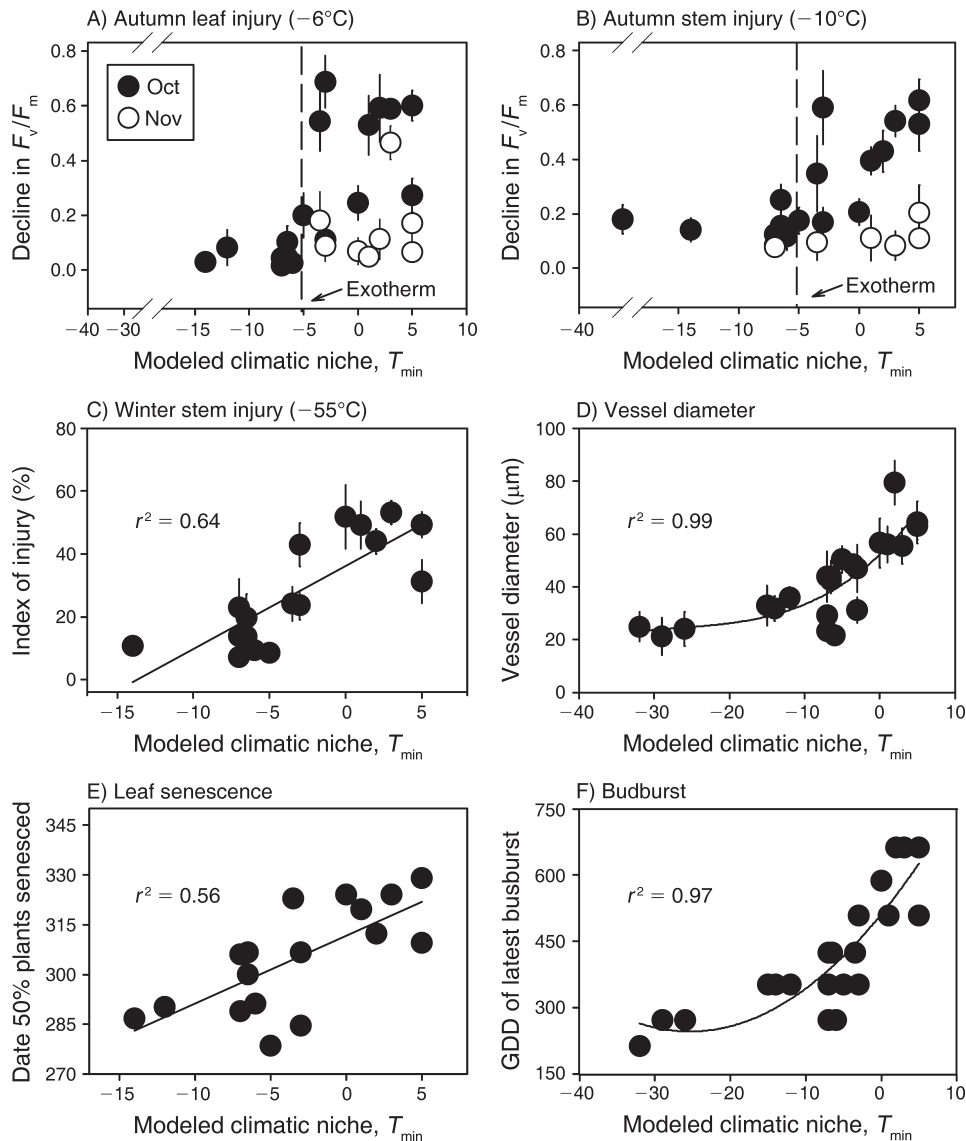


FIG. 3. Correlations between species' modeled climatic niche ( $T_{min}$ ) and traits related to cold tolerance including (A, B) leaf and stem freezing injury following exposure to  $-6^{\circ}\text{C}$  and  $-10^{\circ}\text{C}$ , respectively, in October (solid circles) and November (open circles); (C) stem freezing injury resulting from freezing at  $-55^{\circ}\text{C}$  in the late winter; (D) average vessel diameter; (E) timing of senescence; and (F) timing of budburst (growing degree-days, GDD). Freezing injury was assessed based on a loss in maximum quantum efficiency ( $F_v/F_m$ ) in the autumn and electrolyte leakage in the winter. Dashed lines mark the exotherm temperature, indicating ice formation. Error bars indicate  $\pm\text{SE}$ .

was not strongly correlated with growth rate in the temperate treatment (Appendix D: Fig. D2).

Leaf freezing tolerance in the autumn correlated with species' modeled climatic niche in both climate treatments ( $T_{min}$ ; Fig. 3A and Appendix D: Fig. D3). In general, species native to colder climates ( $T_{min} < -5^{\circ}\text{C}$ ) demonstrated little to no decline in maximum quantum efficiency ( $F_v/F_m$ ) in their leaves after freezing to  $-6^{\circ}\text{C}$  in October, while the warm climate species demonstrated declines of 0.1–0.7. By November, many of the cold climate species had dropped their leaves, and the warm climate species had demonstrated an increase in their

leaf freezing tolerance (paired  $t$  test,  $t = -7.6$ ,  $df = 58$ ,  $P < 0.0001$ ). Of these species, all but one (*S. sitchensis*) was able to maintain an  $F_v/F_m$  within 0.2 of its pre-freezing level. Measurements were taken in the dark 25 hours after the freezing treatment but the same trend was observed after 0, 3, and 7 hours (data not shown). Similar to the leaf freezing tests, species from colder climates exhibited less freezing injury in October in their stem than warm climate species (Fig. 3B). However, the majority of the latter plants did increase their freezing tolerance in November (paired  $t$  test,  $t = 5.93$ ,  $df = 37$ ,  $P < 0.0001$ ). In general, stem  $F_v/F_m$  correlated with



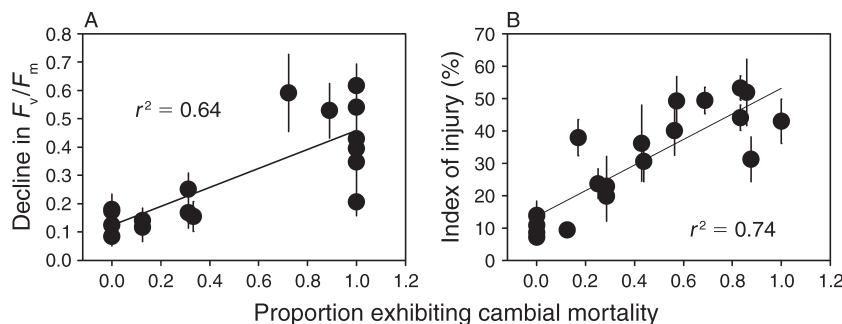


FIG. 4. The relationship between two freezing injury metrics and the proportion of samples that demonstrated cambial mortality: (A) maximum quantum efficiency ( $F_v/F_m$ ) and (B) index of injury from electrolyte leakage. All points represent species means  $\pm$  SE.

cambial death, and a decline of 0.25 resulted in 50% mortality (Fig. 4). After acclimation, species from warm climates continued to exhibit a greater freezing injury ( $-55^\circ\text{C}$ ) than species from cold climates (Fig. 3C). Average vessel diameter correlated with species' climatic niche (LSR,  $F = 4.96$ ,  $df = 1, 19$ ,  $P = 0.04$ ; Fig. 3D), but species did not differ in their average freezing exotherm temperature ( $-5.17^\circ \pm 0.18^\circ\text{C}$ , mean  $\pm$  SE).

Species' phenology varied with their geographic distribution. Species from colder climates exhibited earlier senescence and earlier budburst than species from warmer climates (Fig. 3E, F). A greater proportion of species originating from warmer climates demonstrated dieback in the winter, and the eight species native to the warmest climates (*S. caroliniana*, *S. exigua*, *S. gooddingii*, *S. hookeriana*, *S. nigra*, *S. sitchensis*, *P. fremontii*, and *P. trichocarpa*) exhibited a  $57\% \pm 8\%$  (mean  $\pm$  SE) mortality in the temperate treatment (Appendix D: Table D1).

Growth rate was positively correlated with species' climatic niche ( $T_{\min}$ ) in both treatments (Fig. 5A). Overall, the relative growth rates (GR) observed in the temperate treatment were greater than those in the subtropical treatment. This was an artifact of the timing of our measurements because the plants in the subtropical treatment experienced a subsequent flush mid-winter that was not captured by our data. In both of these treatments, the relative GR of the five most northern species were stunted compared to the long-photoperiod treatment (20.5 hours; Fig. 5B). Overall, there were significant species (ANOVA,  $F = 6.0$ ,  $df = 9, 135$ ,  $P < 0.0001$ ), photoperiod (ANOVA,  $F = 28.4$ ,  $df = 1, 135$ ,  $P < 0.0001$ ), and species by photoperiod effects (ANOVA,  $F = 20.9$ ,  $df = 9, 135$ ,  $P < 0.0001$ ) on relative GR when the temperate and long-photoperiod treatment were compared. Because height and basal growth rate were highly correlated across species (LSR,  $F = 130.5$ ,  $df = 1, 22$ ,  $P < 0.0001$ ), data are only presented in terms of relative height GR.

Species grew more vigorously, in terms of their total height growth, during the experiment under the conditions similar to those experienced in their native habitat

(Fig. 5C). Species from the coldest climates (Group 1,  $T_{\min}$  less than or equal to  $-15^\circ\text{C}$ ) demonstrated limited growth in both the temperate and subtropical treatments. These species have distributions north of  $55^\circ\text{N}$  and occur under climatic conditions different than those in either treatment. Most of the species from intermediate climates (Group 2,  $-15^\circ\text{C} < T_{\min} \leq -5^\circ\text{C}$ ) exhibited greater growth in the temperate treatment, while the species from warmer climates (Group 3,  $-5^\circ\text{C} < T_{\min}$ ) exhibited the greatest growth in the subtropical treatment (Fig. 5C). Species from colder climates also demonstrated greater mortality in the subtropical treatment than species from warmer climates (Appendix D: Table D1).

#### DISCUSSION

It has long been hypothesized that the costs associated with freezing tolerance result in a trade-off between freezing tolerance and growth rate that has important implications for species geographic distributions (MacArthur 1972, Loehle 1998). In the family Salicaceae, species' freezing tolerance is inversely related to their growth rate under certain environmental conditions (Fig. 2), but contrary to expectations, this apparent trade-off results from variation in species' photoperiod cues for growth and not differences in their inherent growth capacity (Fig. 5). Northern species only exhibit slower growth rates than their southern counterparts when grown under summer day lengths shorter than those experienced in their native range (Fig. 5B). These data suggest that there is not a direct growth cost associated with freezing tolerance but there is divergence in the phenological strategies required for surviving in different climates (in terms of timing of budburst, growth up-regulation, and cold acclimation). As a result of these contrasting strategies, there is a strong association between growth phenology and cold tolerance that gives rise to an apparent trade-off between freezing tolerance and growth rate across species (Fig. 2).

Species' growth rates were highly influenced by climate and photoperiod, and species grew faster under

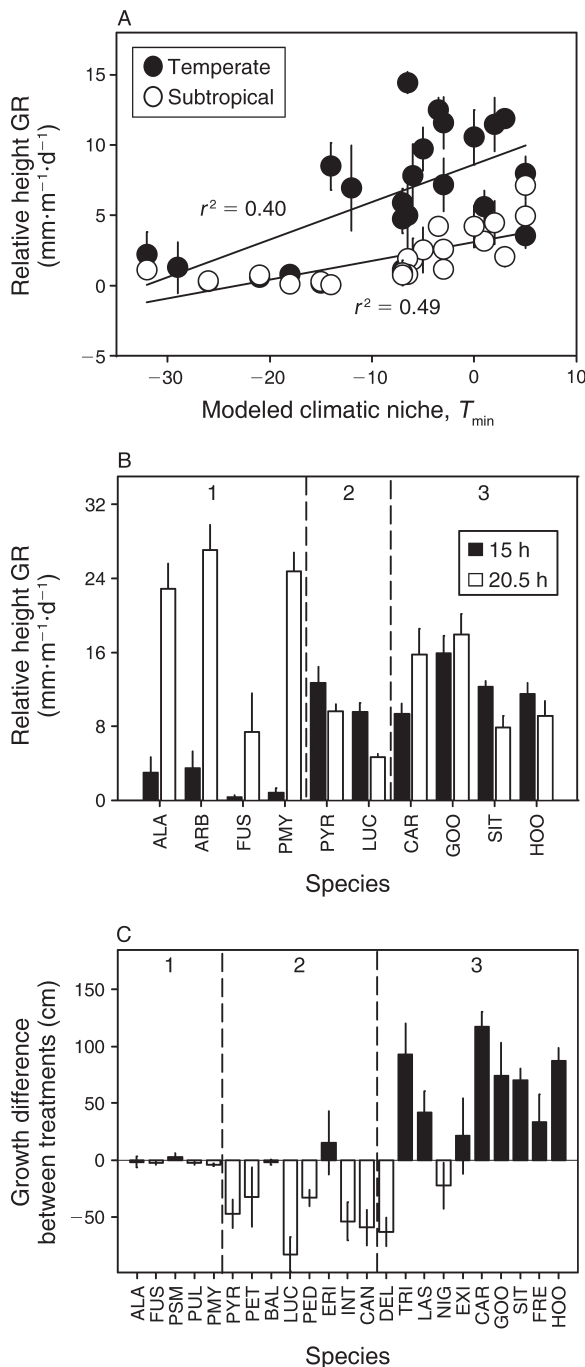


FIG. 5. (A) Correlation between species' relative height growth rate (GR) and their modeled climatic niche ( $T_{min}$ ) in the temperate (solid circles) and subtropical (open circles) treatments. (B) Differences in species' growth rates when summer day length is 15 (solid bars) and 20.5 hours (white bars). (C) The relationship between  $T_{min}$  and species' growth response to the subtropical treatment (difference between total growth in the subtropical and temperate treatments). Positive values indicate that species demonstrated greater growth in the subtropical treatment. In panels (B) and (C), species are ordered based on  $T_{min}$ , with the species from the coldest climates on the left. The plants are grouped (dashed vertical lines) by their growth response. All growth measurements are

climate conditions more similar to those experienced in their native range (Fig. 5C). This in turn led to a strong correlation between species' growth rate and their climatic niche within treatments (Fig. 5A). There are two phenological cues that appear critical for proper growth and development in willows and poplars. First, similar to many other temperate species, they require chilling temperatures in the winter (Heide 1993, Pop et al. 2000, Petri and Leite 2004). In the absence of these temperatures, plants experience stunted growth and high mortality, two responses that were observed in the subtropical treatment (Appendix D: Table D1). Second, our data indicate that northern species require long photoperiods to stimulate growth in the summer. This is consistent with previous work on willows indicating that long day lengths are a cue for gibberellin production and stem elongation (Junttila and Jensen 1988, Olsen et al. 1995). This type of growth regulation could explain why the northern species exhibited slow growth rates in the temperate and subtropical treatments (Fig. 5A). Taken together, these data demonstrate some of the complex ways that environmental conditions can impact growth phenology in plant species (Larcher et al. 1973, Tanino et al. 2010, Cooke et al. 2012).

As expected, species from colder climates demonstrated earlier cold acclimation and higher cold-acclimated freezing tolerance than species from warmer climates (Fig. 3). Despite this trend, species had higher cold-acclimated freezing tolerance than expected based on their geographic distribution (Fig. 3C). A similar observation was made by Sakai and Weiser (1973), who found that in contrast to 68 other species in North America, two species in the family Salicaceae exhibited a higher freezing tolerance than required in their native range. In an earlier paper, Sakai (1970) noted that tropical willow species can acclimate to temperatures as low as  $-30^{\circ}\text{C}$ . He explained this pattern in terms of phylogenetic inertia and proposed that many willows retain the ability to acclimate to colder temperatures because of their freezing tolerant ancestors (Argus 1997, Skvortsov 1999). As a result, there may be a hysteresis in the relationship between species distributions and their cold-acclimated freezing tolerance depending on their ancestral climatic origin. This could explain why willow and poplar species appear to be outliers in Loehle's (1998) original analysis of the freezing tolerance–growth trade-off. Strong phylogenetic inertia would indicate that freezing tolerance is not costly because if it were, selection would have eliminated it when it was no longer necessary (warmer climates).

If phylogenetic inertia explains high freezing tolerance in willows and poplars, then cold-acclimated freezing

← reported in terms of height, and species' means with SE are reported. Species names (see Table 1 for species list) are abbreviated by the first three letters of their specific epithet except for *S. pseudomonticola* (PSM) and *S. pseudomyrsinites* (PMY).

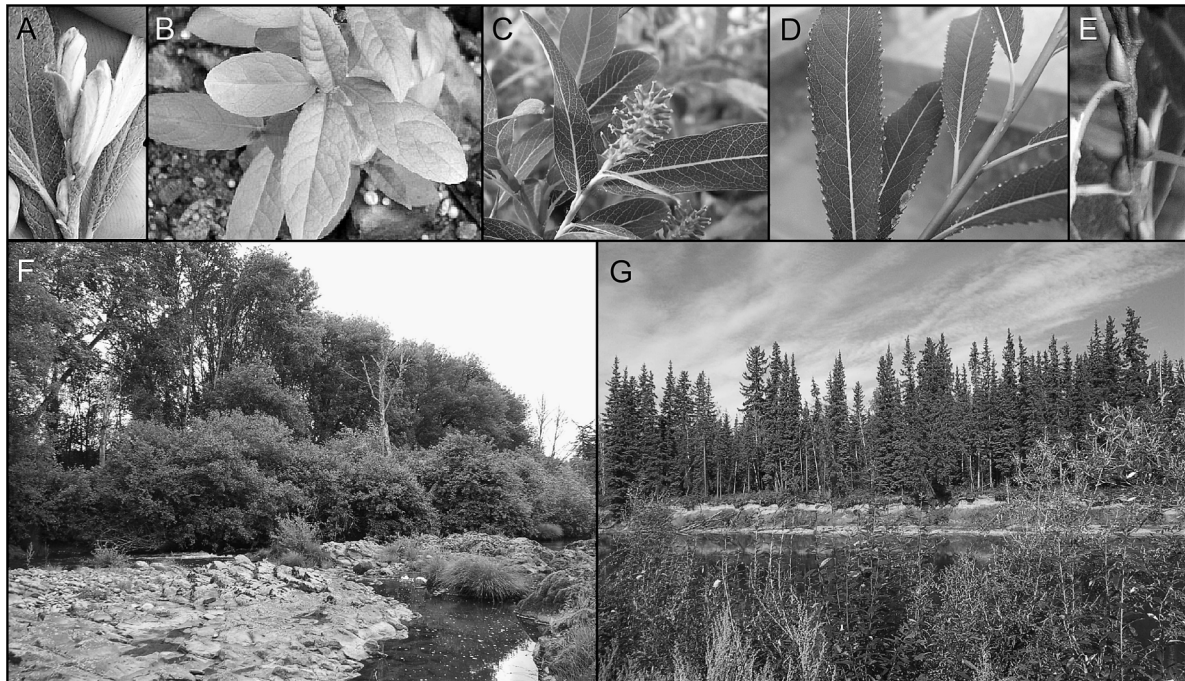


PLATE 1. The top images illustrate stages of leaf and flower development in willows during the year: (A) an actively growing shoot apex of *Salix candida*, (B) immature leaves of *S. pseudomonticola*, (C) a female flower of *S. pedicellaris*, (D) mature leaves of *S. petiolaris*, and (E) mature buds of *S. petiolaris*. The bottom two images show willow thickets in two different climate zones: (F) *S. sitchensis*, *S. hookeriana*, and *S. lasiandra* by the edge of Mosby Creek in Cottage Grove, Oregon, USA, and (G) *S. arbusculoides*, *S. alaxensis*, and *S. lasiandra* by the Tanana River in Fairbanks, Alaska, USA. Photo credits: J. A. Savage.

tolerance should not directly limit species' northern distributions. Instead, we propose that northern migration is prevented by a syndrome of traits associated with freezing tolerance, including the timing of senescence, cold acclimation, de-acclimation, and budburst. These traits are heritable (Heide 1993, Lennartsson and Ögren 2002) and critical in preventing loss in productivity on willow plantations (Christersson et al. 1983, Verwilt et al. 1996).

Although the apparent trade-off is an artifact of differences in species' photoperiod cues, it could limit species distributions if species exhibit slower growth rates along their southern boundaries because of shorter day lengths. Several recent studies have highlighted the importance of phenology in determining species distributions (Morin et al. 2007, Chuine 2010, Tanino et al. 2010), but there is limited research examining trade-offs between different phenological traits (e.g., flowering, growth, and senescence). This is an important area of future research that could prove critical to understanding species distributions.

There are a couple of important issues to consider when examining whether differences in species' photoperiod cues could be important in determining their distributions. First, intraspecific variation could modify the apparent trade-off. Many widely distributed species demonstrate intraspecific variation in both freezing tolerance and growth (Oleksyn et al. 1992, Rossi et al.

2006, Cavender-Bares 2007, Friedman et al. 2008), but this pattern is not ubiquitous (Koehler et al. 2012). We found that two widely distributed willow species differed in their level of intraspecific variation (see Appendix D: Table D2). Second, previous research on willows suggests that their local and continental distributions are influenced by water availability (Amlin and Rood 2002, Savage et al. 2009, Savage and Cavender-Bares 2011, 2012), herbivory (Maschinski 2001), maximum summer temperature (Myklestad and Birks 1993), and flowering phenology (Chuine and Beaubien 2001). Therefore, more research is needed to explore whether a large combination of factors could be influencing geographic distributions in the family.

### Conclusions

In the family Salicaceae, there is evidence that selection for specific phenological strategies at different latitudes has led to an apparent trade-off between species' freezing tolerance and their growth rate under specific environmental conditions. These results emphasize the importance of considering seasonality, plasticity, and lineage-specific effects when examining the implications of large trade-offs in species' environmental tolerances. Phenology is especially important considering that southern species are predicted to encroach on northern habitats causing them to compete with northern species in novel environments. Although some



northern species in the family Salicaceae have higher growth rates in their native range, they rely on photoperiod cues for growth regulation, which may put them at a disadvantage if they have to compete with southern species that can begin growing earlier in the season. However, more research is needed to better understand how phenology, trait plasticity, and intra-specific variation might impact species' responses to future climate conditions and also understand whether patterns can be generalized across lineages.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Information on plant collections ([Ecological Archives E094-156-A1](#)).

### Appendix B

Methods and data on characterizing species distributions ([Ecological Archives E094-156-A2](#)).

### Appendix C

Methods for measuring freezing tolerance ([Ecological Archives E094-156-A3](#)).

### Appendix D

Additional results from climate treatments ([Ecological Archives E094-156-A4](#)).