

# Population variation in cold hardiness of Pacific dogwood (*Cornus nuttallii*)

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## Abstract

Climatic variables and environmental conditions such as precipitation, sunlight, and soil are all important to the longevity of a species, but cold winter temperatures are one of the most limiting factors of species range. Pacific dogwood (*Cornus nuttallii*) is a beautiful tree found along most of the west coast of North America, from British Columbia to southern California. Despite its long range, previous studies have reported low local adaptation. In order to determine the population variation in cold hardiness, twigs were sampled in November 2008 and January 2009 from nine populations of Pacific dogwood planted in a common garden. Samples were analyzed for their Index of Injury at  $-18^{\circ}\text{C}$  and  $-25^{\circ}\text{C}$ , using the electrolyte leakage method (Hannerz *et al.*, 1999). Statistical analyses showed that there was some population variation at  $-18^{\circ}\text{C}$  in November, but no population variation at any of the other test temperatures or dates. This suggests that Pacific dogwood is not very locally adapted in its level of cold hardiness. This may be the result of high gene flow among populations due to seed dispersal by birds, as well as low genetic diversity caused by a population bottleneck during the Pleistocene glaciation. Therefore, this tree may not be well equipped to handle the environmental changes coming with climate change, but could perhaps be a candidate for facilitated migration.

Keywords: climate change, facilitated migration, gene flow, genetic variation, local adaptation

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## Introduction

The ability of a tree species to withstand cold is an important limiting factor of its geographical range (Stushnoff & Junttila, 1986). While there are several other factors to consider such as soil, precipitation, and biotic interactions, the climate of the site, especially during the coldest months of winter, is key to the survival of a species. Local adaptation to these conditions can enable a population to thrive in their habitat. With the rapid increase in atmospheric CO<sub>2</sub> over the last century, many tree species are beginning to suffer in their current locations due to the onset of environmental conditions they are not accustomed to (Aitken *et al.*, 2008). The climate of the geographical range of *Cornus nuttallii* (Pacific dogwood) is changing (Hamann & Wang, 2006), and like for many tree species, it is not yet clear whether this tree will adapt, migrate, or suffer from localized extinctions. Recent studies indicate that Pacific dogwood has low genetic diversity throughout its range (Keir & Aitken, 2008). Therefore, it is of interest to determine how much local adaptation Pacific dogwood exhibits in cold hardiness and the variation in cold hardiness among populations. This information could ultimately be utilized for predicting how this tree species may react to climate change, and possibly in forming seed transfer guidelines for facilitated migration in the future.

Pacific dogwood is a small tree growing up to 20 m tall, sometimes resembling a shrub, which grows mainly along the west coast of North America from southern British Columbia,

Canada, to southern California, United States (Gucker, 2005). It is more commonly found west of the Cascade Mountains, although a small population exists in northern Idaho (Gucker, 2005). Incidence of Pacific dogwood increases along streambanks and in coniferous, hardwood, and mixed low-elevation forests in climates that are temperate to mesothermal.

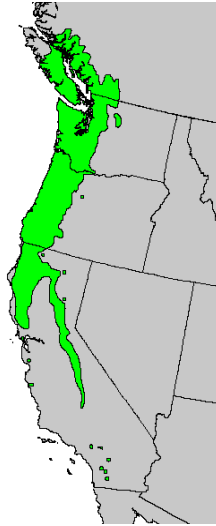


Figure 1. Range of Pacific dogwood along west coast of North America, in green color (Little, 1976).

Pacific Dogwood is most easily identified in the spring by its 4-7 large, white showy floral bracts surrounding an inflorescence of several, hemispheric clusters with no stalks (Farrar, 2006). Each inflorescence produces 20-60 head flowers and each of these produces a small, red fleshy drupe containing one or sometimes two seeds (Gucker, 2005). These fruits are attractive to many birds such as band-tailed pigeons and pileated woodpeckers (Gucker, 2005). Small mammals have also been known to eat the fruit and along with birds aid in the long distance seed dispersal of this species. This sub-canopy tree also provides habitat and cover for large ungulates and small mammals (Gucker, 2005). Although the wood of Pacific dogwood has been used in the past by First Nations as well as for commercial tools and parts such as piano keys, it is not currently valued for its lumber (Gucker, 2005). However, ornamental varieties of this

species are popular with gardeners. Pacific dogwood is also the provincial flower of British Columbia, underlining the importance of this small tree to the province.

Among the threats to Pacific dogwood populations, the introduced fungal disease dogwood anthracnose (*Discula destructiva*) is one of the most important (Redlin, 1991). First noticed in 1976 in Clark County, Washington, the disease was almost simultaneously found on the Northeastern side of the United States on *Cornus florida* (flowering dogwood). Since then, it has spread rapidly through populations of both species of flowering dogwood. This disease has been quite devastating for populations of flowering dogwood which make up a core component of eastern deciduous forests, however, less so for Pacific dogwood (Redlin, 1991). Although dogwood anthracnose has been found in British Columbia, Washington, Oregon, and California, the most damage has occurred to populations in Idaho which have suffered extensive mortality (Redlin, 1991). Other threats to Pacific dogwood include habitat loss through deforestation, as well as the potential for climate change to alter the natural climatic range of the species (Keir, 2008).

The ability of a species to survive cold temperatures is directly related to its long-term success. Without cold acclimation resulting in the development of mechanisms such as deep supercooling or intracellular freezing, plants would not be able to survive the cold winter months characteristic of more temperate climates. Cold injury can result in several complications including dehydration, retarded metabolism, membrane instability, and under certain conditions plant death (Sakai & Larcher, 1987). Therefore, local adaptation of tree populations to their environment plays a large role in their long-term success.

The degree of cold hardiness exhibited by a plant depends on several characteristics such as its age, developmental stage, the rate of cooling, duration of frost, and the season. The development of cold hardiness usually begins in early fall and consists of two phases. The first phase begins after growth has stopped, and is usually induced by short photoperiod in woody plants (Sakai & Larcher, 1987). Organic substances, such as starch and lipids, are stored for use in the metabolic changes associated with the second phase of cold acclimation. The second phase of cold-acclimation is induced by low temperatures of less than 5°C, but especially by subzero temperatures. At this point, membrane proteins and lipids undergo structural changes that allow the plant to achieve maximum cold hardiness (Sakai & Larcher, 1987). Prior to cold acclimation, plants may only be able to withstand temperatures near 0°C, however, by the time they have reached their maximum cold hardiness, some plant species can be well protected to temperatures as low as -196°C (Stushnoff & Junttila, 1986). Although many plants are locally adapted to develop the cold hardiness necessary only to survive in their own environment, some plants such as *Salix nigra* have been capable of hardening to much lower temperatures than expected for individuals coming from a moderate climatic region (Stushnoff & Junttila, 1986).

Information about the cold hardiness of a tree species can be extremely beneficial with the shift in species habitat ranges occurring as a result of climate change. The climatic range of Pacific dogwood is shifting (Hamann & Wang, 2006), but the relatively high speed of this shift suggests that the natural migration of this species may not be fast enough to keep up with its climate. Aitken *et al.* (2008) review three scenarios for tree populations in the face of climate change: trees can adapt to the conditions at hand, migrate with their range, or become



extirpated from their environment. If a species is unable to adapt to the changes in climate, facilitated migration is an option that could be employed to avoid extirpation. Facilitated migration will only work in combination with well developed seed-transfer guidelines (Aitken *et al.*, 2008). Part of these guidelines is ensuring that trees from various provenances are being moved to locations where they will be able to survive the initially colder winter temperatures and any other unfavorable growing conditions, while the climate undergoes changes.

Pacific dogwood has a long range spanning much of the west coast of North America, but not a great deal is known about the extent of local adaptation in this species. A recent study by Keir and Aitken (unpublished) suggests that local adaptation of Pacific dogwood is low, possibly due to high gene flow between populations and low genetic diversity in the species as a whole. Therefore, the focus of this study is to determine how much local adaptation exists in Pacific dogwood when examining the variation in cold hardiness among populations from various parts of the species range. This information will build upon previous studies at UBC in the Centre for Forest Conservation Genetics of population genetics in Pacific dogwood, a tree for which there has been little research, as well as provide some insight into how this tree may react to climate change, and what can be done to ensure it can endure this period of uncertainty.

## **Materials and Methods**

### **Source Materials**

During the fall of 2006, University of British Columbia graduate student Karolyn Keir collected seeds from 164 individuals spanning 11 populations of Pacific dogwood to be used in

establishing a common garden experiment (Keir, 2008). The populations encompassed the entire range of Pacific dogwood and included two isolated populations: one at the southern edge of the species range in San Bernardino, California and one in northern Idaho. All populations sampled in the United States, except the one in Idaho, occurred in various National Forests situated in Washington, Oregon, and California, while the remaining three populations were sampled in British Columbia, Canada. Each population had at least 10 trees sampled and all trees were located 30 m apart or greater. Each tree had its fruit, containing one seed, collected from several flowers and stored in a breathable mesh bag.

**Table 1. Geographic regions where Pacific dogwood populations were sampled**

Abbrev.	Population	Lat	Long	Elev	MAT	MWMT	MCMT	MAP	MSP	FFP
SQ	Sequoia NF*, California	35.7	118.5	529	17.4	27.9	8.3	399	35	283
SB	San Bernadino NF, California	34.2	117.2	523	16.9	26.8	8.2	718	55	296
MD	Mendocino NF, California	39.4	123.8	206	11.4	14.4	8.5	1099	81	365
KL	Klamath NF, Oregon	41.9	123.4	163	13.6	21.1	7.3	1609	146	365
UM	Umpqua NF, Oregon	43.4	122.8	165	13.8	21	7.8	1609	253	365
BL	Buttle Lake, BC	49.8	125.6	74	9.5	17.6	3	2021	349	201
CW	Cowichan Lake, BC	48.8	123.9	10	10	17.6	3.3	1566	221	205
PM	Pemberton, BC	50.3	122.8	123	7.3	18	-3.2	1412	275	136
CL	Clearwater NF, Idaho	46.2	115.5	162	11.8	23.6	0	1054	311	177
SI	Siuslaw NF, OR	44.5	123.5	103	11.9	19.1	5.7	2181	270	286
SC	Sechelt, BC	49.5	123.8	28	10.1	17.5	3.7	1363	299	243

\* NF = National Forest (in USA)

Lat=Latitude

Long=Longitude

Elev=Elevation

MAT=mean annual temperature (Celsius)

MWMT=mean warmest month temperature

MCMT=mean coldest month temperature

MAP=mean annual precipitation (mm)

MSP=mean summer precipitation (mm)

FFP=average frost-free period (days)

In order to prepare the seeds for the common garden, the fruits were first immersed in concentrated sulphuric acid for two hours (Keir, 2008). The seeds were then transferred into screen bags and placed under running water for 48 hours at which point they were relocated to dampened peat at room temperature and stored there for a period of 30 days. Next, the seeds were cold stratified at 4°C for 30 days. In January of 2007 a total of 5407 seeds from the 11 populations were planted two per cone in research greenhouse facilities at the University of British Columbia. After a heavy rodent infestation, 940 seedlings survived, including representatives of all 11 populations. The seedlings were planted in a randomized block design with 12 blocks and single-tree plots in two raised beds at Totem Field, University of British Columbia.

### **Freeze-testing**

In November of 2008, after the seedlings had undergone two growing seasons, lateral branches were sampled from 405 individuals. The method used for freeze-testing followed the procedure in Hannerz *et al.* (1999) with modifications for deciduous species. Instead of sampling foliage, small slices of twigs approximately 1 mm in length were cut using a razor blade. Two slices from each twig were placed into each of four vials. Each set of vials was exposed to one of four maximum temperatures: 4°C (control), -11°C, -18°C, or -25°C. The control vials were kept in a standard refrigerator at 4°C for 24 hours. The other three sets of vials were placed into a programmable Tenney Environmental Chamber (T20C-3) initially set to 4°C. The freezer was programmed to decrease its temperature by 4 degrees every hour. Once the freezer reached the first temperature (-11°C), it was held there for one hour. At this point, one set of vials was removed. This process was repeated for each of the next two

temperatures, with one set of vials being removed at the end of each one hour holding period. Vials were then transferred to the refrigerator and kept at 4°C overnight to thaw. Once thawed, each set of vials was topped off with deionized water, and put on a shaker (VXR Basic IKA Vibrax) for one hour. Next, the electrolyte leakage of the dogwood cells was measured via a conductivity meter (VWR® Portable Conductivity Meter, Model 2052). After all vials were measured they were placed in a 95°C hot water bath (Fisher Scientific Isotemp 220) for one hour, then removed and placed in the 4°C refrigerator overnight. Next the vials were again shaken for 1 hour and their conductivity was measured once more.

This procedure was repeated in January 2009 only this time 120 individuals were sampled. Additionally, the temperatures of interest in this experiment were 4°C (control), -18°C and -25°C. Although there were 405 trees sampled in November, only the samples corresponding with the 120 individuals sampled in January were analyzed, for a total of 120 samples in November and January respectively.

### Statistical Analysis

In order to assess the level of cold hardiness exhibited, an Index of Injury ( $I$ ), as described by Flint *et al.* (1967), was calculated for each sample:

$$I_t = \frac{100(R_t - R_o)}{(1 - R_o)}$$

where  $I_t$  is the Index of Injury, stated as a percentage, resulting from exposure to temperature  $t$ .  $R_t = L_t/L_k$  is the relative conductance of the sample exposed to temperature  $t$ .  $L_t$  is the conductivity of the sample frozen at temperature  $t$ , and  $L_k$  is the conductivity of the leachate

frozen at temperature  $t$  after being heat killed.  $R_o = L_o/L_d$  is the ratio of the conductivity of the unfrozen control.  $L_o$  is the conductance of the leachate from the unfrozen control and  $L_d$  is the conductance of the heat-killed unfrozen control.

The SAS System for Windows Version 9.1 was used for all statistical analyses. In order to determine the difference in cold-hardiness among populations, an ANOVA was performed using PROC GLM to compare Index of Injury at  $-18^{\circ}\text{C}$ ,  $-25^{\circ}\text{C}$  and the average of the two temperatures, for each date separately. Individual values from all seedlings were used in this analysis. All data was analyzed using an  $\alpha$  of 0.05. A t-test for pairs of population means was used to see which populations differed from each other. This was executed using SAS statements TDIFF and PDIFF within the LSMEANS statement of PROC GLM. Although the common garden experiment was a randomized complete block design, because of the imbalanced subsampling for the cold hardiness testing it was in reality an incomplete block design. In order to make the statistical analysis simpler, two populations were left out since there was not enough data for all 11 populations to be analyzed as a randomized complete block design. The two populations dropped were: Sequoia National Forest, California and Pemberton, British Columbia, leaving 9 populations to be analyzed. Using the average Index of Injury for each date, the  $LT_{50}$  was calculated and plotted in Figure 1. This resulted in analysis of 106 tree samples in each of November and January instead of 120.

PROC REG was used to determine if there was any correlation between the Index of Injury and various climatic and geographic variables such as latitude, longitude, elevation, mean temperature of the coldest month (MCMT), mean temperature of the warmest month

(MWMT), mean annual precipitation (MAP), mean summer precipitation (MSP) and average frost free period (FFP). Population least square means from the test temperature of -18°C in both November and January were used for these analyses. A second regression was run using individual tree data. The data from this test temperature were chosen because it was the most variable.

## Results

The regression analysis using population least square means was not significant and showed only moderate positive correlation of cold hardiness with any of the climatic or geographic variables (Table 1). In both November and January, cold hardiness was most strongly positively correlated with MSP. The  $r^2$  was 0.38 in November and 0.31 in January; therefore the correlation was not very strong. The second highest positive correlation was seen with latitude, resulting in an  $r^2$  of 0.26 in November and 0.31 in January. All other climatic and geographic variables had  $r^2$  values of less than 0.22. After running another regression analysis including all the data from individual trees, two of the climatic variables, MCMT and MSP, had significant regressions (Table 1). However, the  $r^2$  values were 0.0524 and 0.0440, respectively, suggesting only a weak positive correlation.

No significant differences in cold hardiness were detected among populations in January at -18°C ( $p=0.0631$ ), -25°C ( $p=0.0877$ ), or by averaging the results from the two temperatures ( $p=0.0503$ ), although this last result was close to being significant (Table 2). Therefore, overall there was no variation among populations in cold hardiness at any of the test temperatures for the month of January. The ANOVA performed on the data from sampling in November provided

slightly different results. Although there was no significant variation among populations in cold hardiness at -25°C (p=0.2067) or when the two temperatures were averaged (p=0.0716), there was a significant difference at -18°C (p=0.0215) (Table 2). However, by using t-tests to compare pairs of population means, the only differences found occurred between the disjunct population in Clearwater, Idaho and the populations in San Bernadino National Forest and Mendocino National Forest, both in California. All other population pairs showed no significant difference in cold hardiness.

**Table 2. Results of regression analysis at test temperature -18°C using population least square means and individual tree data, respectively. All climatic and geographic variables were positively correlated with cold hardiness.**

	Population LS Means			Individual trees		
	r <sup>2</sup>	F statistic	p value	r <sup>2</sup>	F statistic	p value
<b>November</b>						
Latitude	0.2563	2.07	0.2005	0.0227	2.42	0.1231
Longitude	0.0946	0.63	0.4587	0.0149	1.57	0.2129
Elevation	0.2061	1.56	0.2585	0.0122	1.28	0.2607
MWMT	0.0136	0.08	0.7835	0.0093	0.98	0.3247
MCMT	0.0913	0.60	0.4670	0.0524	5.75	0.0183
MAP	0.2197	1.69	0.2414	0.0013	0.13	0.7417
MSP	0.3848	3.75	0.1008	0.0440	4.79	0.0309
FFP	0.0003	0.00	0.9663	0.0160	1.70	0.1957
MAT	0.0114	0.07	0.8016	0.0023	0.24	0.6265
<b>January</b>						
Latitude	0.3121	2.72	0.1501	0.0220	2.34	0.1288
Longitude	0.1334	0.92	0.3737	0.0358	3.86	0.0521
Elevation	0.2102	1.60	0.2532	0.0186	1.97	0.1630
MWMT	0.1190	0.81	0.4027	0.0288	3.08	0.0822
MCMT	0.1287	0.89	0.3827	0.0003	0.03	0.8650
MAP	0.0033	0.02	0.8918	0.0021	0.22	0.6413
MSP	0.3135	2.74	0.1489	0.0147	1.55	0.2156
FFP	0.0466	0.29	0.6075	0.0005	0.05	0.8191
MAT	0.1831	1.35	0.2902	0.0186	1.98	0.1629

Table 3. Analysis of variance results.

	Block		Population		Block X Population	
	F statistic	p value	F statistic	p value	F statistic	p value
<b>November</b>						
-18	2.88	0.0631	3.25	0.0215	0.96	0.5115
-25	1.24	0.2955	1.58	0.2067	1.33	0.2062
Average	1.93	0.1532	2.33	0.0716	1.01	0.4531
<b>January</b>						
-18	3.62	0.0319	2.42	0.0631	0.79	0.6908
-25	0.49	0.6148	2.18	0.0877	0.92	0.5556
Average	3.07	0.0529	3.01	0.0289	0.86	0.6164

Trees in January were more cold hardy than those in November. As visible in Figure 1, the LT<sub>50</sub> (lethal temperature at which 50% injury occurs) is slightly lower in January (-20.5°C) than in November (-18.5°C). However, when looking at the difference between the testing dates via Figure 2, it can be seen that some populations decrease in cold hardiness, while others increase, even though there is an overall increase in cold hardiness from November to January. Figure 3 and 4 show that the populations again vary in whether they increase or decrease in cold hardiness from November to January, but the average variation in cold-hardiness in January appears to be smaller than that seen in November.

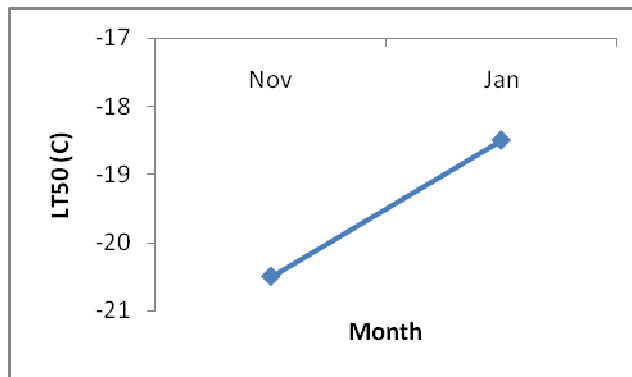


Figure 2. Temperature causing 50% lethality in November 2008 and January 2009.



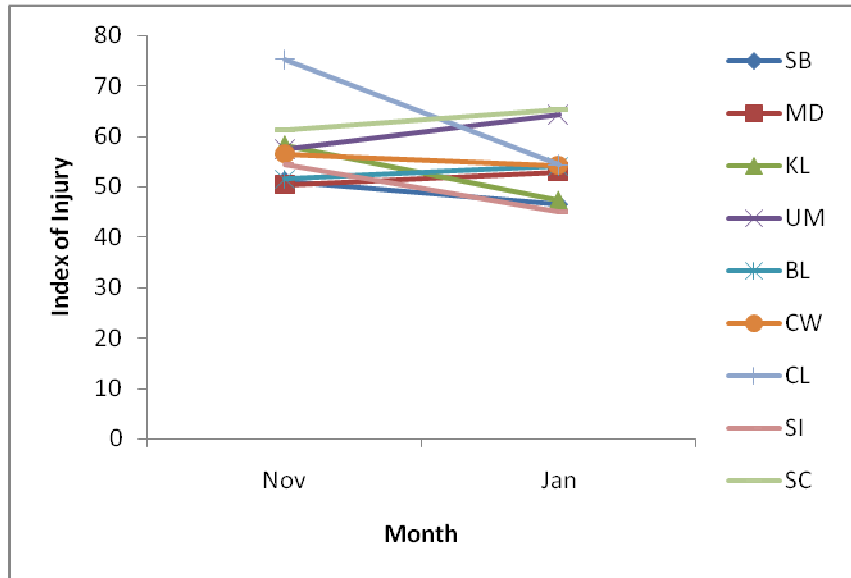


Figure 3. Average change in Index of Injury for each population from November 2008 to January 2009.

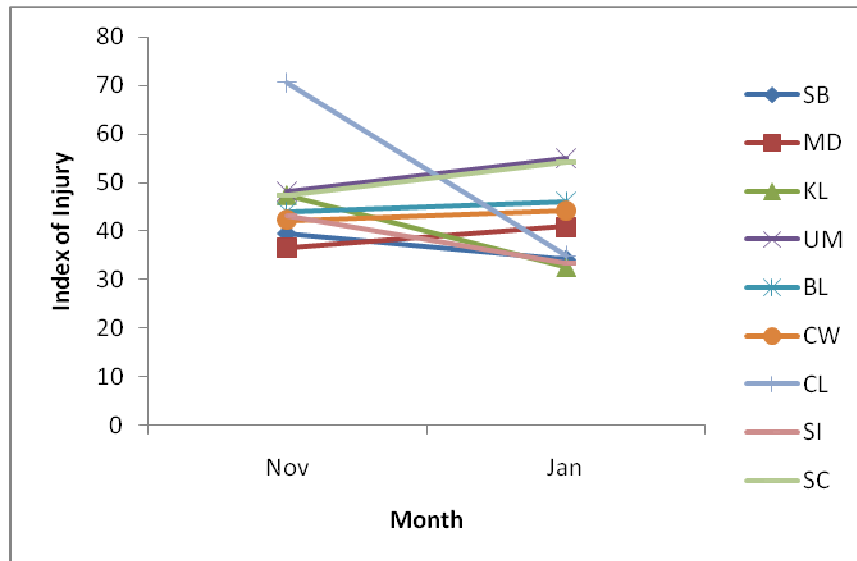


Figure 4. Change in Index of Injury of each population tested at -18°C from November 2008 and January 2009.

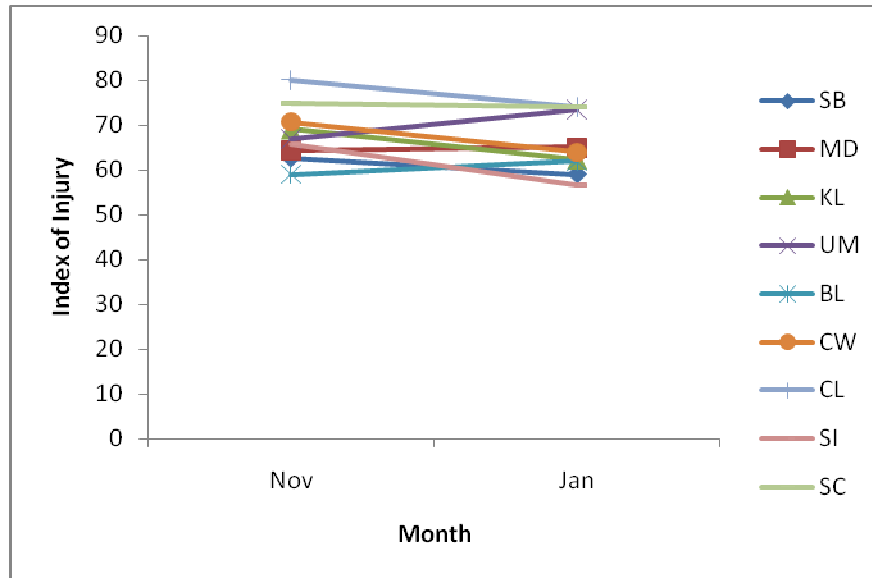


Figure 5. Change in Index of Injury of each population tested at -25°C from November 2008 to January 2009.

## Discussion

In general, the development of cold hardiness is a result of the adaptation of trees to cold winter temperatures. Therefore, it may be expected that certain climatic or geographic variables such as latitude and elevation are correlated with an increase in resistance to cold injury (Bower & Aitken, 2006). However, the results of this study suggest that Pacific dogwood does not follow this general model. There were only weak correlations between the Index of Injury and the various climatic and geographic variables investigated. Regression using population means, showed that cold injury actually increased in populations from higher latitudes and elevations, but again, these relationships were weak (Table 2). This suggests that gene flow may be substantial between central and northern populations, introducing alleles that are deleterious to populations living in a colder climate. Perhaps most interesting is that the strongest positive correlation was seen with provenance mean summer precipitation. Although drought conditions during summer can weaken a plant and make it more susceptible

to cold injury in the winter, a gradual decrease in water content can be favorable to the development of cold hardiness (Sakai & Larcher, 1987). This is likely because mechanisms of frost tolerance in plants usually involve the desiccation of cells so that they are less capable of freezing due to an increase in their solute content (Sakai & Larcher, 1987). From a genetic standpoint, cold response genes are often similar to those upregulated under drought conditions (Holliday *et al.*, 2008). This is likely because cold injury involves desiccation of cells, simulating drought conditions. The gene dehydrin, has been linked to response mechanisms for both cold injury and drought stress (Holliday *et al.*, 2008). Therefore, provenances may show a positive correlation of Index of Injury with MSP simply because some of the genes related to drought stress are involved in cold tolerance as well.

Local adaptation can be described as the ability of an organism to exhibit better performance in its home range than a migrant (Lenormand, 2002). Such adaptations help ensure the long-term success of populations by providing them with the necessary qualities to thrive in their habitat (Slatkin, 1987). Many forest tree populations exhibit local adaptation to their climate in several different variables such as bud flush, bud set, or in their degree of cold hardiness (Howe *et al.*, 2003). This has been documented in several cases such as by Bower and Aitken (2006) who found that whitebark pine (*Pinus albicaulis*) showed significant variation in cold hardiness among regions in both fall and spring cold injury. Another case described by these authors involved the variation in cold hardiness among provenances of mountain hemlock (*Tsuga mertensiana*). The results of Pacific dogwood freeze testing in November and January suggest that these populations do not show much local adaptation in cold hardiness, which is not consistent with patterns of local adaptation of forest tree populations in many other

species. However, midwinter cold hardiness usually shows less variation among populations than in the fall (Howe *et al.*, 2003), which is consistent with this study.

Although local adaptation is common, it may not always occur if conditions for the development of local adaptation are not present. The degree of local adaptation which populations exhibit depends on several features such as natural selection, isolation of populations, genetic drift, gene flow, and the genetic variation of the species as a whole (Slatkin, 1987). Presence of local adaptation can be the result of a number of factors. Populations that have been isolated for some time, or that have low gene flow may show more locally adapted phenotypes. Genetic drift can be a big contributor to genetic divergence of small populations, and natural selection is the main driving factor of adaptation which is especially important when the environmental contrast among populations is high (Slatkin, 1987). These mechanisms frequently operate in conjunction with one another. Nonetheless, the extent of local adaptation is determined primarily by the balance between gene flow and selection (Savolainen, Pyhajarvi, & Knurr, 2007). Conversely, local adaptation can also be limited by many of the factors described above. High gene flow can counteract natural selection and genetic drift occurring in local populations (Slatkin, 1987). By introducing alleles which natural selection may be working against, the process of local adaptation can be slowed. If gene flow is occurring consistently and over a long time period, this can ultimately result in a population with little local adaptation (Slatkin, 1987). However, this depends on both the force of selection and the force exerted by gene flow. A lack of genetic diversity can also be a hindrance to the development of local adaptation. Low genetic diversity can be the result of various events in the history of a species. For example, if a population has been through a

'bottleneck' where few members of the species are left for re-colonization, subsequent populations will lack genetic variation. Thus, if a species does not have very much genetic diversity to begin with, it may not have the necessary variation present in the population that enables some individuals to have higher fitness under certain environmental conditions than others, and consequently increase the frequency of their genes, ultimately resulting in a more locally adapted population (Savolainen *et al.*, 2007).

A recent study on the population genetics of Pacific dogwood by Keir and Aitken (unpublished) based on Keir (2008) and subsequent research suggests that this tree does not have a lot of genetic variation. Their analyses indicate that populations of Pacific dogwood developed in two major ways. During the Pleistocene glaciation, many tree species survived in a southern glacial refugium. Post-glacial re-colonization of this species has had a northward 'leading edge' away from the southern refugium. A 'leading edge' refers to populations that slowly move into new habitat as it becomes available. Below the refugium, populations most likely developed via dispersal events after glacial retreat. Keir and Aitken (unpublished) also note that the disjunct population in Idaho is likely the result of a more recent dispersal event. This pattern of spread is substantiated by their finding that northern populations have slightly less genetic variation than southern populations. Therefore, evidence of Pacific dogwood surviving in a glacial refugium suggests that this species encountered a bottleneck which has resulted in a decrease in its genetic diversity (Keir & Aitken, unpublished). Although all indicators are that this species has low genetic diversity, subsequent generations of these tree populations could still have developed some local adaptation utilizing the limited variation present if populations were exposed to the conditions necessary for the evolution of local

phenotypes such as isolation, strong natural selection, and limited dispersal. However, the findings of this study as well as those of Keir and Aitken (unpublished) suggest this was not the case.

An interesting feature of Pacific dogwood which distinguishes it from many commercially important forest trees, is that seed dispersal involves small mammals and birds (Keir & Aitken, unpublished). In particular, seed dispersal via birds can result in seeds travelling long distances from their host populations, possibly enabling a high degree of gene flow to occur among populations. Consequently, this phenomenon may be contributing to homogenizing the low genetic diversity within the species and continuing local adaptation throughout most of its range. If high levels of gene flow have been maintained for an evolutionarily significant amount of time, this may have been enough to counteract any selection for local adaptations. This may also explain why the population in Clearwater, Idaho is the most genetically divergent from the other populations, as determined by Keir and Aitken (unpublished) and as seen in this study, since it is the most geographically isolated. In summary, Pacific dogwood populations may not display any local adaptation to cold hardiness for a combination of reasons including a past bottleneck resulting in low species genetic diversity, and the maintenance of high gene flow via long distance seed dispersal.

Because Pacific dogwood has likely had low genetic diversity since the Pleistocene glaciation, it is clear that this forest tree has persisted in spite of lacking variation. However, future climatic conditions may make this tree more vulnerable to extirpation or extinction if it lacks the genetic variations that could enable it to adapt to the changing environment. Climate

change is occurring as a result of an increase in carbon dioxide concentrations over the last century, and it is threatening to change species habitat ranges and disrupt the structure of ecosystems (Aitken *et al.*, 2008). With the rate of increase in carbon dioxide concentrations quickening in recent decades, there has been an increasing amount of research in trying to determine where the range of forest tree species will move (Hamann & Wang, 2006). Whether or not trees will be able to adapt to the conditions at hand, migrate with their range, or become extirpated is difficult to predict (Rice & Emery, 2003). Several characteristics of tree species can help predict the outcome such as the amount of genetic variation the species has, dispersal distance, and rate of establishment (Savolainen *et al.*, 2007). The lack of genetic variation for Pacific dogwood may make it difficult for it to weather the changes occurring in its habitat. Although its dispersal distance is wide, it is unclear how quickly this tree may be able to establish itself in a new habitat, especially if there is competition from species it has not encountered before.

The idea of facilitated migration has been explored for several commercial tree species in British Columbia. Facilitated migration involves the movement of seeds or seedlings from the climate that they are grown in currently, to the location where their climate may shift to in the future (Aitken *et al.*, 2008). Facilitated migration is an interesting idea, but it comes with many issues. Although trees may be adapted to the climate of the new site in the future, they may suffer for years or even die if they are introduced there too soon (Aitken *et al.*, 2008). For commercial tree species, this is a big risk to take, as forestry companies rely on good harvest from their plantations. Even if trees suffered at first and were to thrive late in their life, their growth up to that point could be slow, and full of signs of stress and damage, decreasing the

quality of the wood. They also might be outcompeted early by trees adapted to colder conditions and be excluded from stands by density-dependent competition. Pacific dogwood is not considered a tree of commercial interest, so facilitated migration may be a welcome option that can be used simply to conserve this tree species, and keep it from going extinct during the ongoing changes in climate. Nonetheless, seed sources need to be carefully chosen. Rice and Emery (2006) suggest that seed sources be comprised of seeds from different climate zones, but also from different microenvironments within those zones. In this manner, seeds are selected to match climate but the mix of genotypes may also carry enough genetic variation to promote adaptation in the future. Because Pacific dogwood does not show much population differentiation in cold hardiness, moving southern populations slightly further north may not be an issue. Of course, the species as a whole will still have a limited range due to its  $LT_{50}$  of approximately  $-20.5^{\circ}\text{C}$ . Other aspects of the tree and its environmental requirements need to be studied before this method can be employed.

In summary, results of freeze testing in the months of November and January suggest that the nine populations in this study do not vary significantly in their cold hardiness. This result is consistent with the finding by Keir and Aitken (2008, unpublished) that Pacific dogwood has little local adaptation, and has suffered a population bottleneck during the Pleistocene glaciation. The combination of low genetic variation and high gene flow among populations via biological vectors has likely contributed to the lack of local adaptation seen. However, there are more traits that need to be investigated before firm conclusions can be drawn about the local adaptation of this species. The lack of variation in cold hardiness among these populations may be of benefit when considering the use of facilitated migration to ensure the species can



survive climate change. However, the most important tool a species has that will enable it to adapt to change is genetic variation – a quality that Pacific dogwood is deficient in.

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