Adaptation and acclimation of red alder (*Alnus rubra*) in
two common gardens of contrasting climate

by

Brendan Porter
B.Sc., University of Victoria, 2007

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of the Requirements for the Degree of

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Supervisory Committee

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Abstract

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Red alder (*Alnus rubra* Bong.) is the only tree in British Columbia and the Northwest US to engage in actinorhizal symbiosis to fix atmospheric nitrogen. This study was conducted to explore the plasticity in growth and physiology among 58 17-year-old red alder families in response to variation in climate in two common garden plots, one at Bowser, BC and one at Terrace, BC. Physiological assessments included height and diameter growth, bud flush, water use efficiency as measured by $\delta^{13}C$, cold hardiness as measured by controlled freezing and electrolyte leakage, autumn leaf senescence, and instantaneous and seasonally integrated rates of nitrogen fixation as measured by acetylene reduction and natural abundance $\delta^{15}N$ isotope analysis, respectively. Significant differences were identified among families for growth (height and diameter), bud burst stage, leaf senescence, cold hardiness, and bud nitrogen content. No significant differences among families were identified for water use efficiency as measured by $\delta^{13}C$, or for rates of nitrogen fixation as measured by either acetylene reduction or natural abundance $\delta^{15}N$. This study identified possible adaptive differences among red alder genotypes, especially in traits such as bud flush timing, cold hardiness, or nitrogen fixation and their respective contributions to growth. These differences often reflected a tradeoff between growth and the ability to tolerate an extreme environment. Cold hardiness results indicate that red alder families are well adapted to their climate of origin, and may not be able to acclimate sufficiently to a northward assisted migration of genotypes. Nitrogen fixation results demonstrated gaps in our current knowledge of *Frankia* distribution and impact on the actinorhizal symbiosis in British Columbia.

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Introduction

Background

Red alder (*Alnus rubra* Bong. syn. *A. oregona* Nutt.) is the most commonly occurring broadleaf tree in British Columbia (BC) and the Northwest United States (US) (Burns and Honkala 1990, Farrar 1995). A coastal species, it ranges from northern California to southern Alaska, generally within approximately 200 km of the coast, though scattered populations exist in Idaho (Burns and Hokala 1990). Red alder is especially common in riparian or disturbed sites, with some disturbance of the soil being required for successful establishment (Haeussler *et al.* 1995). Red alder establishes quickly following disturbance on these sites and then, as with all members of *Alnus*, fixes atmospheric nitrogen into more biologically accessible forms through a symbiotic association with the actinomycete bacteria *Frankia* (Bousquet *et al.* 1989). As little nitrogen is reabsorbed from the foliage in autumn, much of this nitrogen is added to the soil following leaf drop (Coté *et al.* 1989). This input of nitrogen can increase productivity and functionally change the ecosystem (Binkley 1983).

In BC, the primary focus of forestry activities has traditionally been native conifers, and only since approximately 1990 have broadleaved trees been viewed as more than a nuisance to foresters (Vyse and Simard 2009). As red alder has frequently been treated as an undesirable species in plantations, regulations reflect a view that does not recognize the benefits of planting red alder (Vyse and Simard 2009, Burns and Honkala 1990). Even so, some researchers have found that red alder's genetic variability, rapid growth, and early, prolific seed production present an opportunity for selective breeding programs to increase growth and yield of a potentially economically valuable species (Xie *et al.* 2002). The wood is valued for furniture, fiber-based products, and potential bioenergy applications, while the addition of nitrogen to soils represents a valuable ecosystem service (Burns and Honkala 1990).

Red alder populations in British Columbia have been divided into distinct groups based primarily on isozyme studies: one major group on the Haida Gwaii and Vancouver Island, and another consisting of a mainland population, further subdivided at approximately 52°N into northern and southern sections (Hamann *et al.* 2000, Xie *et al.*

2002, Xie 2008). The division between mainland and island populations is believed to be due to repopulation from two separate refugia following the most recent glaciation of coastal BC, one to the south of the province, and the other to the west, near the current location of the Haida Gwaii (Hamann *et al.* 1998). The most recent work, based on a meta-analysis of isozyme and physiological variables, has divided red alder in the province into six regional groups (Fig. 1), with the Haida Gwaii separated from a divided eastern and western Vancouver Island, and the addition of a Bella Coola (or mid mainland) region between the northern and southern mainland regions (Hamann *et al.* 2011).

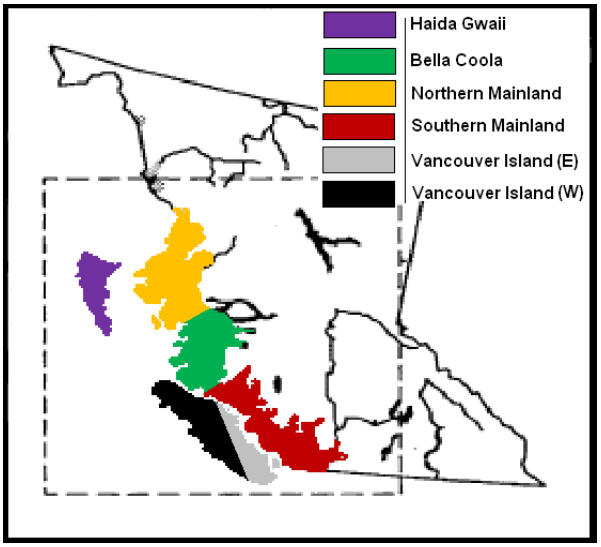

Figure 1: Regional groupings of provenances of red alder (*Alnus rubra*), adapted from Hamann *et al.* 2011.

Some have suggested that geographic origin of red alder genotypes at the regional scale may have less influence on growth than the micro-environment from which the seed originates (Dang *et al.*, 1994). This may have been, in part, due to initial observations that *A. rubra* demonstrated uniform height growth among all populations (Ager *et al.* 1993). More recent isozyme studies have since found clinal variation from the southeast of BC to the northwest (Hamann *et al.*1998). Growth has been demonstrated to vary strongly in relation to the distance between the origin of the seed and the site of planting. Provenances grown in a common garden close to their location of seed origin had higher

growth and survival rates at both 2 years and 6 years than did provenances originating from a greater distance away (Hamann *et al*. 2000, Xie 2008). Elevation has not been found to correlate with provenance growth (Hamann *et al.* 2000); the authors believe this may be due to high gene flow over short distances preventing differentiation due to elevation. However, elevation has been found to weakly predict bud burst in a common garden (Ager *et al.* 1993). Other traits known to vary among genotypes of red alder include midday xylem water potential, transpiration rate, stomatal conductance, response to flooding, and onset of cold hardiness (Dang *et al.* 1994, Hook *et al.* 1987, Cannell *et al.* 1987).

Although red alder has received little serious consideration for use in forest plantations, it has several advantages over coniferous species, particularly in the face of global change. Red alder is able to tolerate or even improve disturbed sites without requiring nitrogen fertilization, while its short lifespan means that climate change is less of a consideration when selecting families for planting in a specific area (McKenney *et al.* 2009). In order to select best-adapted families, physiological variation must be measured and catalogued; to this point red alder physiology has been little studied. As an early successional species with abundant regeneration following disturbance., red alder is more likely to survive a changing climate through a shift in species range, possibly to higher latitudes or altitudes (Aitken *et al.* 2008, Valle-Diaz *et al*. 2009). However, the early successional life history has disadvantages: small, isolated populations may lag behind in their adaptation to new conditions (Aitken *et al.* 2008). In order to better evaluate whether movement of genotypes based on their adaptive traits is necessary, or even possible, this study analyzed a number of key physiological traits with the objective of determining the degree of variation in traits among families or regions, and the strength of red alder's adaptation to local conditions.

Phenology: Bud Burst and Leaf Senescence

The timing of emergence from winter dormancy is of critical importance to temperate trees. To emerge too early risks exposure to late spring frosts, while beginning the growing season late is a disadvantage in the competition for light and other early growing season resources (Beaubien and Hamann 2011). Similarly, extending the

growing season in the autumn allows additional growth, but exposes the tree to potential damage from the initial frosts of winter. As the onset of spring and winter occurs at different times for trees from different latitudes, it is expected that populations will vary in their response to environmental cues for these crucial events. For example, in a common garden plot, northern ecotypes of English oak (*Quercus robur*) tended to flush bud earlier in spring, and responded more readily to cues such as changes in day length and temperature than did southern ecotypes (Jensen and Hansen 2008). The same was true of *Betula pendula*, a relative of red alder in the family Betulaceae (Li *et al.* 2003). These differences are not always large: differences in bud flush among populations of *B. pendula* separated by over 1000 km were on the order of days, while the onset of cold hardiness in autumn was over a month earlier in northern genotypes (Li *et al.* 2003). Similarly, red alder seedlings planted in a common garden in Britain demonstrated buds that burst almost synchronously, with less than a one week difference in the timing between families whose origins spanned from Washington to Alaska (Cannell *et al.* 1987).

The timing of the end of the growing season has been found to vary among ecotypes of trees in the Betulaceae, with *B. pendula* originating from the north ceasing growth earlier than those from the south (Li *et al.* 2003). Extending growth late in the season may increase the total growth of the southern provenances, and it is more often the timing of the end of the growing season that distinguishes among ecotypes with variable growing season lengths, rather than the time of the start of the growing season (Jensen and Hansen 2008, Vitasse *et al.* 2009). However, late senescence may be associated with either higher or lower growth, depending on species (Vitasse *et al.* 2009).

Many temperate trees show significant differences among provenances in growth and phenological characteristics, and though the trends are not always similar, they are often consistent within a species between sampling years (Vitasse *et al.* 2009). With data on bud flush and leaf senescence, combined with cold hardiness and height growth data, I hope to better understand the relationship between growing season length and growth in red alder families, and compare observed patterns to those reported for other temperate hardwood trees. I expected, based on the literature presented above, that red alder families would differ significantly in their timing of bud development over the period of

bud flush, but that the differences would not be very great. Red alder families from the south of the province were expected to show a longer growing season, primarily by delaying leaf senescence in autumn.

Drought Hardiness

Water, despite being abundant in many habitats, is frequently in insufficient supply for optimal plant growth (Taiz and Zeiger, 2010). Projected climate models for coastal British Columbia show a drying trend in many regions which is likely to have a strong impact on the flora of those regions (Hamann and Wang 2006). In order to better predict the effects of this projected drying trend on red alder, the current study examines family differences in drought hardiness. An additional concern is that when breeding for specific traits, such as growth or yield, there is a possibility of decreased stress tolerance. This has been observed for drought hardiness in agricultural species that have undergone artificial selection over many generations (Kumar *et al.* 2011). Identification of drought hardy families of red alder would provide a valuable resource for the breeding program.

Alders in general do not exhibit strong stomatal control of water loss (Borghetti *et al.* 1989), and this holds true for red alder, specifically (Pezeshki and Hinckley 1982). The lack of stomatal control leads to a reasonably constant rate of transpiration in *A. rugosa* (Ewers *et al.* 2007). While this represents a risky strategy during periods of drought, maintaining open stomata under slight to moderate stresses does allow for increased growth compared to co-occurring species exhibiting similarly low drought hardiness, such as black cottonwood (*Populus trichocarpa*), which closes its stomata at a higher plant water potential than red alder when grown on seasonally dry sites (Pezeshki and Hinckley 1982). Pre-exposure to drought conditions does increase the ability of *A. glutinosa* to tolerate drought, likely due to a reduction in leaf osmotic potential, increase in root:shoot ratio, and a thicker cuticle (Seiler 1985); however, Hawkins and McDonald (1994) found no evidence of osmotic adjustment in red alder, nor any other evidence of conditioning to drought conditions.

Provenances of *A. cordata* were found to vary in their ability to tolerate drought, despite the fact that none of the provenances were observed to exhibit strong stomatal control of water loss (Borghetti *et al.* 1989). A study of 40 provenances of red alder seedlings grown in a common garden found statistically significant differences among

provenances in many drought-related characteristics, including mesophyll conductance, transpiration rate, midday xylem water potential, and even stomatal conductance (Dang *et al.* 1994). However, no differences in water use efficiency among provenances were found, possibly because the stomata of red alder are not sensitive enough to allow more efficient use of water under drought conditions (Dang *et al.* 1994).

Seedling shoot biomass was reduced under drought conditions in the European *A. glutinosa*, the North American *A. serrulata* and *A. maritima*, as well as the Asian *A. nitida*, but not the Himalayan *A. nepalensis* (Schrader *et al.* 2005). *A. glutinosa*, the most closely related alder in this study to *A. rubra,* showed a decreased shoot biomass during drought (Schrader *et al.* 2005, Bousquet *et al.* 1989). Even a 3-week drought period without significant precipitation is sufficient to cause significant decreases in growth rates of 4-year old red alder (Giordano and Hibbs 1993). These effects were different between four tested provenances, but no easily detected geographic pattern existed, possibly due to the low number of provenances used (Hibbs *et al.* 1995). Shoot growth is affected more strongly by drought than is root growth in *A. glutinosa*, leading to an increased root:shoot ratio with long term drought (Seiler and Johnson 1984).

As with many stresses, drought stress can decrease photosynthetic rate in alder species (Schrader *et al.* 2005). This decrease in photosynthetic rate could potentially lead to a decrease in the instantaneous rate of nitrogen fixation, as the fixation process requires a constant source of photosynthate (Sundström and Huss-Danell 1987). This effect will be discussed in more detail in the nitrogen fixation section.

Most work on drought hardiness in alders has used seedlings under 3 years old (Schrader *et al.* 2005, Borghetti *et al.* 1989, Seiler 1985, Pezeshki and Hinckley 1982, Pezeshcki and Hinckley 1988). The current study examines potential differences in drought hardiness among mature alder families planted in two common garden plots, with an emphasis on the geographic origins of the families. While previous work on seedlings has shown no significant differences in water use efficiency among families of red alder, differences in other drought characteristics suggest that mature alder families may show some significant difference in their water use efficiency or drought tolerance.

Drought Hardiness and Stable Carbon Isotopes

Carbon in terrestrial C_3 plants has long been known to be isotopically light when compared to atmospheric CO_2 (Bender, 1968). In order to quantify this difference, the isotope ratio for carbon-13 ($\delta^{13}C$) is commonly used (Close *et al.* 2011, Aspelmeier and Leuschner 2004, Sun *et al.* 1996). $\delta^{13}C$ is a specific application of the general isotope ratio formula:

$$\delta X_{std} = \left(\frac{R_{sam}}{R_{std}} - 1\right) * 1000$$

Where:

δX_{std} = isotope ratio relative to a specific standard, expressed as parts per thousand (‰)

R_{sam} = isotope abundance ratio of sample

R_{std} = isotope abundance ratio of standard

(Pearcy *et al.* 1989, Farquhar *et al.* 1982).

For analysis of ^{13}C, samples are compared to the Pee Dee Belemnite standard as, unlike nitrogen, the isotope ratio of carbon in the atmosphere has changed over time, slowly becoming more negative due to dilution of ^{13}C from the burning of fossil fuels (Pearcy *et al.* 1989, Farquhar *et al.* 1989). In terrestrial C_3 plants, values of $\delta^{13}C$ commonly range from -30 to -25‰ (Close *et al.* 2011, Aspelmeier and Leuschner 2004, Lambers *et al.* 1998).

C_3 plants are depleted in ^{13}C relative to the atmosphere because discrimination against the heavy isotope occurs. The primary site of discrimination against heavy isotope incorporation is by the enzyme ribulose bisphosphate carboxylase/oxygenase (RuBisCO) (Farquhar *et al.* 1982). However, the $\delta^{13}C$ of most plants is significantly less negative than predicted (i.e.: the discrimination is less), were RuBisCO the only factor involved (Farquhar *et al.* 1982). This is because the partial pressures of ^{12}C and ^{13}C within the leaf are not exactly equal to those of the atmosphere; assimilation and diffusion together alter the balance (Farquhar *et al.* 1989). The diffusion of gases through the boundary layer and stomata of the leaf causes a weaker discrimination between the isotopes of carbon than does the enzyme (Farquhar *et al.* 1982). This effect becomes more pronounced when resistance to diffusion is higher, for example due to the closing of stomata. Thus, the $\delta^{13}C$ of a plant reflects the relative contributions of the discrimination of both processes: when the stomata are open, and the primary site of discrimination is

RuBisCO, the resulting $\delta^{13}C$ is more negative (isotopically light). When stomata are closed, causing diffusion of the gases to become limiting, RuBisCO must then fix whatever limited carbon is available to it, and $\delta^{13}C$ is less negative (Farquhar *et al.* 1982). In practice, values are normally intermediate between these two, and serve as an indication of which of the two processes are dominating over time (Lambers *et al.* 1998)

Analysis of $\delta^{13}C$ provides a fast measure of water use efficiency (amount of carbon fixed per water lost) that has commonly been used in tree breeding programs, as well as in other applications (Close *et al.* 2011, Aspelmeier and Leuschner 2004, Sun *et al.* 1996). The concept is simply that a more negative value of $\delta^{13}C$ indicates a plant that has spent more time with open stomata, thus using water less efficiently. However, caution must be used in interpreting results from $\delta^{13}C$ data, as observed variation may be due to other factors, such as variation in assimilation rates of carbon (Sun *et al.* 1996). In *B. pendula*, however, water use efficiency was found to depend mainly on stomatal conductance and provenance, with carboxylation efficiency not contributing significantly to the variation (Aspelmeier and Leuschner 2004). A less common concern arises in experimental designs where variation in atmospheric isotope ratio between the lower and upper canopy may require a more complex method to account for these differences (Farquhar *et al.* 1989, Pearcy *et al.* 1989). In the current study, as all samples were collected at a point high in the canopy (see Methods) exposed to well-mixed atmosphere, this is not a serious concern.

Cold Hardiness

In general, trees originating from more northerly provenances show earlier development of cold hardiness when grown in common garden trials (Friedman *et al.* 2008, Weng and Parker 2008, Jensen and Deans 2004, Li *et al.* 2003). For some species, more northerly or more inland populations show greater cold tolerance than southern or coastal populations, even at midwinter (Friedman *et al.* 2008, Jensen and Deans 2004). Other studies, especially those on species in the Betulaceae, show that while provenances may vary in the onset of and emergence from cold hardiness, all populations achieve approximately the same level of hardiness by midwinter (Li *et al.* 2003, Taulavuori *et al.* 2004). This equality is observed even in populations separated by over 1000 km (Li *et al.* 2003). The only study of geographically widespread provenances of *A. rubra* found

variation among provenances in the onset of cold hardiness in seedlings; but due to methodological limitations, the investigators were unable to compare damage sustained at midwinter among provenances (Cannell *et al.* 1987). Tremblay and Lalonde (1987) also were unable to resolve differences among populations, likely due to the physical proximity of the populations used. Between-family variation in cold hardiness was shown to be greater in autumn than in midwinter for *A. sinuata* (Benowicz *et al.* 2000a).

Populations of red alder have been observed to be hardy to temperatures far below those commonly occurring in much of the range of the species, with tissues collected in midwinter suffering less than 50% mortality at -30°C (Cannell 1987). Other deciduous species have also been found to exhibit hardiness to temperatures far below those commonly occurring in their range (Friedman *et al.* 2008, Li *et al.* 2003). This illustrates the importance of hardiness at the onset of and emergence from cold hardiness in autumn and spring, as much of the risk to trees is not due to extreme midwinter cold, but rather to early autumn or late spring frost events (Beaubien and Hamann 2011).

This susceptibility to early or late frost events is of special concern, as current data predicts an increase in the frequency of such events (Beaubien and Hamann, 2011). Many species, including *B. pendula*, use changes in photoperiod to signal the onset of cold hardiness (Li *et al.* 2003). Northern provenances of *B. pendula* are more sensitive to changes in photoperiod than are southern provenances (Li *et al.* 2003). While some alders, such as the northern *A. crispa* show strong sensitivity to changes in day length, low temperature is the more important signal for development of cold hardiness in *A. rubra* (Tremblay and Lalonde 1987). Temperature is also the primary cue for resumption of growth in spring (Ager *et al.* 1993). Warming climates are expected to increase the risk of premature dehardening of trees, and thus increase the likelihood of exposure to unseasonable frost events occurring in late spring (Beaubien and Hamann, 2011).

An earlier emergence from winter dormancy might be expected to be associated with an earlier bud flush in spring. While this trend does not always hold (Weng and Parker 2008), it is exhibited by *A. sinuata* (Benowicz *et al.* 2000a). Red alder has been found to have drastically decreased cold hardiness following bud flush in spring compared to midwinter hardiness (Cannell *et al.* 1987). In autumn, it has been shown in *Quercus* that there is no strong correlation between the end of the growing season (bud

set) and autumn cold hardiness (Jensen and Deans 2004). While red alder has been found to show variation among families in date of bud set (Cannell 1987), it remains to be demonstrated whether earlier bud set is correlated with earlier onset of cold hardiness.

Much of the damage sustained by plant cells during freezing temperatures is due to dehydration of the cell (Close 1997), and there are many similarities in the response of plants to both freezing and drought stresses (Siddiqua and Nassuth 2011, Wang *et al.* 2011). A signal for the initiation of both drought and frost hardening is abscisic acid (Li *et al.* 2003, Seiler and Johnson 1984). Drought stress has been found to slightly increase freezing tolerance and plant sensitivity to both short days and low temperatures; however, its effect was less than either day length or temperature (Li et al 2002).

A lack of significant correlation between frost hardiness and biomass for *A. sinuata* led Benowicz *et al.* (2000a) to suggest that it is possible to find red alder families showing both high tolerance to cold and increased growth. Such families would be of particular value in any red alder breeding program looking to improve yield.

It is also possible that rates of nitrogen fixation might be correlated with cold hardiness. High instantaneous nitrogen fixation rates are dependent on increased photosynthate availability (Sundström and Huss-Danell 1987) which might also increase resources available for cold hardening. Midsummer photosynthetic rates have been found to be higher in *A. sinuata* and paper birch (*B. papyrifera*) originating from areas with colder winters or shorter growing seasons, and showing higher cold hardiness in the previous November (Benowicz *et al.* 2000b).

In my study, I expected red alder to show significant differences in mean family cold hardiness throughout the winter, with families originating from farther north developing cold hardiness earlier in the autumn and maintaining greater hardiness through the winter. At Bowser, I expected genotypes from farther north to deharden earlier in the spring.

Cold Hardiness and Electrolyte Leakage

Cold hardiness is commonly evaluated either by visual assessment of damage after cold treatment (Tremblay and Lalonde 1987, Cannell *et al.* 1987, Deans *et al.* 1992, Jensen and Deans 2004) or by measuring electrolyte leakage after treatment (Friedman *et al.* 2008, Taulavuori *et al.* 2004, Li *et al.* 2002, Hannerz *et al.* 1999). Of these methods,

electrolyte leakage is more labour-intensive, but has been found to yield more consistent statistical results (Jensen and Deans 2004). Electrolyte leakage is usually measured by controlled freezing of tissues to a specific temperature (or temperatures), then adding a set volume of water and measuring the conductivity of the resultant solution. In order to provide a control against which to compare these conductivity values, the tissues are then entirely killed by heating and the conductivity measured again (Flint *et al.* 1967). The two values, plus the values from an unfrozen sample, are combined to provide a single value, the index of injury. This index is a relative value of electrolytes leaked, expressed on a scale of 0-100, where 100 is the value for the heat-killed tissue. A higher index of injury indicates tissues that have sustained more damage due to the freezing treatment, and are thus less cold hardy. Index of injury can be compared directly (Weng and Parker, 2008), or interpolated to generate the temperature at which a given percentage of mortality has occurred (frequently 50% of maximum, expressed as LT_{50}) (Li *et al.* 2002, Taulavuori *et al.* 2004, Deans *et al.* 1992).

While deciduous leaves do show some ability to develop frost hardiness (Li *et al.* 2002), the use of deciduous leaves as a sample tissue is limiting, as it precludes mid-winter measures. For the current study, stem tissue was used to evaluate cold hardiness via the electrolyte leakage method. It is known that stems develop freezing tolerance later than leaves or buds (Li *et al.* 2002); however, stem tissue can still be used to detect differences in cold hardiness among provenances (Weng and Parker 2008).

Nitrogen Fixation: Background and Benefits

Terrestrial environments are bathed in an ocean of nitrogen gas; however, in most natural ecosystems, lack of available nitrogen is a major limitation to plant growth (Taiz and Zeiger 2010). This apparent contradiction is due to the nature of atmospheric N_2: the triple bond between the two atoms is difficult to break, and only a select few organisms, of which none are plants, are capable of the feat. The fixing of atmospheric dinitrogen into a more biologically useful form is performed primarily by prokaryotes, including cyanobacteria, *Rhizobium*, and *Frankia*. Both *Rhizobium* and *Frankia* form tight symbiotic associations with plants in the form of root nodules. These symbioses occur in many families of angiosperms, but DNA evidence suggests that all families known to

engage in nitrogen fixing symbioses belong to the same clade within the eurosids I, together with some families unable or not known to fix nitrogen (Soltis *et al.* 1995). Sequence analysis of the large subunit of RuBisCO suggests that legumes (which associate with *Rhizobium*) are in one subclade, while actinorhizal plant species (which associate with the nearly-ubiquitous *Frankia*) are in three other related subclades (Soltis *et al.* 1995).

Because nitrogen can often be limiting, one would expect that the addition of nitrogen fixing organisms to an area would alleviate this restriction on plant growth. Since the early 1980s, evidence of the actinorhizal association's positive effects on both the plant symbiont and the surrounding vegetation has been accumulating. Alders exhibiting high rates of nitrogen fixation also have large leaf and total biomass (Bormann and Gordon 1984, Hawkins and McDonald 1994). Perhaps surprisingly, increased nitrogen fixation rates were not associated with an increase in foliar N in the alder, but due to increased biomass, the total amount of N in the plant was higher (Hawkins and McDonald 1994). In contrast, the presence of alder is associated with an increase in the foliar N concentration in nearby Douglas-fir (Binkley 1983), and in the understory vegetation beneath it (Rhoades *et al.* 2001). This increase in foliar N can be linked by ^{15}N analysis to the nitrogen fixed in the alder nodules (Rhoades *et al.* 2001). It has also been demonstrated that alder litter can increase soil N levels (Rhoades *et al.* 2001, Son *et al.* 2007). This increase in available N may cause other nutrients to become limiting, though experimental evidence is mixed. While some have found a decrease in foliar phosphate (P) concentrations in surrounding vegetation (Binkley 1983), others have shown a significant increase in soil P levels under alder (Rhoades *et al.* 2001).

The nitrogen from alder litter fall can allow surrounding Douglas-fir to divert resources from root development to shoot growth (Binkley 1984). This increased investment in shoot growth may be of particular value in an economically important tree such as Douglas-fir. Ecologically, the total biomass and productivity of an ecosystem can be increased by the presence of red alder (Binkley 1983); however, this effect may be negated if the system is already rich in nitrogen, as fixation rates are low when the energy would be more efficiently spent on absorption rather than fixation (Son *et al.* 2007).

Nitrogen fixation is an energetically expensive process. The enzyme that catalyzes the reaction, commonly referred to as "nitrogenase", is, in fact, two proteins: dinitrogenase and dinitrogenase reductase. Dinitrogen reductase provides reducing power to dinitrogenase in order to fuel the enzymatic fixation (Burris 1991). However, the reaction wastes a significant proportion of its reducing power converting protons to H_2. Under normal conditions, 20-30 ATP are required to convert a single N_2 molecule to $2NH_3$ (Burris 1991). Despite differences in endosymbiont and nodule morphology, the levels of nitrogenase activity as well as the energy requirement for that activity are similar between legumes and non-legumes (Tjepkema and Winship 1980).

The general reaction carried out by nitrogenase is as follows (Burris 1991):

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$$

The enzyme's activity is inhibited by O_2, and H_2. Oxygen can also inhibit the synthesis of the nitrogenase enzymes (Huss-Danell 1997). Because of these limitations, both plant and bacteria have developed methods of protecting nitrogenase. In the nodules of alder, *Frankia* develops vesicles: specialized cells similar to the heterocysts of cyanobacteria. These vesicles have a thick envelope consisting mainly of lipids, which presumably serves to slow the diffusion of O_2 into the vesicle (Huss-Danell 1997). While many plants' nodules contain hemeoproteins to scavenge O_2, high concentrations are not required for nitrogen fixation in actinorhizal associations, and the nodules of alder contain little hemeoprotein (Tjepkema and Asa 1987). It appears that, in alder, the vesicle envelope is the major mechanism for limiting the exposure of nitrogenase to oxygen (Silvester *et al.* 1988, Rosendahl and Huss-Danell 1988). Hydrogen inhibition may be minimized by hydrogenase as it helps keep H_2 concentrations low; however, it does not appear to be required for proper function of nitrogenase (Huss-Danell, 1997).

While oxygen can inhibit nitrogenase, large amounts of energy, generated by aerobic metabolism, are required by the enzyme (Winship and Tjepkema 1985). Therefore, the nodule cannot exclude oxygen entirely, but must keep concentrations within a range between inhibition and oxygen starvation. The declines in nitrogen fixation under stressed conditions may, in many cases, be due to physical or chemical

damage to the vesicle membrane, disrupting the balance of O_2 concentrations (Wheeler *et al.* 1978).

Measurement of Nitrogen Fixation

In order to measure rates and absolute amounts of nitrogen fixation, several methods have been devised, each with associated advantages and limitations. For the purposes of this study, the two methods selected were the acetylene reduction assay (ARA) and natural abundance $\delta^{15}N$ analysis. Other methods, such as total nitrogen difference, the xylem-solute method, or ^{15}N enrichment, were either too inaccurate, or impractical to apply to a large number of mature alder trees (Danso, 1995).

Nitrogen Fixation: The Acetylene Reduction Assay (ARA)

Nitrogenase is capable of reducing a wide range of substrates (Hardy *et al.* 1968). One of the most scientifically useful alternative reactions is the reduction of acetylene to ethylene. The acetylene reduction assay takes advantage of nitrogenase's equal response to nitrogen and acetylene (Hardy *et al.* 1968). Both reactions use ATP and reductant, and most reaction characteristics and optima are similar (Hardy *et al.* 1968). Very little acetylene is required to completely saturate the enzyme (reported initially by Hardy *et al.* (1968) as 3-10%, v/v). Since 1968, many studies have used ARA to estimate nitrogen fixation rates either of individual trees or entire plots of land (Tripp *et al.* 1979, Anderson *et al.* 2004, Son *et al.* 2007). While initially, it was known that high concentrations (0.5 atm) of acetylene were inhibitory to nitrogenase (Hardy *et al.* 1968), the assay conditions were such that this was not believed to be an obstacle. However, while measuring *Rhizobium*-legume symbionts, Minchin *et al.* (1983) reported a flaw in the closed container ARA typically performed until that point. Using an open flow system which constantly measures ethylene production, they demonstrated that nitrogenase activity declines over time once exposed to concentrations of acetylene commonly used in the assay, and this decline can occur on the order of minutes post-exposure (Minchin *et al.* 1983). As the standard closed-container ARA is generally performed over longer time scales, this result suggested that any calculations made using these methods was likely an

underestimate of actual nitrogenase activity. The interpretation of the assay is further complicated. While Hardy *et al.* (1968) initially suggested a conversion factor of 4 acetylene reduced per nitrogen (3 for the bonds of nitrogen, and 1 for a "wasted" reduction of two protons), this value has been found to vary, either downward due to underestimation from acetylene-induced decline, or upward due to increased fixation of H_2, possibly due to stress (Schwintzer and Tjepkema 1994). The conversion factor has also been found by Anderson *et al.* (2004) to vary by season or successional stage (the two factors could not be separated). Thus, the values of acetylene reduction rates from a single site measured at the same time are comparable to one another, but converting those values to an absolute amount of nitrogen fixed may be problematic.

Further concerns were raised by Minchin *et al.* (1986) when it was found that physical disturbance to nodules, including simply shaking off loose soil, decreased the measured rate of acetylene reduction. This augmented work from eight years earlier, reporting that removing the shoot and transferring the root system to a new container for analysis could decrease the ARA-measured nitrogenase activity by up to 50% (Wheeler *et al.*1978). Minchin *et al.* (1986) investigated the decline in more detail in two *Rhizobium*-associated species, and found that physical disturbance of the nodule (shaking or brushing away loose soil) caused a greater decrease in activity than the removal of shoot tissues. These effects were less significant at lower growth and incubation temperatures (Minchin *et al.* 1986). This last result led the researchers to suggest that any environmental stress (including drought) may cause nodules to become insensitive to physical disturbance which may lead to a change in ranking between families or individuals, should they vary in their ability to tolerate or alleviate the stress. Indeed, when the first report of the effects of drought stress on the time course of acetylene reduction was produced, it was found that drought caused a deeper acetylene-induced decline, and a decreased rate of recovery (Schwintzer and Tjepkema 1994), though variation between individuals or families was not measured. Independent of the time-course data, it had previously been found that water potentials of -0.6 to -0.8 MPa (designated "moderate stress") caused acetylene reduction to decline by half in *A. incana*, while *A. glutinosa* was able to maintain near maximal rates until a sharp decline at -1.30

MPa, a level at which there was no nitrogen fixation activity in *A. incana* (Seiler and Johnson 1984, Sundström and Huss-Danell 1987).

Nodules of actinorhizal species respond less to acetylene than do legumes, with a much smaller decline in nitrogenase activity (Tjepkema *et al.* 1988). Furthermore, rates have been found to recover spontaneously within minutes to near-maximal in *A. incana* (Silvester *et al.* 1988), as well as other actinorhizal species (79%-98% of maximum rate) (Tjepkama *et al.* 1988). *A. incana* was found to experience little acetylene-induced decline in activity (14% decline), with rates remaining stable at the lower value (Rosendahl and Huss-Danell, 1988). Red alder was found to decline to a minimum rate of 47% of maximum approximately 1-5 minutes post-exposure, followed by recovery to 87% of maximum within 10 minutes post-exposure when no other stresses or disturbances were present (Tjepkema *et al.* 1988). This recovered rate was maintained to at least 60 minutes post-exposure (Tjepkama *et al.* 1988). Both studies found a smaller acetylene-induced decline in respiration rates than has been found in legumes (Rosendahl and Huss-Danell 1988, Tjepkema *et al.* 1988). Overall, the acetylene-induced decline in nitrogenase activity appears to be smaller in actinorhizal species than in legumes, and fixation rates in actinorhizal species (including red alder) may spontaneously recover to near-maximum values (Tjepkema *et al.* 1988).

Weather, too, may affect the results of ARA. Increased air temperature (both daily mean and at 1 pm), increased soil temperature and daily sunshine hours have been found to correlate with increased rates of acetylene reduction (Ekblad *et al.* 1994, Son *et al.* 2007). These factors may explain, in part, the seasonal variation in acetylene reduction: highest in early spring and summer, and declining from late summer into late fall (Son *et al.* 2007). Even over the course of a single day, rates can vary: maximum rates were observed to be later in the day in August when compared to May or October (Lee and Son, 2005). In contrast with temperature and light, humidity was found to be negatively correlated with acetylene reduction rates (Ekblad *et al.* 1994). While minimum temperature and rainfall events were not found to significantly affect acetylene reduction rates, very few rainfall events occurred during the test period, and so their importance cannot be ruled out (Ekblad *et al.* 1994). Weather up to two days before measurement has been found to significantly affect the ARA, and it has been suggested that the decline in

acetylene reduction in late summer can be explained by weather-based models (Ekblad *et al.* 1994). These models emphasize the importance of humidity, sunshine hours, and mean as well as maximum air temperature on the rates of acetylene reduction (Ekblad *et al.* 1994).

Despite complications, ARA continues to be widely used, especially for relative estimates of nitrogen fixation rates (Markham 2008a,b, Lee and Son 2005, Anderson *et al.* 2004, Batzli and Dawson, 1999, Hibbs *et al.* 1995, Hawkins and McDonald 1994). Even critics of the method, when discussing the assessment of legumes that are less suited to the method, admit that it is a useful tool for relative ranking of nitrogen fixers (Minchin *et al.* 1983, Danso 1995, Vessey 1994). Incubation times can be kept short to avoid effects of acetylene-induced decline (Anderson *et al.* 2004, Vessey 1994), and are commonly run to at least 60 minutes when *A. rubra* is being measured (Batzli and Dawson 1999, Hibbs *et al.* 1995, Hawkins and McDonald, 1994). Though it may not be an appropriate measure for absolute values of nitrogen fixation (Vessey 1994), some researchers continue to use closed container ARA to estimate nitrogen fixation over large areas of land (Lee and Son 2005).

ARA allows relatively rapid sample collection, and gas samples can be stored for long periods prior to analysis (Bormann and Gordon 1984). In a study such as mine, where the emphasis is on relative differences rather than absolute volume of nitrogen fixed, ARA remains a valuable tool. While it has been rarely used on large trees, the method has been demonstrated to work under such conditions (Lee and Son 2005).

Because the results of the ARA often appear to be affected by the stress status of the individual tree, I expected that those trees best suited for each site (as determined by other physiological traits) would show the greatest rates of nitrogen fixation. I expected families originating from the region near each test site to fix nitrogen at a higher rate than genotypes originating from farther away.

Nitrogen Fixaton: Natural Abundance $\delta^{15}N$

While acetylene reduction provides a method to measure instantaneous rates of nitrogen fixation, it is of interest to test how well this measure compares to estimates of nitrogen fixed over the entire growing season. Extrapolation of ARA measured at a

number of time points is possible, but of limited value (Danso 1995). By taking advantage of a variance in nitrogen isotope ratio between soil and atmosphere, it is possible to measure the percentage of a plant's nitrogen that has been fixed from the air. The amount of ^{15}N present in the atmosphere is constant at 0.3663 atom %. Due to discrimination in plant uptake, bacterial nitrification and subsequent loss of N_2O, as well as other soil processes, soils generally have a higher ratio of ^{15}N:^{14}N than air, as the heavier isotope is left behind (Pérez *et al.* 2006, Högberg *et al.* 1999). Thus N-fixers usually have lower isotope ratio (a more negative $\delta^{15}N$) than do non-fixers in the same environment (Shearer and Kohl 1986). $\delta^{15}N$ is another specific application of δX_{std} discussed above, using the constant nitrogen isotope ratio of the atmosphere as the standard against which samples are compared (Pearcy *et al.* 1989). This measure of natural abundance has several advantages, in that it involves no addition of N to the soil (as is done by fertilization in dilution-style ^{15}N experiments) and therefore causes no inhibition of N-fixation by fertilization, there is no disturbance of the natural system (minimized but not entirely avoided with ARA), the estimate is long-term (over an entire growing season if desired), and finally, tissues may be dried in the field and processed at a later time.

While rankings of ^{15}N:^{14}N ratios are valuable for relative comparisons (Danso 1995), in order to estimate the absolute amount of nitrogen fixed on a site, isotope analysis methods require a non-nitrogen-fixing plant as a control. Ideally, this control plant will have growth characteristics nearly identical to the species being tested, and will co-occur on the test site. While it is possible to simply measure the ^{15}N present in the soil, a control plant takes into account the amount of ^{15}N present at various depths and what proportion of this nitrogen is taken up from each depth (Shearer and Kohl 1986). Control plants also integrate nitrogen uptake over a period of time and, to some degree, compensate for the isotope discrimination of the roots, more closely replicating the conditions experienced by the test plant (Shearer and Kohl 1986). While finding a co-occuring control plant that perfectly mimics the test plant is, in many cases, impossible, Busse (2000) has found that for plants fixing large amounts of nitrogen (up to 80% of total nitrogen), the control plant's growth characteristics have less impact on the estimate than if the test plant has low rates of nitrogen fixation. Therefore, exact matching of

growth characteristics between control and test plants becomes less critical for more efficient nitrogen-fixers. Even for less efficient N-fixers, the use of several species of reference plants may increase confidence in the estimate of the non-fixing ratio of ^{15}N:^{14}N (Shearer and Kohl 1986). Finally, one cannot assume that the soil is higher in ^{15}N than the atmosphere at all test sites, thus this assumption must be demonstrated in each test area and if possible, one should use reference plants from throughout the test area (Shearer and Kohl 1986).

The primary disadvantage of the natural abundance $\delta^{15}N$ method arises due to differences in concentration of ^{15}N between atmosphere and soil potentially being very small, and so differences may be lost in the error inherent in measuring isotope concentration. Similarly, the values attained may not be as precise as other methods (such as ARA). Within a single plant, the distribution of ^{15}N is fairly uniform, with the exception of nodule tissues (Shearer and Kohl 1986). In order to best represent differences in stable N isotope ratio due to nitrogen fixation rates, my study compared the same non-nodule tissues across all test plants.

Nitrogen Fixation and Abiotic Stresses

While individual abiotic stresses have their own set of impacts on the entire tree, the manner in which they impact the nodules and nitrogen fixation processes is not entirely clear. Two proposed mechanisms of action are 1) changes in vesicle membranes in the nodules cause a disruption in the ability of the membrane to exclude O_2 (Wheeler *et al.* 1978) and 2) decreased photosynthetic activity due to damage elsewhere in the plant inhibits the energetically expensive nitrogen fixation process (Sundström and Huss-Danell 1987). The two stresses studied are discussed in more detail below.

Drought

As with any symbiosis, the pair of species involved is limited in its ability to function in stressful or extreme environmental conditions by the more restricted of the two. In the case of *Alnus* and *Frankia*, the drought tolerance of the host is limiting, rather than that of the endosymbiont (Shipton and Burggraaf 1982, Hennessey *et al.* 1989). However, nitrogenase activity can continue (albeit at very low rates) even in very severe

drought conditions (Hennessey *et al.* 1989, Son *et al.* 2007). The decrease in nitrogenase activity is largely due to the high energy demands of the process; indeed, it has been found that nitrogenase activity drops concurrently with stomatal closure, suggesting that photosynthates are required continuously (Sundström and Huss-Danell 1987). While the decline in activity was quite rapid, recovery after re-watering was found to be much slower, on the order of a few days, with the more highly stressed treatment showing the slowest recovery (Sundström and Huss-Danell 1987). Reduced photosynthate availability does not appear to be the sole cause of the observed decline. When plants were dark-treated, to similarly reduce photosynthate availability to the nodules, the decline in nitrogenase activity was much slower (>3 days). The authors suggest that drought may directly damage the nitrogenase system, or at least affect the functioning of the system in different ways (Sundström and Huss-Danell 1987, Schwintzer and Tjepkema 1994). Damage to the nitrogenase system may be averted so long as some roots have access to water, however. Split root experiments have shown a net movement of water from wetted roots to dry roots sufficient to maintain approximately 70% of maximal nitrogenase activity, even in dry nodules (Sundström and Huss-Danell 1995).

Over the long term, drought stress alone does not influence nodule biomass (Hibbs *et al.* 1995), but the interaction of high temperature and slight drought has been found to decrease ARA values (Hawkins and McDonald 1994). The impact of slight drought was found to be increasingly significant at higher temperatures despite the treatment being mild enough that no significant differences were observed in transpiration, photosynthesis, or stomatal conductance (Hawkins and McDonald 1994). These declines in ARA do not appear to be prevented by drought conditioning of *A. glutinosa* (Seiler and Johnson 1984).

The effect of drought on nitrogen fixation tends to be negative, with stressed trees less able to fix nitrogen. As the drought tolerance of the symbiosis is driven primarily by the alder host (Shipton and Burggraaf 1982), I expected that more water use efficient families would show higher rates of nitrogen fixation.

Temperature

As mentioned above, high temperature and drought interact in red alder to decrease measured rates of acetylene reduction (Hawkins and McDonald, 1994). High temperature (>30°C) alone has been found to be sufficient to reduce acetylene reduction without drought (Winship and Tjepkema 1985), while lowered temperatures (15°C) were found to cause an increase in root:shoot biomass, while simultaneously decreasing nodule mass (Hawkins and McDonald 1994). As far as we are aware, no study has analyzed the correlation between winter frost hardiness and summer nitrogen fixation in *Alnus*.

Objective

The objective of this research was to explore the plasticity of growth and physiological responses among red alder families in two climates. My approach was to identify differences in adaptation and acclimation to climate among genotypes from across the range of red alder by assessing select key physiological factors contributing to the growth and performance of red alder on two contrasting sites.

Methods

Site Background

The physiology of red alder from BC was studied at two provenance-progeny test trials established by the BC Ministry of Forests in early 1994. One trial site was located on the east coast of Vancouver Island near Bowser (49°29'N, 124°40'W, elevation 50m); the other was located near Terrace, BC (54°27'N, 128°8'W, elevation 200m) (Fig. 2, Table 1). Alder families established from open-pollinated seed collected from wild stands and grown in a nursery in Surrey, BC for one year were planted at each site (Xie, 2008). Each site was divided into 3 blocks; however, one of the blocks planted at Bowser suffered high mortality well before the start of this experiment and so was not used. Within each block, open-pollinated families were planted in rows of 5 trees, located randomly within the block. A total of 116 families from 36 provenances (sites of origin) remained at both the Bowser and Terrace site in the spring of 2010. At Bowser, the understory plants included salmonberry (*Rubus spectabilis*), bracken fern (*Pteridium aquilinum*), sword fern (*Polystichum munitum*), Oregon grape (*Mahonia nervosa*), salal (*Gaultheria shallon*), and vanilla leaf (*Achlys triphylla*). The understory vegetation at the Terrace site was primarily hardhack (*Spirea douglasii*), red elderberry (*Sambucus racemosa*), red raspberry (*Rubus idaeus*), lady-fern (*Athryium filix-femina*), false lily of the valley (*Maianthemum dilatatum*), fireweed (*Epilobium angusifolium*), and bunchberry (*Cornus canadensis*).

Table 1: Mean annual temperature (T), mean temperature of the warmest month (T_w), mean temperature of the coldest month (T_c), mean annual precipitation (p), mean summer (June-August) precipitation (p_{summ}) and mean precipitation as snow (p_{snow}) for the Bowser and Terrace test sites. Weather data is from 1971-2000, obtained from the National Climate Data and Information Archive available online from Environment Canada (climate.weatheroffice.gc.ca). All data shown are from the single nearest weather station to each test site: Terrace A (54°27' N, 128°34' W, elevation 217 m) for Terrace, Qualicum River (49°23' N, 124°37' W, elevation 8m) for Bowser.

	T (°C)	T_w (°C)	T_c (°C)	p (mm)	p_{summ} (mm)	p_{snow} (mm)
Bowser	9.3	16.8 (Jul)	3 (Jan)	1314.2	112.2	50
Terrace	6.3	16.4 (Jul)	-4.3 (Jan)	1322.4	166.7	375.4

Family Selection

In an attempt to include families with a greater and lesser ability to acclimate to climatic differences, I selected families that did or did not differ in their relative performance between the two climatically different test sites. For all 116 families planted at both Bowser and Terrace, the difference in height rank between the two sites was calculated for each family using height data 10 years after planting from the BC Ministry of Forests. Initially, families were selected whose difference in height rank was 20 or less and 50 or more. From this list of 69 families, 30 were selected from each of the two groups (large or small differences in height rank between the two sites after 10 years). Families with high mortality (fewer than two surviving individuals in any block) were eliminated. Priority was given to selection of geographically separated families, as well as pairings of families from the same provenance showing large and small differences in height ranking. This selection reduced the total to 58 families in 35 provenances (Table 2). Families in each provenance were assigned to one of the 6 regions outlined by Hamann et al. (2010), based on their geographic origin: east or west Vancouver Island, Haida Gwaii, Bella Coola, northern mainland or southern mainland (Fig. 2). Due to logistical limitations, the 50 families (25 from each of the upper and lower limits of rank difference) were used for assessments of cold hardiness, acetylene reduction, and isotope analyses, while all 58 families were used for bud burst and canopy cover estimates.

Table 2: Seed origin for each of the selected families planted at Bowser and Terrace. Seed collected by the BC Ministry of Forests. Family number was used to distinguish families within the same provenance. Latitude in °N (Lat) and Longitude in °W (Long), as well as Elevation in metres (Elev) are given for each family.

Provenance Name	Provenance	Family	Lat (°N)	Long (°W)	Elev (m)
Port Renfrew	4	4	48°36’	124°14’	20
Port Renfrew	4	5	48°36’	124°14’	20
Klanawa #2	6	1	48° 46	124 58	40
Nitinat Flats	8	3	48°50’	124°40’	30
Nitinat Flats	8	4	48°50’	124°40’	30
Sarita Lake	11	3	48°55’	124°52’	40
Sarita Lake	11	4	48°55’	124°52’	40
Between the Lakes	13	3	48°58’	124°43’	200
Between the Lakes	13	5	48°58’	124°43’	200
Ucluelet	14	2	49°00’	125°34’	40
Cassidy	15	2	49°03’	123°56’	107
Cassidy	15	8	49°03’	123°56’	107
Britannia Creek	21	5	49°07’	123°07’	660
China Creek #2	23	5	49 10	124 41	400
Indian River	30	1	49°34’	122°56’	190
Indian River	30	3	49°34’	122°56’	190
Indian River	30	4	49°34’	122°56’	190
Indian River	30	5	49°34’	122°56’	190
Pender Harbour	31	2	49°39’	124°02’	150
Pender Harbour	31	3	49°39’	124°02’	150
Mamquam River	32	1	49°43’	123°07’	100
Mamquam River	32	2	49°43’	123°07’	100
Culliton Creek	35	1	49°53’	123°11’	250
Culliton Creek	35	2	49°53’	123°11’	250
Woss #2	37	1	49°58’	126°15’	1000
Woss #2	37	5	49°58’	126°15’	1000
Roberts Lake	43	5	50°13’	125°33’	700
Bigtree #1	44	1	50°14’	125°43’	250
Bigtree #2	45	2	50°14’	125°43’	300
Bigtree #2	45	3	50°14’	125°43’	300
Ronning Main	48	2	50°36’	128°15’	30
Ronning Main	48	4	50°36’	128°15’	30
San Josef Main	50	1	50°40’	128°04’	20
San Josef Main	50	4	50°40’	128°04’	20
NE 62	51	2	50°43’	127°59’	170
NE 62	51	3	50°43’	127°59’	170
Kingcome Inlet	52	1	51°30’	126°08’	30

Poole Inlet	53	2	52°21’	131°21’	1
Poole Inlet	53	3	52°21’	131°21’	1
Hagensborg	54	4	52°22’	126°35’	40
Hagensborg	54	5	52°22’	126°35’	40
Bachelor Bay	55	3	52°22’	126°55’	30
Salloomt River	56	1	52°26’	126°33’	150
Salloomt River	56	2	52°26’	126°33’	150
Salloomt River	56	4	52°26’	126°33’	150
Copper Bay	57	3	53°07’	131°40’	10
Channel	58	5	53°08’	132°15’	20
Rennell Sound	59	2	53°22’	132°27’	100
Rennell Sound	59	3	53°22’	132°27’	100
Masset	61	3	54°03’	132°00’	10
Masset	61	5	54°03’	132°00’	10
Kitimat	62	5	54°15’	128°30’	60
Snow Creek	63	2	54°15’	129°33’	10
Snow Creek	63	4	54°15’	129°33’	10
Rainbow Summit	64	1	54°15’	130°02’	160
Prince Rupert	65	4	54°16’	130°16’	46
Shames River	68	2	54°26’	128°55’	100
Oliver Lake	71	3	54°00’	130°00’	45
Oliver Lake	71	4	54°00’	130°00’	45

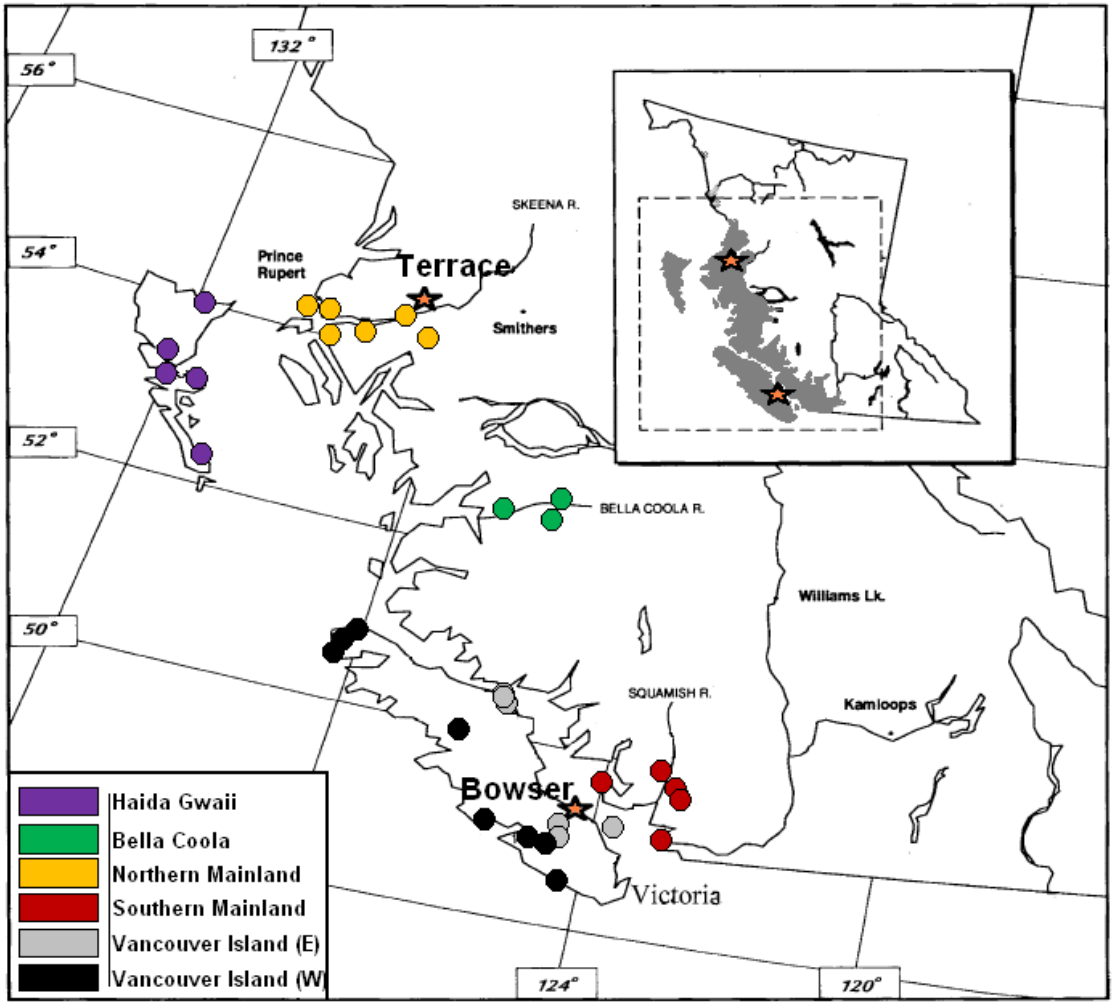

Figure 2: Provenance source locations for families planted at two common garden sites (Bowser and Terrace, indicated by stars on the map). Provenances are colour coded to indicate the region of origin to which they were assigned. Inset at top right is the range of red alder in British Columbia. Modified from Xie *et al.* (2008)

Diameter and Height

Diameter at breast height (DBH) and height of all trees in the 58 selected families were measured in two blocks at Terrace on April 20-22, 2010 and in two blocks at Bowser on May 13, 2010. Height was measured by clinometer at 10m horizontal distance.

Bud Burst

Bud burst was assessed for each tree from the 58 families in two blocks at Bowser on March 20 and April 10, 2010, and at all three blocks at Terrace on April 21, 2010. One assessment was carried out in the following spring in both blocks at Bowser on April 11,

2011. Assessment of the leader was performed using binoculars, after preliminary trials of binocular assessments followed by pruning and scoring of assessed branches confirmed the viability of the method. Buds were scored using the following scale (modified from Murray *et al.* 1989): 1=not swollen, 1.5=slightly swollen, 2=swollen, 2.5=swollen with distinct asymmetrical bulge, 3=green foliage showing, 3.5= emergent foliage, and 4=emergent, unfolded foliage.

Electrolyte Leakage

Cold hardiness was assessed by electrolyte leakage after controlled freezing for samples collected at Bowser on September 28 and December 1, 2010, and January 29, and March 25, 2011. Samples collected from Terrace were assessed on October 15, 2010. For each collection, two trees per family per block were sampled, for two blocks at each site. Live twigs were collected from the mid to upper canopy on the south side of each tree using a 10m pole pruner, and then stored on ice for transport to the laboratory. Samples were rinsed in distilled water and cut into 0.5cm sections, excluding buds. Six of these sections were added to each of four scintillation vials with 0.2mL of distilled water. Each section of stem had both ends freshly cut at the time of processing. The four replicate vials prepared from each tree were allocated to each of three freezing temperatures and one refrigerated control. The three treatment vials were frozen in a programmable freezer (Caltech Scientific Ltd. Richmond, BC; Lab Chest Freezer Model 8458, Forma Scientific, Walton, MA) to -8, -12, or -16°C for September and October samples, -12, -16 or -20°C for December samples, -16, -20 or -24°C for January samples, and -12 or -20°C for March samples. The rate of temperature decline during freezing was a maximum of 5°C/h, and samples were held at each test temperature for one hour prior to removal. Immediately following removal from the freezer, samples were stored in a 4°C refrigerator, where they remained overnight. The following morning, 10mL of distilled water was added to each of the vials, which were then left on an orbital shaker at room temperature for 18 hours at 75RPM. Conductivity (L_t) was then measured (Jenway Ltd. 4020 Conductivity Meter, Staffordshire, UK), after which samples were heated to 100°C in an oven to heat kill the tissues, then once again shaken overnight. Conductivity (L_k) was remeasured the following morning.

Index of injury (I_t) was calculated for each tree at each temperature using the following formula:

$$I_t = \frac{100(R_t - R_0)}{1 - R_0}; R_t = \frac{L_t}{L_k}; \; R_0 = \frac{L_0}{L_d}$$

Where:

I_t = The index of injury due to freezing at temperature t

L_t = Conductance of solution from sample frozen at temperature t

L_k= Conductance of solution from sample frozen at temperature t, then heat-killed

L_0= Conductance of solution from unfrozen sample

L_d= Conductance of solution from unfrozen, heat-killed sample

(Flint *et al.* 1967).

Acetylene Reduction

Assessment of nitrogen fixation by acetylene reduction was carried out from June 28-July 10, 2010 in Bowser, and July 23-August 2, 2010 in Terrace. Pits were dug approximately 1m from the base of the stem to a depth of 11cm, for each of two trees per family per block for two blocks at each test site. One nodule >0.5cm in diameter was gently removed from a root and placed in a 125mL glass jar with a customized lid featuring a perforable membrane. 12.5mL of air was removed from the jar and replaced with acetylene (atomic absorption grade, Praxair Inc. Danbury, CT, USA) to create a 10% v/v atmosphere of acetylene. Samples were incubated in a covered, 15-25cm deep pit for 60 minutes to minimize any change in temperature due to removal from the soil. After incubation, 12mL of sample gas was removed from the jar and stored in evacuated vials (12mL Exetainer, Labco Ltd. Buckinghamshire, UK) for transport to the laboratory. Each sampling day, any rainfall and the temperature of the incubation pit were recorded. The location of each nodule collected for acetylene reduction was marked for later nodule density estimates.

Gas samples were analyzed for concentrations of acetylene and ethylene using a flame ionization detector (FID) (Varian 3800 Gas Chromatograph, Varian Inc, Palo Alto, CA). 0.5mL subsamples were injected for each gas sample collected in the field.

Nodule density was estimated by collecting all nodules found in four pits of fixed size (22cm long x 22cm wide x11cm deep). The location of the nodule used for acetylene reduction measurement was used as the centre point of the first pit (thus, the nodule collected for acetylene reduction was counted as being within the first pit); the other three pits were evenly spaced around the tree, approximately 1m from the stem. Pits were dug to a depth of 11cm, as preliminary testing indicated no nodules below this depth. All nodules collected were oven dried and weighed. Nodule density was then calculated as oven dry weight of nodules per unit volume of soil excavated (g/m^3).

At the end of each acetylene reduction assessment period, soil samples were collected from throughout the sample sites, to estimate soil nitrogen content. Six to ten cores 10cm in diameter and 12cm deep, approximately evenly spaced throughout the two blocks sampled, were collected. Cores were collected and immediately dried, then packaged for analysis of carbon and nitrogen content using a Flash EA 1112 elemental analyzer (Thermoquest Corp., Milan, Italy).

$\delta^{15}N$ and $\delta^{13}C$ Isotope Analyses

$\delta^{15}N$ was measured to estimate the proportion of nitrogen derived from nodule activity by each of 50 families over the growing season. $\delta^{13}C$ was measured to estimate the water use efficiency of the same 50 families. Bud samples were collected from the mid to upper canopy on the south side of each of two trees per family per block for two blocks, then oven dried at 50°C to constant weight. The individual trees sampled at each site were those used to measure acetylene reduction that summer. Collections were performed on September 21-22, 2010 in Bowser and October 7-8, 2010 in Terrace. Dried samples were homogenized using a ball mill and packaged as 4.0mg of sample in 8x5mm tin capsules (Elemental Microanalysis Ltd. Cambridge, UK.) for analysis at the UC Davis Stable Isotope Facility in Davis, California.

Finding control plants for $\delta^{15}N$ analysis was a significant challenge in the current study. In order to limit interspecific competition, the test sites were routinely thinned and brushed after establishment of the alder seedlings. Collection of bud or leaf tissue from control plants was thus limited to small, understory plants that shared little in the way of rooting profile or uptake discrimination processes with the alder. Due to these difficulties,

$\delta^{15}N$ values are presented here as relative comparisons among families or regions, and not as absolute values of total nitrogen fixed by individual trees, as suggested by Danso (1995).

Canopy Cover

Autumn canopy cover was visually estimated to the nearest 10% for each surviving tree in the 58 selected families in two blocks on each test site. Estimates were made on September 23 and November 2, 2010 in Bowser, and on October 9, 2010 in Terrace. All estimates were performed by the same experimenter and were calibrated using images of known proportions of canopy cover (BC Ministries of Forest and Environment 1998) in order to maintain consistency.

Data Analysis

Data were analyzed by analysis of variance to examine the significance of regional and family variation, and by correlations to examine relationships among latitude, longitude and elevation of family origin and all measured phenology, cold hardiness, acetylene reduction, and isotope variables (Appendix A, B). SAS 9.1 software (SAS Institute Inc., Cary, NC, USA) was used for analyses; specifically, PROC MIXED (using the REML estimation method), PROC CORR and PROC REG (for latitude, longitude and elevation comparisons). A p-value of 0.05 was used as the limit of significance, while p-values from 0.05 to 0.07 were treated as "weakly significant", and are presented here primarily as trends within the data. Individual family and regional means were compared with the SAS LSMEANS function.

Families were considered a fixed effect because they were specifically selected based on changes in height rank between sites using 10-year height data, as well as to represent a spread of provenances in coastal British Columbia. Temperature was considered a fixed effect in cold hardiness analyses as controlled freezing temperatures were selected, based on past experience in the lab, with the intention to create a range of damage in the frozen tissues.

ANOVAs to examine family differences in phenology, acetylene reduction and isotope data were performed separately by site with the following model:

$$y_{ijk} = \mu + b_i + F_j + bF_{ij} + \varepsilon_{ijk}$$

where:

y_{ijk} = performance of the k^{th} tree of the j^{th} family in the i^{th} block,

μ = the overall mean,

b_i = the random effect of the i^{th} block (i=1,2,3 in Terrace for bud flush, i=1,2 in Terrace for other data, i=1,2 in Bowser),

F_j = the fixed effect of the j^{th} family (j=1...50 for acetylene reduction and isotope analysis, j=1...58 for bud flush and leaf senescence),

bF_{ij} = the random effect of the i^{th} block by the j^{th} family,

ε_{ijk} = the random error associated with the k^{th} tree from the j^{th} family in the i^{th} block.

ANOVAs to examine family differences in cold hardiness were performed separately by site with the following model:

$$y_{ijk} = \mu + b_i + F_j + T_l + bF_{ij} + bT_{il} + TF_{jl} + \varepsilon_{ijk}$$

where:

y_{ijk} = index of injury of the k^{th} tree of the j^{th} family in the i^{th} block at the l^{th} temperature,

μ = the overall mean,

b_i = the random effect of the i^{th} block (i=1,2),

F_j = the fixed effect of the j^{th} family (j=1...50),

T_l = the fixed effect of the l^{th} temperature (l=1,2 for March assessment, l=1,2,3 for all other assessments)

bF_{ij} = the random effect of the i^{th} block by the j^{th} family,

bT_{il} = the random effect of the i^{th} block by the l^{th} temperature,

TF_{jl} = the random effect of the j^{th} family by the l^{th} temperature,

ε_{ijkl} = the random error associated with the k^{th} tree from the j^{th} family in the i^{th} block at the l^{th} temperature.

ANOVAs to examine regional differences in phenology, acetylene reduction and isotope data were performed separately by site with the following model:

$$y_{ijk} = \mu + b_i + R_j + bR_{ij} + \varepsilon_{ijk}$$

where:

y_{ijk} = performance of the k^{th} tree of the j^{th} region in the i^{th} block,

μ = the overall mean,

b_i = the random effect of the i^{th} block (i=1,2,3 in Terrace for bud flush, i=1,2 in Terrace for other data, i=1,2 in Bowser),

R_j = the fixed effect of the j^{th} region (j=1…6),

bR_{ij} = the random effect of the i^{th} block by the j^{th} region,

ε_{ijk} = the random error associated with the k^{th} tree from the j^{th} region in the i^{th} block.

ANOVAs to examine regional differences in cold hardiness were performed separately by site with the following model:

$$y_{ijk} = \mu + b_i + R_j + T_l + bR_{ij} + bT_{il} + RT_{jl} + \varepsilon_{ijk}$$

where:

y_{ijk} = index of injury of the k^{th} tree of the j^{th} region in the i^{th} block at the l^{th} temperature

μ = the overall mean,

b_i = the random effect of the i^{th} block (i=1,2),

R_j = the fixed effect of the j^{th} region (j=1...50),

T_l = the fixed effect of the l^{th} temperature (l=1,2 for March assessment, l=1,2,3 for all other assessments),

bR_{ij} = the random effect of the i^{th} block by the j^{th} region,

bT_{il} = the random effect of the i^{th} block by the l^{th} temperature,

RT_{jl} = the random effect of the j^{th} region by the l^{th} temperature,

ε_{ijkl} = the random error associated with the k^{th} tree from the j^{th} region in the i^{th} block at the l^{th} temperature.

Results

Height and Diameter

Mean height differed significantly among regions of origin at each test site (Bowser (BW) $p=0.0090$, Terrace (TR) $p<0.0001$, see Appendix A for ANOVA tables). At both sites, trees originating from the Haida Gwaii were among the shortest, while trees from the other regions varied in their rankings between sites (Fig. 3). Differences among mean family height were also significant at each test site (BW $p=0.0206$, TR $p<0.0001$). At both sites, families from the Haida Gwaii region grouped loosely in the bottom half of height ranking (Fig. 4).

DBH varied significantly among regions at both test sites (BW $p=0.0082$, TR $p=0.0150$). Families were also significantly different at both test sites (BW $p=0.0003$, TR $p=0.0086$), with mean family DBH ranging from 8.1-16.5cm at Bowser and from 8.0-12.1cm at Terrace. No significant correlation was detected for mean family DBH between the two sites. Mean family height and diameter were correlated at both test sites (BW $r=0.7376$, $p<0.0001$; TB $r=0.5913$, $p<0.0001$).

Both mean family height and DBH at the Bowser test site were negatively correlated with family latitude of origin (height $r=-0.3903$, $p=0.0061$, DBH $r=-0.3768$, $p=0.0083$, see Appendix B for correlation table). Correlations between latitude and height or diameter were both positive at Terrace, though not statistically significant.

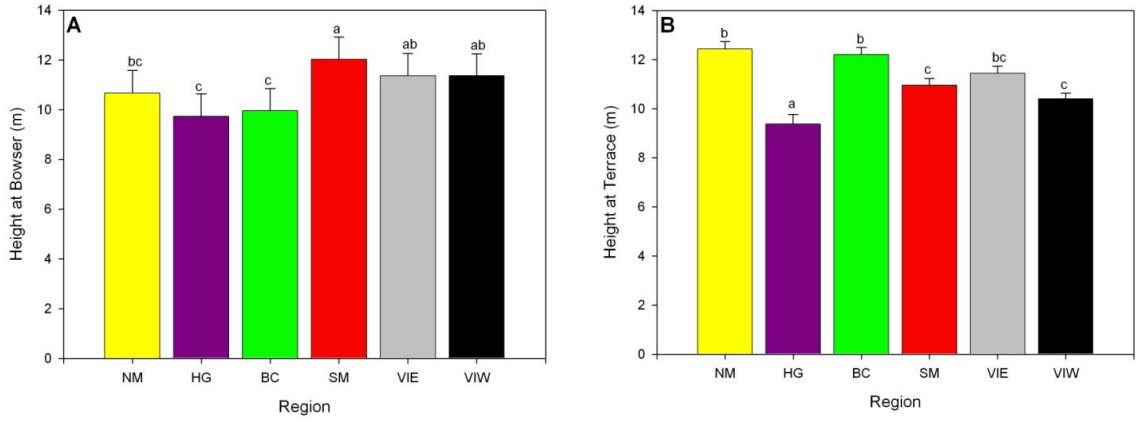

Figure 3: Mean height by region of origin of trees measured at Bowser (A) and Terrace (B) in the winter of 2010. Regional means are shown ± standard errors. Lower case lettering indicates statistically significant groupings of regions. Region abbreviations: Northern Mainland (NM), Haida Gwaii (HG), Bella Coola (BC), Southern Mainland (SM), Eastern Vancouver Island (VIE) and Western Vancouver Island (VIW).

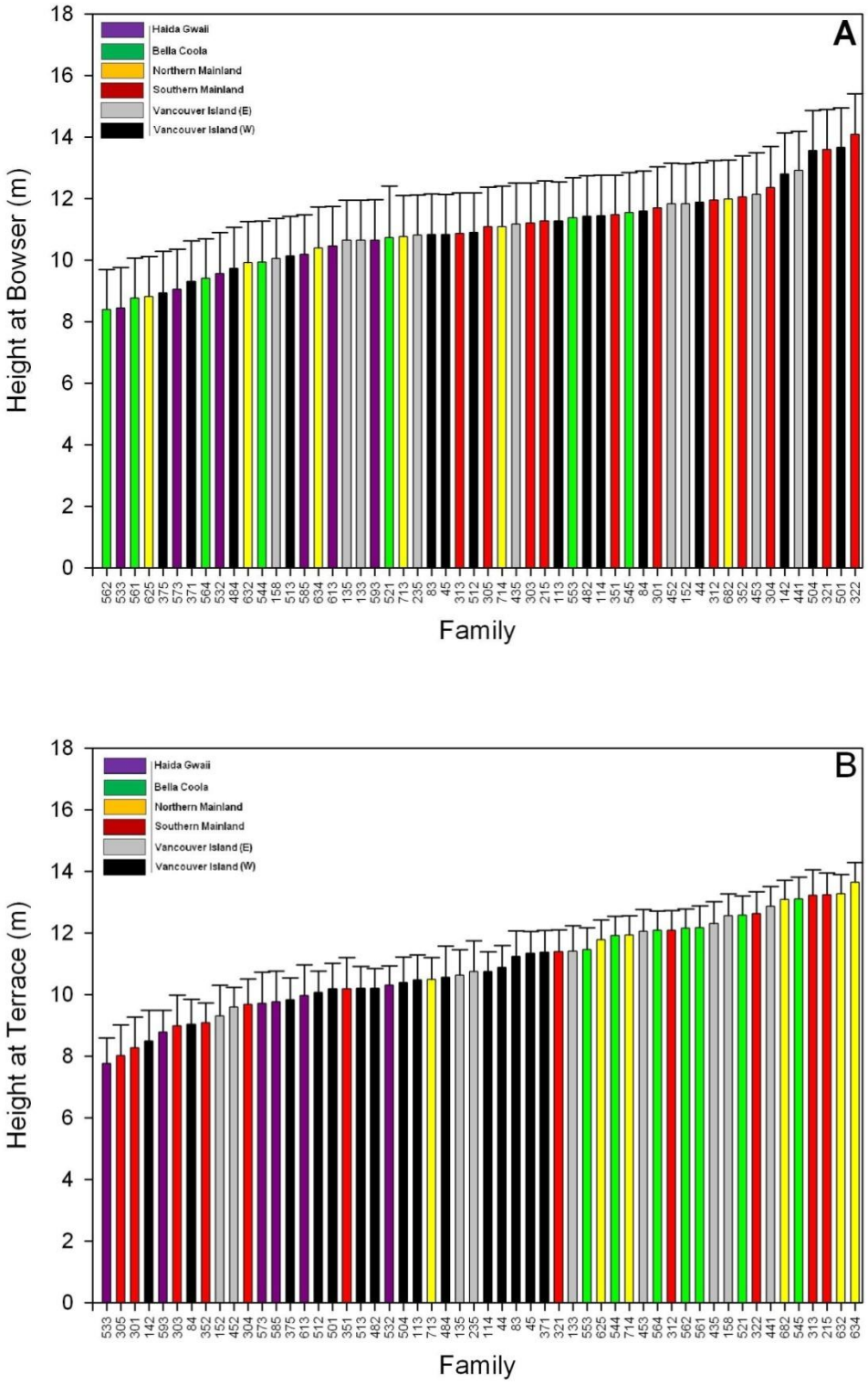

Figure 4: Mean family height for trees measured at Bowser (A) and Terrace (B) in the winter of 2010. Family means are shown ± standard errors and are coloured to indicate region of family origin.

Bud Burst

Bud burst measured in March and April of 2010 in Bowser, and April of 2010 in Terrace was found to vary significantly among regions of origin (Fig. 5). No significant differences among regions were detected in April 2011 in Bowser, although the pattern among regions was notably similar to that observed one year prior (Fig. 5B,D). In general, families from the northern and southern mainland regions, together with the Haida Gwaii, showed more advanced bud development early in the spring, while the Bella Coola and Vancouver Island families formed a second group showing a more delayed bud burst. Although differences among regions were statistically significant, they are biologically small: most mean values were within one quarter of one point on the four-point scale. My data do not allow for an assessment of how long transitions among stages may take, thus differences among stages may represent important increases in growing season length.

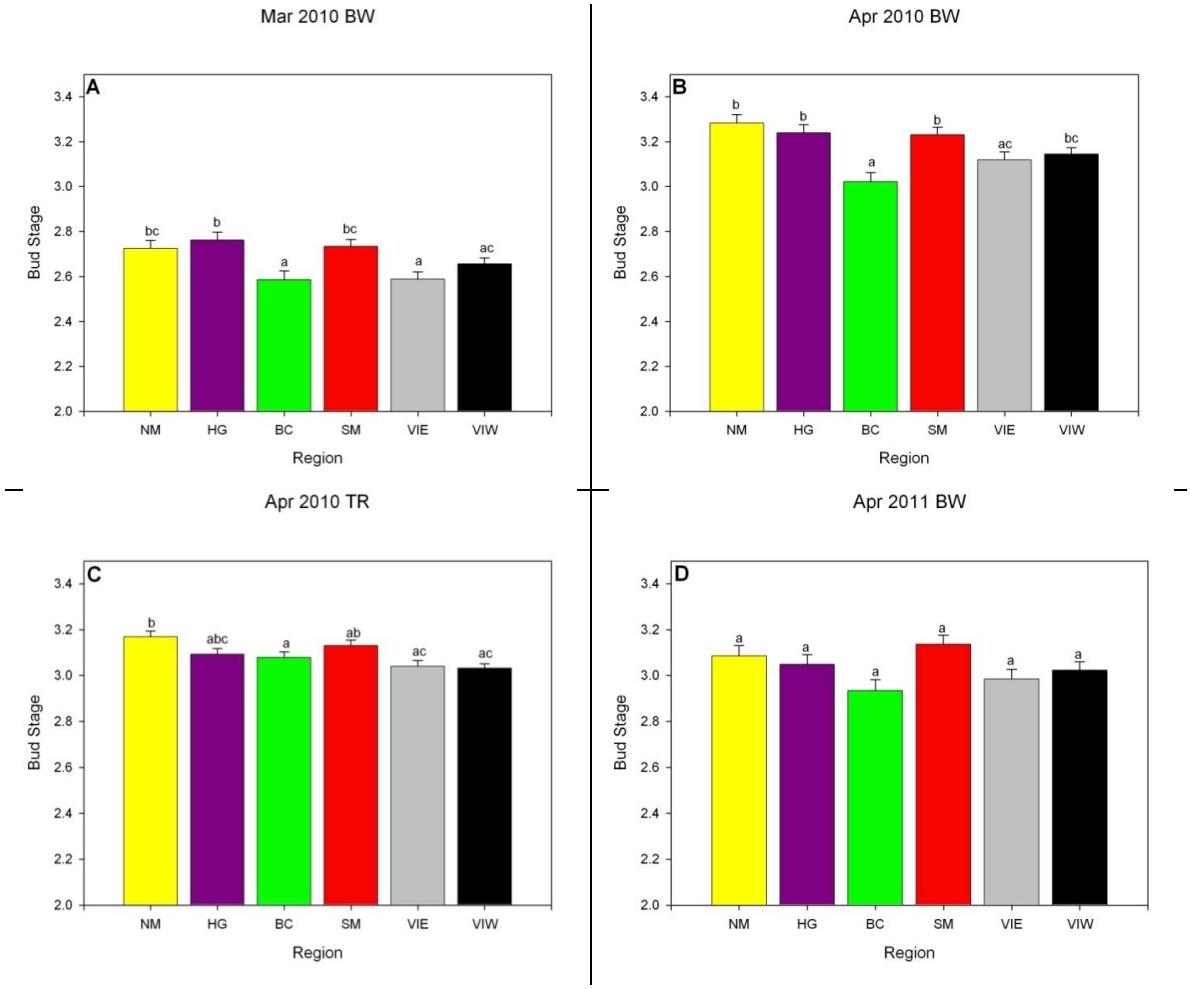

Figure 5: Bud burst at Bowser (BW) and Terrace (TR) in spring of 2010 and 2011. Bud stage was evaluated visually on a 4-point scale with higher values indicating more advanced bud development. Regional means are shown ± standard errors. Lower case lettering indicates statistically significant groupings of regions. Region abbreviations: Northern Mainland (NM), Haida Gwaii (HG), Bella Coola (BC), Southern Mainland (SM), Eastern Vancouver Island (VIE) and Western Vancouver Island (VIW).

Differences among families in bud burst stage were statistically significant at Bowser in April 2010 (e.g. Fig. 6) and April 2011 ($p<0.0001$, and $p=0.0078$, respectively), but not in Bowser in March 2010. Family differences were weakly significant at Terrace in April 2010 ($p=0.0518$). Almost all families were within one half point of each other on the four point scale at each evaluation date (Fig. 6).

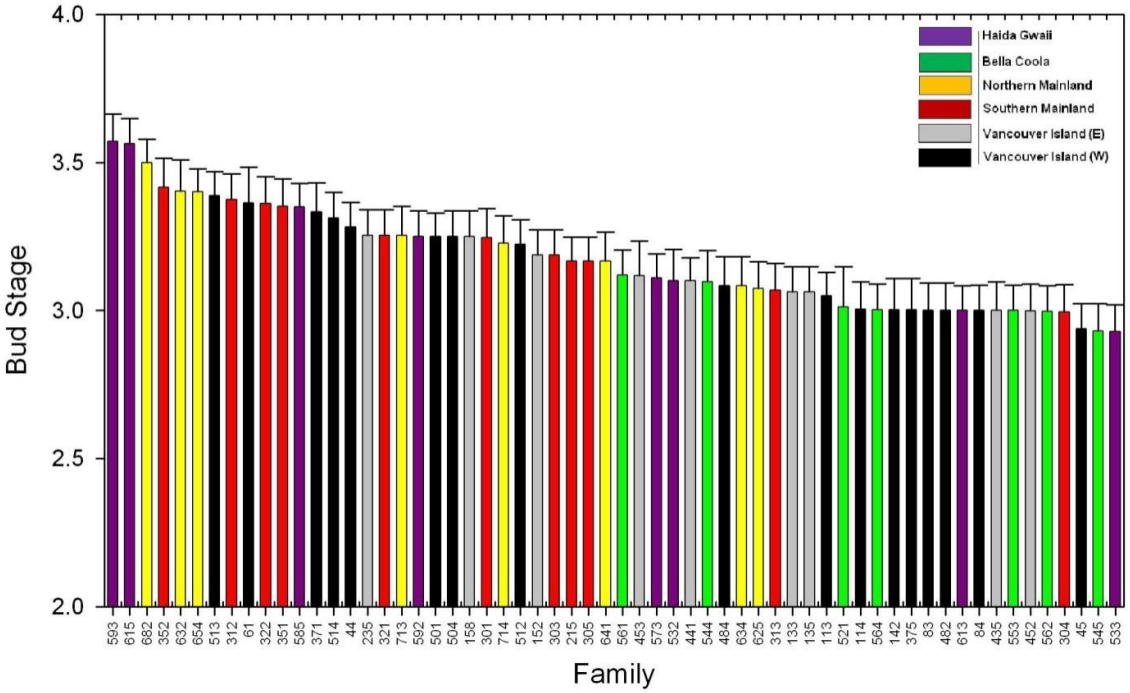

Figure 6: Bud burst at Bowser in April 2010, the evaluation date showing greatest variation among families. Family means ± standard errors for each of 58 measured families, colour coded by region of origin.

Bud Burst Correlations

Mean family bud burst values for each date of assessment were positively correlated with values from all other assessments (Table 3). At Terrace, bud stage was also correlated positively, but weakly, with height ($r=0.2800$, $p=0.0539$). At Terrace, April bud burst was positively correlated with latitude of family origin ($r^2=0.1302$, $p=0.0117$); northern trees were more advanced in bud stage than were southern trees. In April 2010, Bowser bud development was more advanced in those trees showing a lower frost hardiness (higher index of injury) after freezing treatment in March 2011 ($r=0.2969$,

p=0.0404). At Bowser, bud burst in April 2011 showed a similar positive correlation with index of injury in March 2011, but this was not statistically significant (r=0.2204, p=0.1323). Mean family bud burst stage at Bowser in March, 2010 was negatively but weakly correlated with mean family canopy cover in November 2010 at the same site (r=-0.2781 p=0.0556).

Table 3: Pearson correlation coefficients (r) and p-values for mean family bud burst measured at two sites over two years. Measurements were performed at Bowser (BW) on March 20, 2010, April 10, 2010, and April 11, 2011. Trees at Terrace (TR) were evaluated once on April 21, 2010.

	April 2010 BW	April 2010 TR	April 2011 BW
March 2010 (BW)	0.6126 p<0.0001	0.3333 p=0.0206	0.5857 p<0.0001
April 2010 (BW)		0.4878 p=0.0004	0.6295 p<0.0001
April 2010 (TR)			0.3859 p=0.0068

Leaf Senescence (Canopy Cover)

There were significant differences in canopy cover among families from the six regions at each site at each assessment (Sept BW p=0.0290, Oct TR p<0.0001, Nov BW p=0.0104); however, the trends observed among regions were inconsistent among assessments (Fig. 7). In September and October, families from the Bella Coola, southern mainland and eastern Vancouver Island regions had greater canopy cover. By November, the southern mainland and eastern Vancouver Island regions had highest canopy cover. Mean family canopy cover differed significantly at each site at each assessment (Sept BW p=0.0023, Oct TR p<0.0001, Nov BW p<0.0001) (Fig. 8).

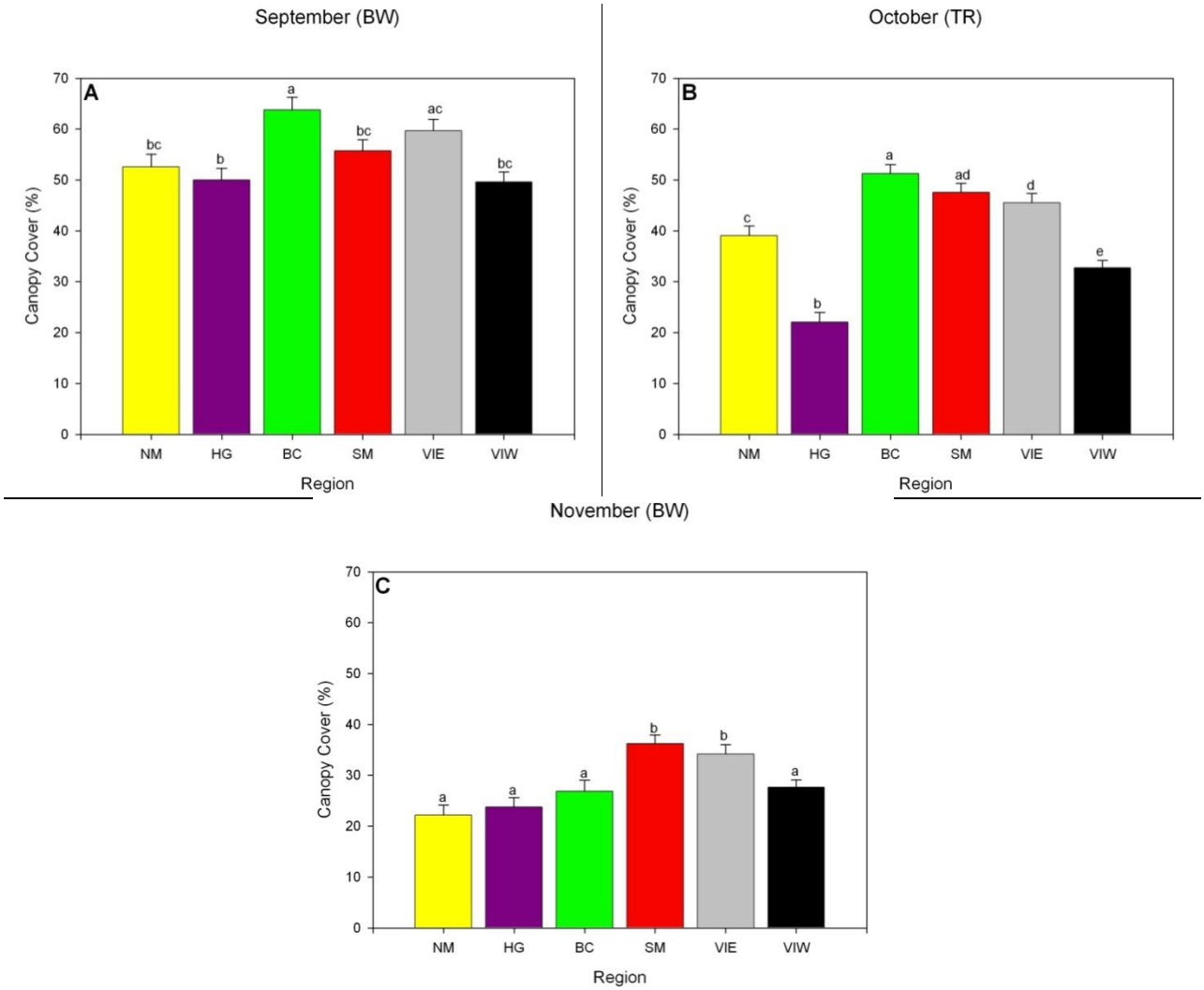

Figure 7: Canopy cover present at Bowser (BW) and Terrace (TR) by region in the autumn of 2010. Assessment was by visual estimate, carried out by a single researcher using photographs to maintain consistency. Regional means are shown ± standard errors for each assessment date. Lower case lettering indicates statistically significant groupings of regions for each assessment date. Region abbreviations: Northern Mainland (NM), Haida Gwaii (HG), Bella Coola (BC), Southern Mainland (SM), Eastern Vancouver Island (VIE) and Western Vancouver Island (VIW).

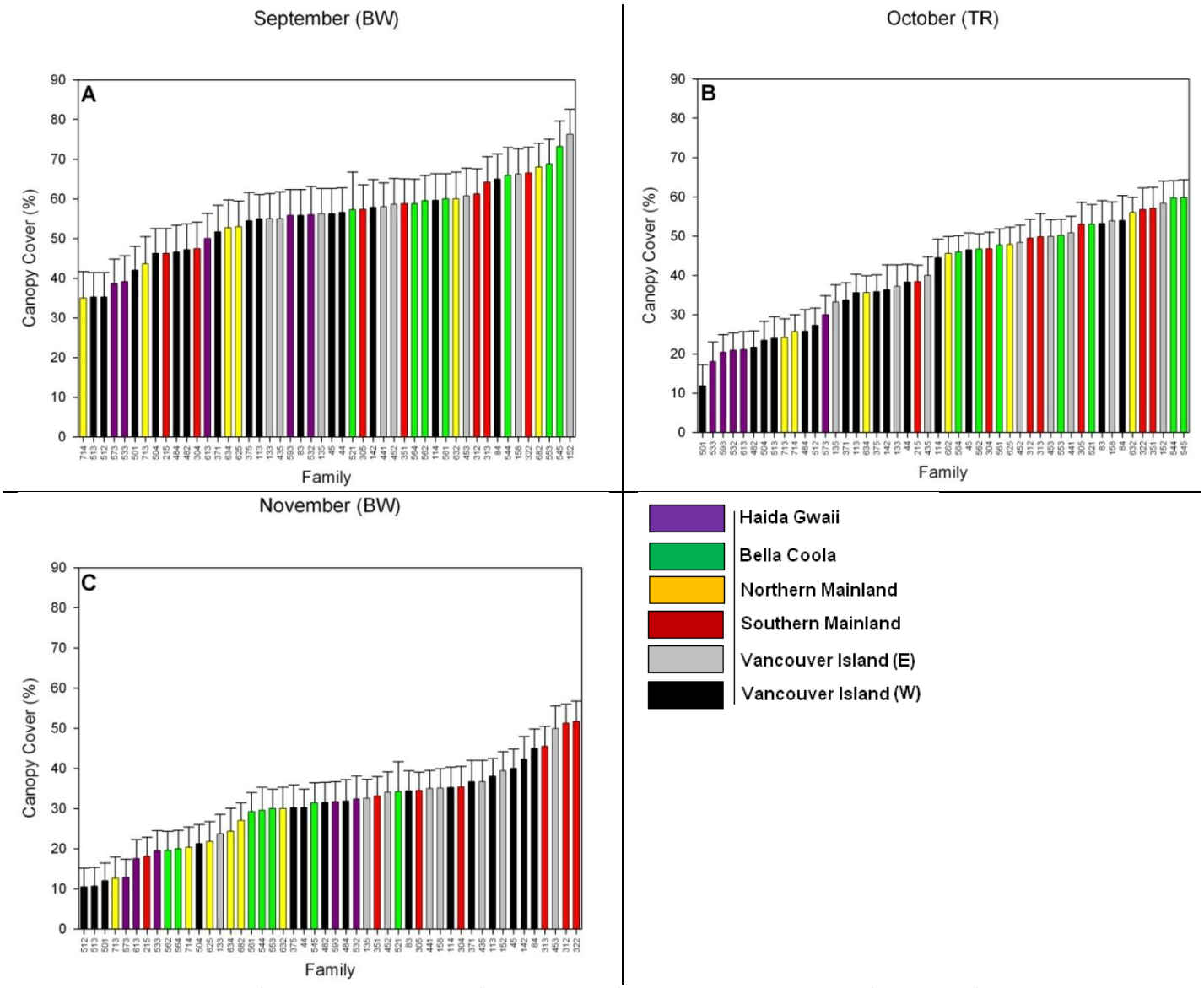

Figure 8: Mean family canopy cover for autumn of 2011 ± standard error for each of 60 measured families, colour coded by region of family origin. Assessments were carried out at Bowser (BW) and Terrace (TR) on Sept 23 (BW), Oct 9 (TR) and Nov 2 (BW).

Senescence Correlations

Mean family percentage of canopy cover on each date of assessment was positively correlated with values for all other assessments (Table 4). Values from each assessment date were negatively correlated with longitude; those families from farther west had lower canopy cover at each assessment date ((Sept r^2=0.2118 p=0.0010, Oct r^2=0.4228 p<0.0001, Nov r^2=0.3365 p<0.0001, Fig. 9). At Bowser, in November, mean family canopy cover was also negatively correlated with latitude of family origin (r=-

0.5151 p=0.0002), indicating northern families had less canopy cover in late autumn in the common garden trial. In October (Terrace) and November (Bowser), canopy cover was correlated positively with both tree height and diameter at the respective site (TR height r=0.4144 p=0.0034, TR DBH r=0.3334 p=0.0206, BW height r=0.3534 p=0.0137, BW DBH r=0.4392 p=0.0018).

The October measure of canopy cover in Terrace showed significant positive correlation with $\delta^{13}C$ values (r=0.3034 p=0.0361), indicating that more water use efficient families had a higher canopy cover later in the year.

Mean family canopy cover in both the October (Terrace) and November (Bowser) assessments was positively correlated with $\delta^{15}N$ (Oct r=0.4375 p=0.0019, Nov r=0.3205 p=0.0264); trees with a higher percentage of canopy cover had less of their bud nitrogen from fixation in the nodules. Similarly, canopy cover at Terrace in October was negatively correlated with the rate of acetylene fixation per gram of nodule tissue in the previous summer (r=0.3774 p=0.0082). Canopy cover was positively correlated with percent nitrogen in bud tissues for each assessment (Sept r=0.5786 p<0.0001, Oct r=0.5939 p<0.0001, Nov r=0.3418 p=0.0174).

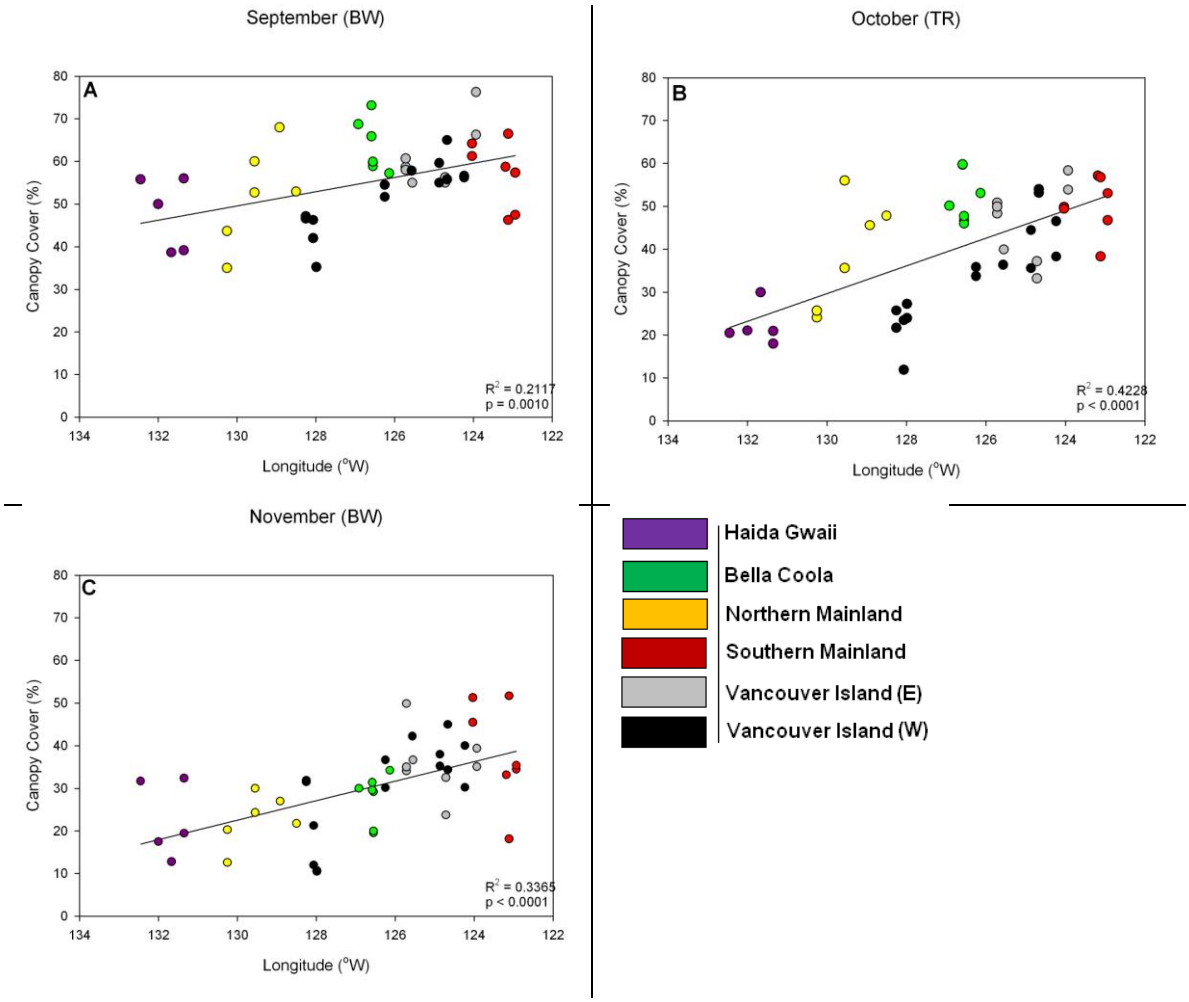

Figure 9: Regressions between mean family canopy cover in autumn 2011 and family longitude of origin for each of three assessments. Assessments were carried out at Bowser (BW) on Sept 23 and Nov 2, and at Terrace (TR) on Oct 9. Points are colour coded by region of family origin.

Table 4: Pearson correlation coefficients (r) and p-values for mean family canopy cover measured at two sites (Bowser-BW and Terrace-TR). Assessments were carried out at Bowser on September 23 and November 2, and at Terrace on October 9, 2010.

	TR Oct 2010	BW Nov 2010
BW Sept 2010	0.7783 p<0.0001	0.6605 p<0.0001
TR Oct 2010		0.5694 p<0.0001

$\delta^{13}C$

Differences in mean values for $\delta^{13}C$ were not found to be statistically significant among families or regions. Mean $\delta^{13}C$ was statistically different between the Bowser and Terrace sites ($p<0.0001$). Mean $\delta^{13}C$ at Bowser was -26.20 ± 0.1015, while the mean at Terrace was -29.09 ± 0.1271 (means ± standard error). Values for individual trees ranged from -31.23 to -23.27 at Bowser, and from -33.10 to -25.22 at Terrace.

$\delta^{13}C$ Correlations

When family $\delta^{13}C$ values were averaged over both sites, $\delta^{13}C$ was weakly negatively correlated with longitude ($r^2=0.0791$ $p=0.0528$) (Fig. 10), indicating that more eastern families were more water use efficient. As mentioned above, $\delta^{13}C$ was positively correlated with the October measure of canopy cover in Terrace ($r=0.3034$ $p=0.0361$).

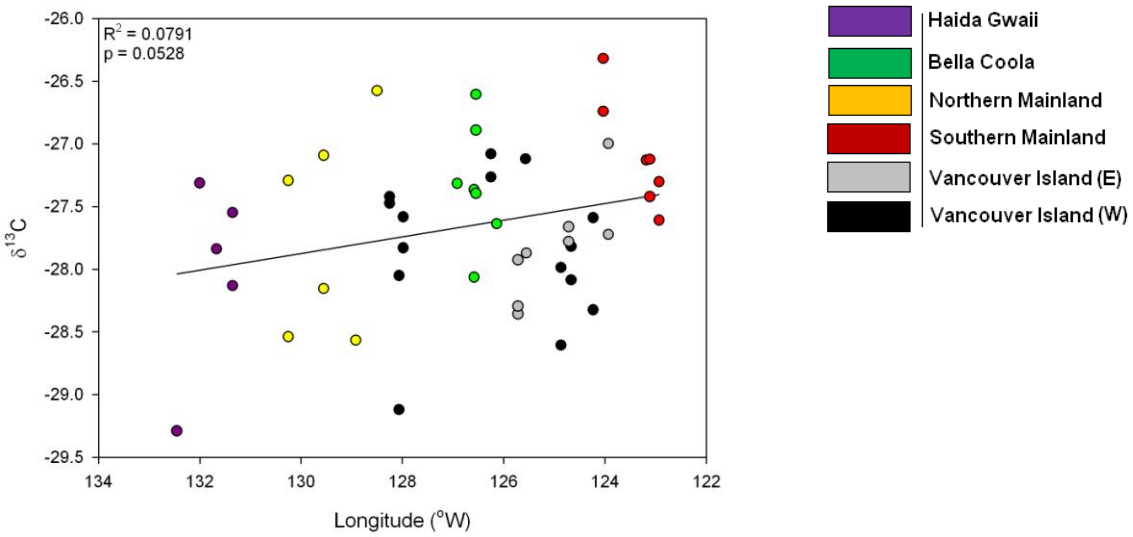

Figure 10: Regression of mean family $\delta^{13}C$ with longitude for each of 48 families. Data shown were averaged over the Bowser and Terrace sites. Points are colour coded by region of family origin.

Cold Hardiness

Cold hardiness measured in September at Bowser varied weakly among regions (p=0.0655). In December, January, and March in Bowser, index of injury varied significantly among regions (p=0.0271, 0.0384, and 0.0123, respectively (Fig. 11B,C,D). Index of injury was also found to vary among regions at Terrace, when measured in October (p=0.0434) (Fig. 11E). At both test sites in autumn, families from the northern mainland and Bella Coola regions showed the greatest level of cold hardiness (lowest index of injury) (Fig. 11A,D). This trend continued throughout December and January at Bowser (Fig. 11B,C). In March, the northern mainland families were significantly less hardy than the Bella Coola families, though they were still hardier than the southern mainland or eastern Vancouver Island families (Fig. 11D). Individual family means were significantly different for all measurement dates (Table 5, Fig. 12).

Table 5: PROC MIXED results for the significance of the family effect on index of injury measured by stem electrolyte leakage at Bowser (BW) or Terrace (TR) over the winter of 2010-2011.

	Family (p>F)
September (BW)	0.0219
October (TR)	0.0119
December (BW)	0.0008
January (BW)	0.0092
March (BW)	0.0184

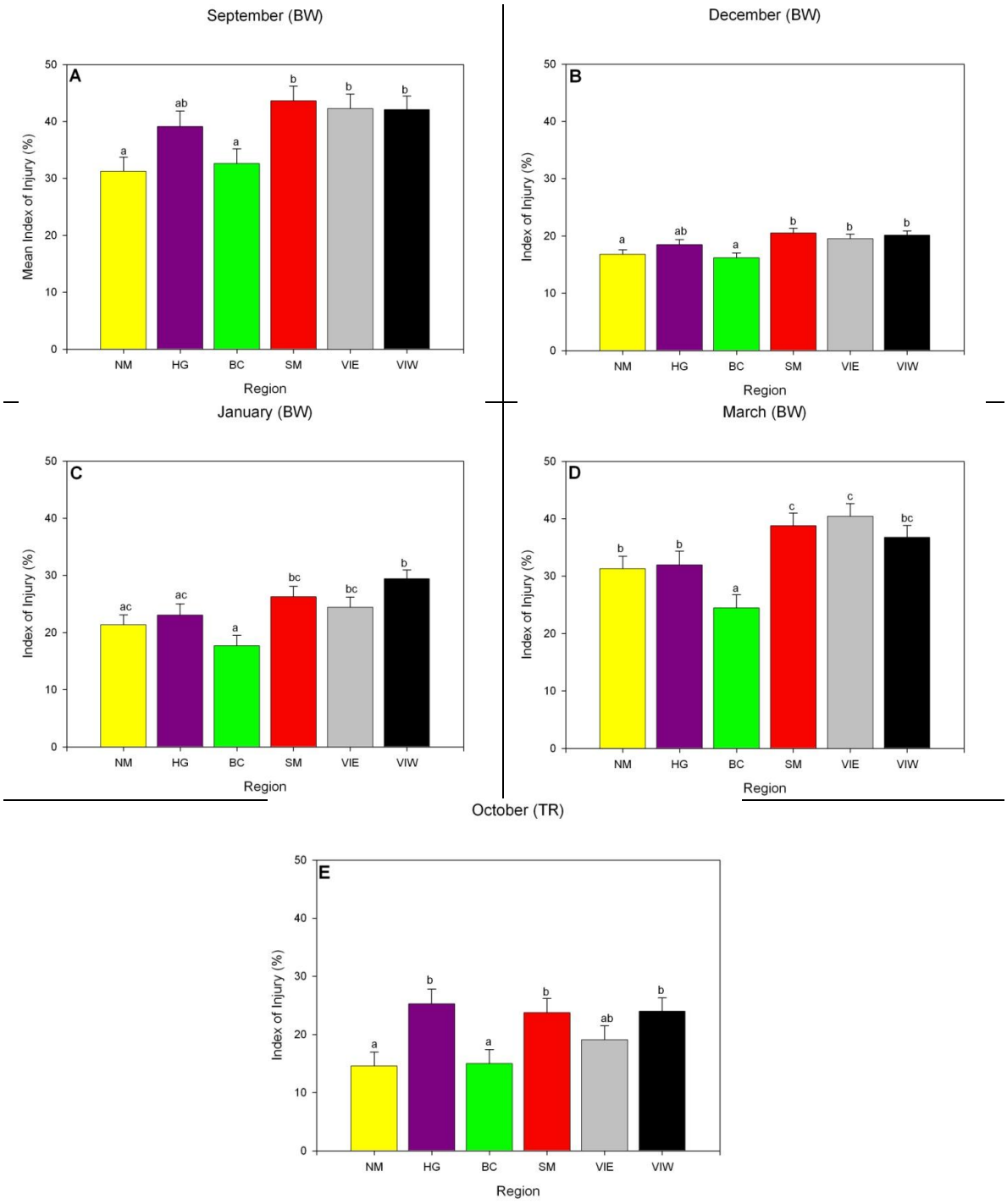

Figure 11: Mean index of injury by region measured by electrolyte leakage at Bowser (BW) and Terrace (TR) over the winter of 2010-2011. Regional means are shown ± standard errors. Lower case lettering indicates statistically significant groupings of regions. Region abbreviations: Northern Mainland (NM), Haida Gwaii (HG), Bella Coola (BC), Southern Mainland (SM), Eastern Vancouver Island (VIE) and Western Vancouver Island (VIW).

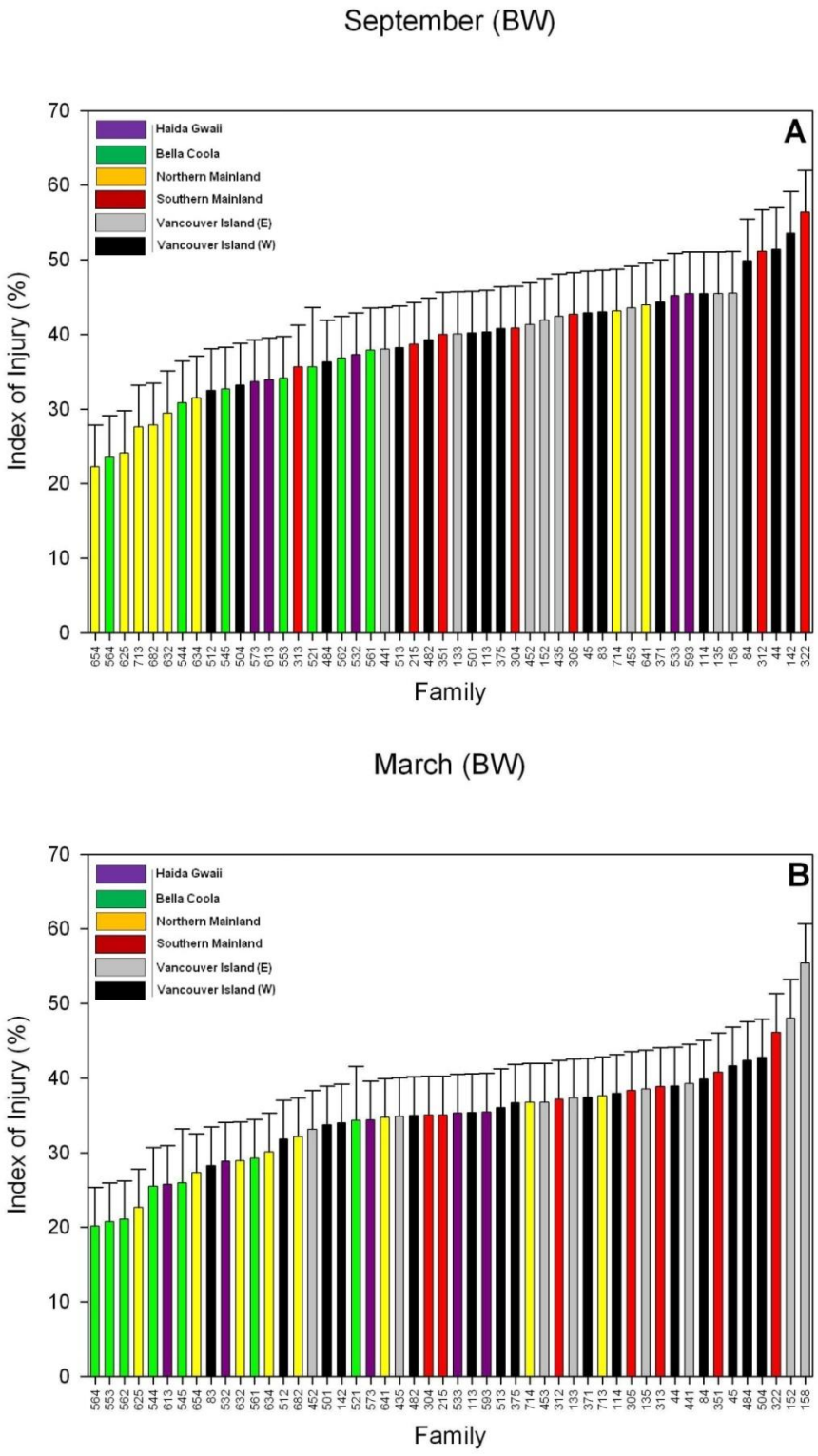

Figure 12: Mean index of injury for individual families planted at Bowser at the onset of (A) and emergence from (B) winter cold hardiness. Family means are arranged from most cold hardy to least cold hardy for that assessment, and are shown ± standard error. Individual family means are colour coded by that family's region of origin.

Cold Hardiness Correlations

Mean family index of injury values for each date of assessment were positively correlated with values from all other assessments (Table 6, Fig. 13). Mean index of injury was negatively correlated with latitude for all dates (Sept r^2=0.4497 p<0.0001, Oct r^2=0.1446 p=0.0077, Dec r^2=0.1528 p=0.0060, Jan r^2=0.1319 p=0.0112, Mar r^2=0.3325 p<0.0001). Northern families were less damaged by controlled freezing than were southern families. Family mean index of injury was negatively correlated with longitude for the September and March measures (r^2=0.1982 p=0.0015, r^2=0.1425 p=0.0082, respectively), thus eastern families were less cold hardy than were western families on those dates. The correlations between mean family height and index of injury were of opposite sign at the two sites. At Bowser, height was positively correlated with index of injury for each assessment date; those families that were least cold hardy tended to be tallest (Sept r=0.3732 p=0.0090, Dec r=0.3403 p=0.0179, Jan r=0.4678 p=0.0008, Mar r=0.3705 p=0.0095) (Fig. 14A). At Terrace, the opposite was true, height and index of injury were negatively correlated (r=-0.3719 p=0.0092) (Fig. 14B).

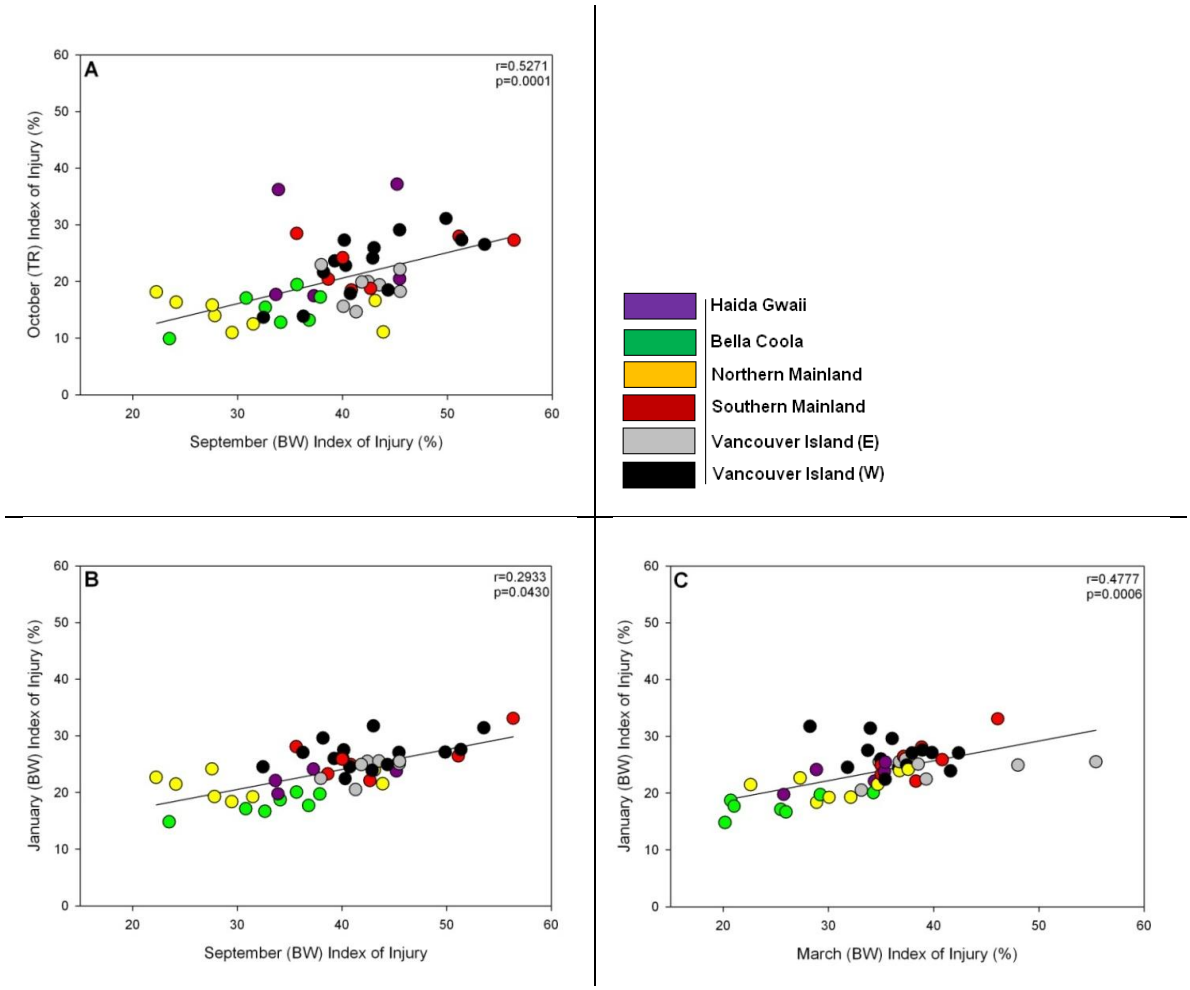

Figure 13: Correlations between assessments of cold hardiness. Family mean index of injury is coloured to reflect region of origin. Correlations between sites (BW=Bowser, TR=Terrace) (A), between autumn and midwinter assessments at Bowser (B), and between midwinter and spring assessments at Bowser (C) are shown.

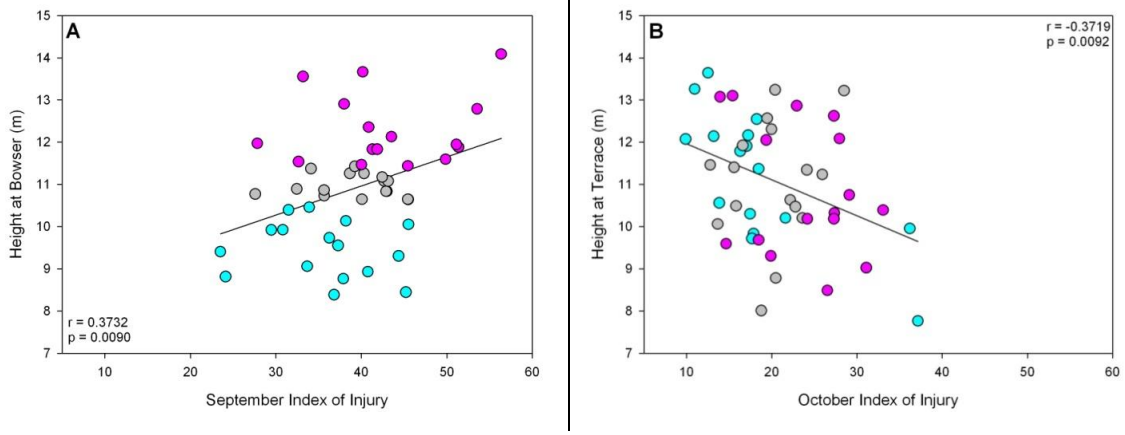

Figure 14: Correlations between mean family height and index of injury, showing opposite trends at Bowser (BW) and Terrace (TR). Points are coloured based on height rankings at Bowser (pink = tallest third of families at Bowser, grey = middle third of families, blue = shortest third of families).

Table 6: Pearson correlation coefficients (r) and p-values for mean family index of injury measured at two sites (Bowser-BW and Terrace-TR) over the winter of 2010-2011.

	Oct (TR)	Dec (BW)	Jan (BW)	Mar (BW)
Sept (BW)	0.5271 p=0.0001	0.5302 p=0.0001	0.2933 p=0.0430	0.5615 p<0.0001
Oct (TR)		0.2887 p=0.0466	0.5263 p=0.0001	0.3492 p=0.0150
Dec (BW)			0.3161 p=0.0286	0.4473 p=0.0014
Jan (BW)				0.4777 p=0.0006

Nitrogen Fixation

Acetylene reduction per gram of nodule tissue or per cubic metre of soil excavated was not found to vary significantly among families or regions. Mean mass of nodules in the density assessment pits was found to be weakly, but significantly different among families (p=0.0594), but only when data was pooled for the two sites (Fig. 15). Soil nitrogen content varied between sites (p=0.0030). Mean soil nitrogen at Terrace was 1.73% ± 0.1547 by mass; while at Bowser mean soil nitrogen was 0.84% ± 0.1958 (mean ± standard error).

$\delta^{15}N$ did not vary significantly among regions. There was a weak but significant difference among families at both sites (BW p= 0.0594, TB p=0.0510, Fig. 16). $\delta^{15}N$ values at Bowser ranged from -4.57 to -0.49, with a mean of -2.73, and at Terrace from -5.58 to -0.39 with a mean value of -2.02.

Percent nitrogen in bud tissue varied significantly among regions at both sites (BW p=0.0512 TR p=0.0397) (Fig. 17), and among families at Terrace (p=0.0433) (Fig. 18). Mean percent nitrogen in bud tissues at both sites was found to be 1.8%. Values for individual trees at Bowser ranged from 1.3-2.4%, while values for individual trees at Terrace ranged from 1.0-2.5%.

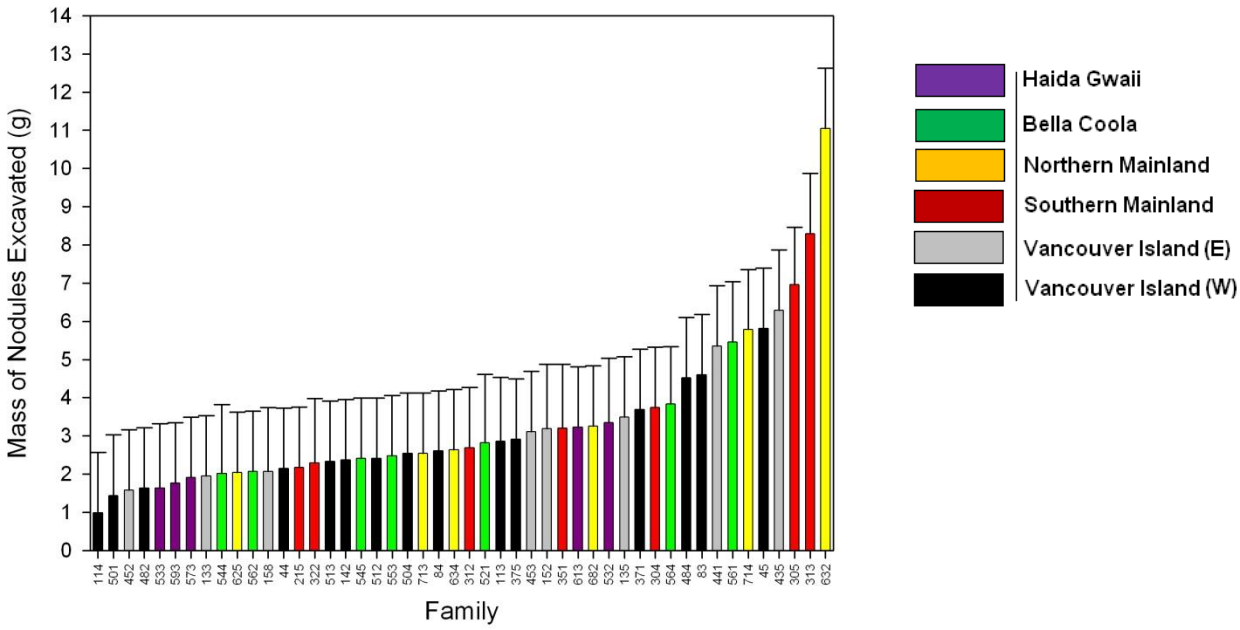

Figure 15: Mean family mass of nodules excavated from four, evenly distributed, 22x22x11cm pits located approximately one metre from the base of each tree. Family means are pooled between the two test sites, and arranged from lowest to highest, and are shown ± standard error. Each family mean is colour coded by the family's region of origin.

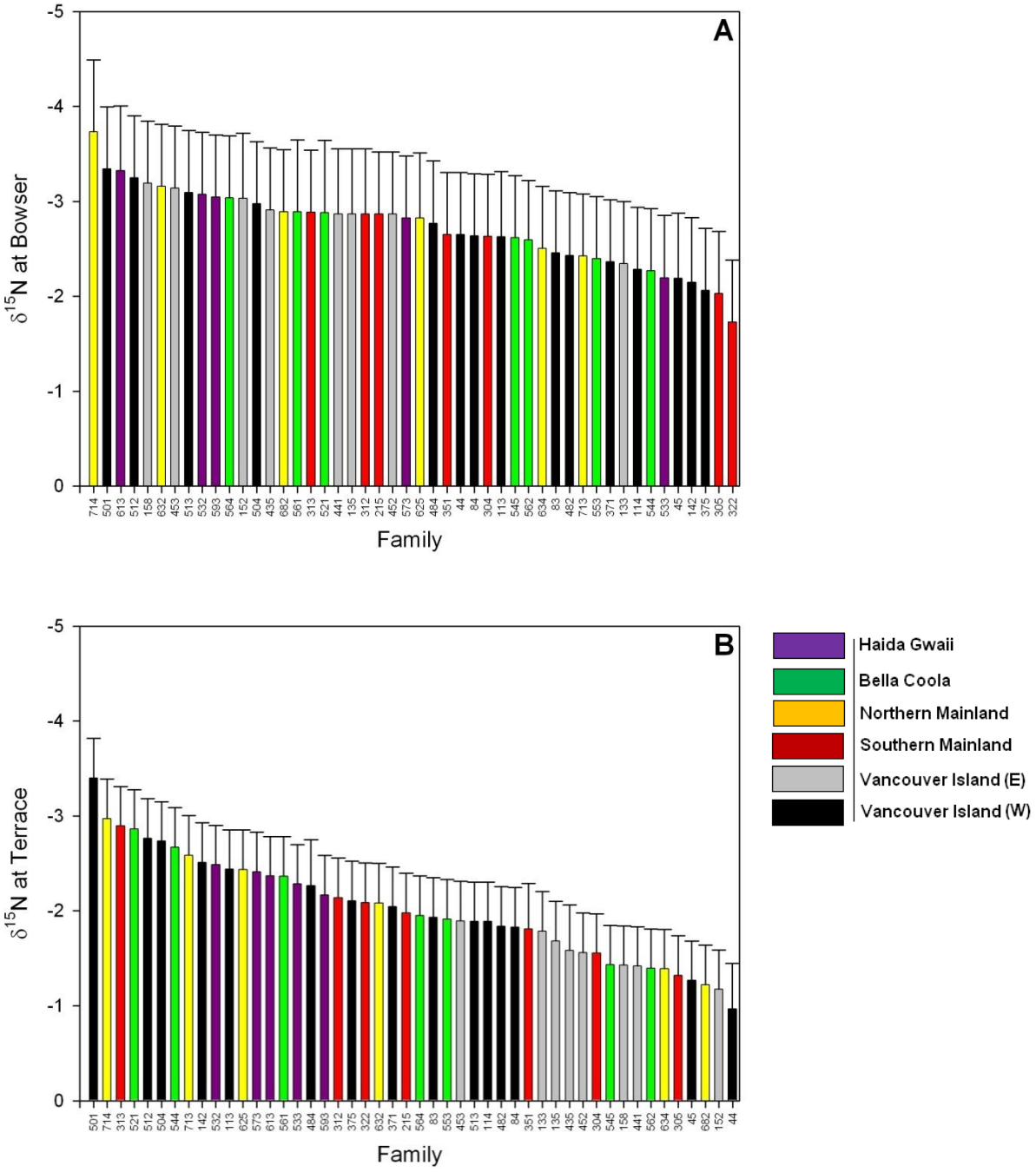

Figure 16: Mean family $\delta^{15}N$ values at Bowser (A) and Terrace (B) for bud samples collected in the autumn of 2010. Family means are arranged from highest to lowest, and are shown ± standard error. Each family mean is colour coded by the family's region of origin.

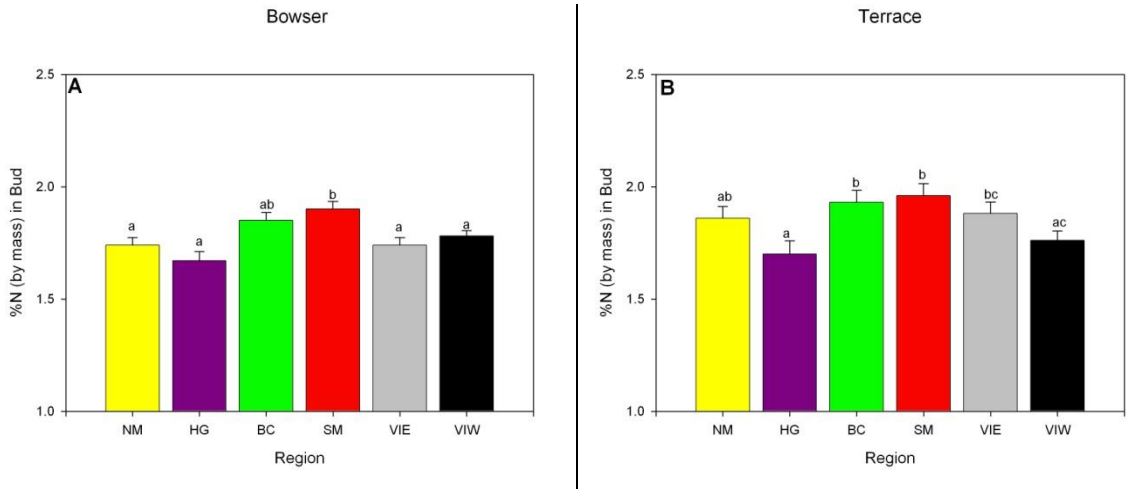

Figure 17: Mean bud nitrogen concentration measured in autumn of 2010 at the Bowser and Terrace test sites. Lower case lettering indicates statistically significant groupings of regions. Region abbreviations: Northern Mainland (NM), Haida Gwaii (HG), Bella Coola (BC), Southern Mainland (SM), Eastern Vancouver Island (VIE) and Western Vancouver Island (VIW).

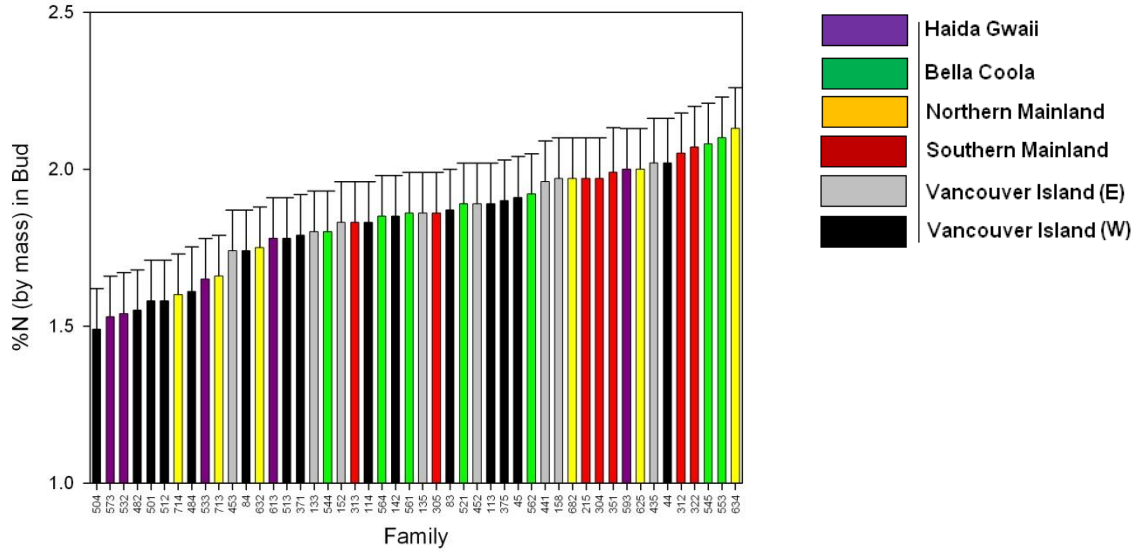

Figure 18: Mean family bud nitrogen concentration for 50 families grown at Terrace. Family means are arranged from lowest concentration of nitrogen to highest, and are shown ± standard error. Individual family means are colour coded by that family's region of origin.

Nitrogen Fixation Correlations

Family means at Bowser for acetylene reduction (per g and per m^3), density of nodules, and $\delta^{15}N$ were not significantly correlated with those measured at Terrace. Family mean percent nitrogen in bud tissues was positively correlated between the two sites (r=0.3323 p=0.0210).

Acetylene reduced per m^3 of soil was negatively but weakly correlated with $\delta^{15}N$ at Terrace (r=-0.2825 p=0.0517), thus those families with a high rate of instantaneous nitrogen fixation in August were also found to have a high proportion of their total nitrogen from fixation on the Terrace site (Fig. 20D). $\delta^{15}N$ was positively but weakly correlated with the percentage of nitrogen in bud tissues for both sites (BW r=0.5775 p<0.0001, TR r=0.5556 p<0.0001), thus families with more nitrogen obtained from fixation in the nodules had a lower total percentage of nitrogen (Fig. 20E).

At Terrace, acetylene reduction per gram of nodule tissue was correlated negatively with both height (r=-0.3789 p=0.0079) (Fig. 20F) and diameter (r=-0.4497 p=0.0013), thus, larger families appeared to be fixing less nitrogen per unit of nodule mass than were smaller families at this site, though the trend with height was primarily driven by two families (Fig. 20F). Neither height nor diameter at Terrace were significantly correlated with acetylene reduction per m^3 of soil (r=0.0919 p=0.5340 and r=-0.1356 p=0.3581 for height and diameter, respectively). Family mean percent nitrogen in buds was positively correlated with height at Terrace (r=0.3951 p=0.0054) (Fig. 20I) and with diameter at Bowser (r=0.3688 p=0.0099).

Mean family $\delta^{15}N$ at Bowser was found to correlate negatively with latitude (r^2=0.0955 p=0.0325) (Fig. 19A); northern families had a higher percentage of their nitrogen sourced from fixation in the nodules than did southern trees. A negative trend was observed for $\delta^{15}N$ and longitude at both sites (BW r^2=0.1236 p=0.0143, TR r^2=0.1627 p= 0.0045) (Fig. 19B). There was an observed negative correlation between mean family percent nitrogen and longitude of family origin on each site (BW r^2=0.2067 p=0.0012, TR r^2=0.2181 p=0.0008) (Fig. 19D). At Bowser, both the mean family acetylene reduced per m^3 of soil and the mass of nodules per unit volume excavated were positively correlated with elevation of family origin (r^2=0.2236 p=0.0009, r^2=0.0913 p=0.0412, respectively) (Fig. 19C).

Mean family $\delta^{15}N$ was negatively but weakly correlated with bud burst at Bowser in April of 2010 (r=-0.2781 p=0.0556) (Fig. 20A); thus families with earlier bud development had a higher percentage of their nitrogen sourced from fixation in the nodules.

Both sites showed a positive correlation between mean family $\delta^{15}N$ and canopy cover in either October (Terrace) or November (Bowser) (TR r=0.4375 p=0.0019, BW r=0.3205 p=0.0264) (Fig. 20C); families with a higher canopy cover later in the year acquired less of their total nitrogen from fixation in the nodules. At Terrace, acetylene reduced per gram of nodule tissue was correlated negatively with canopy cover in October (r=-0.3774 p=0.0082) (Fig. 20H).

Acetylene reduced per gram of nodule tissue at the Terrace site was correlated positively with index of injury in October (r=0.3221 p=0.0256) (Fig. 20G). A positive association was found for $\delta^{15}N$ and index of injury in both September and December at the Bowser site (r=0.2884 p=0.0468, r=0.2999 p=0.0384, respectively) (Fig. 20B), thus less cold hardy families tended to have less of their bud nitrogen sourced from fixation in the nodules.

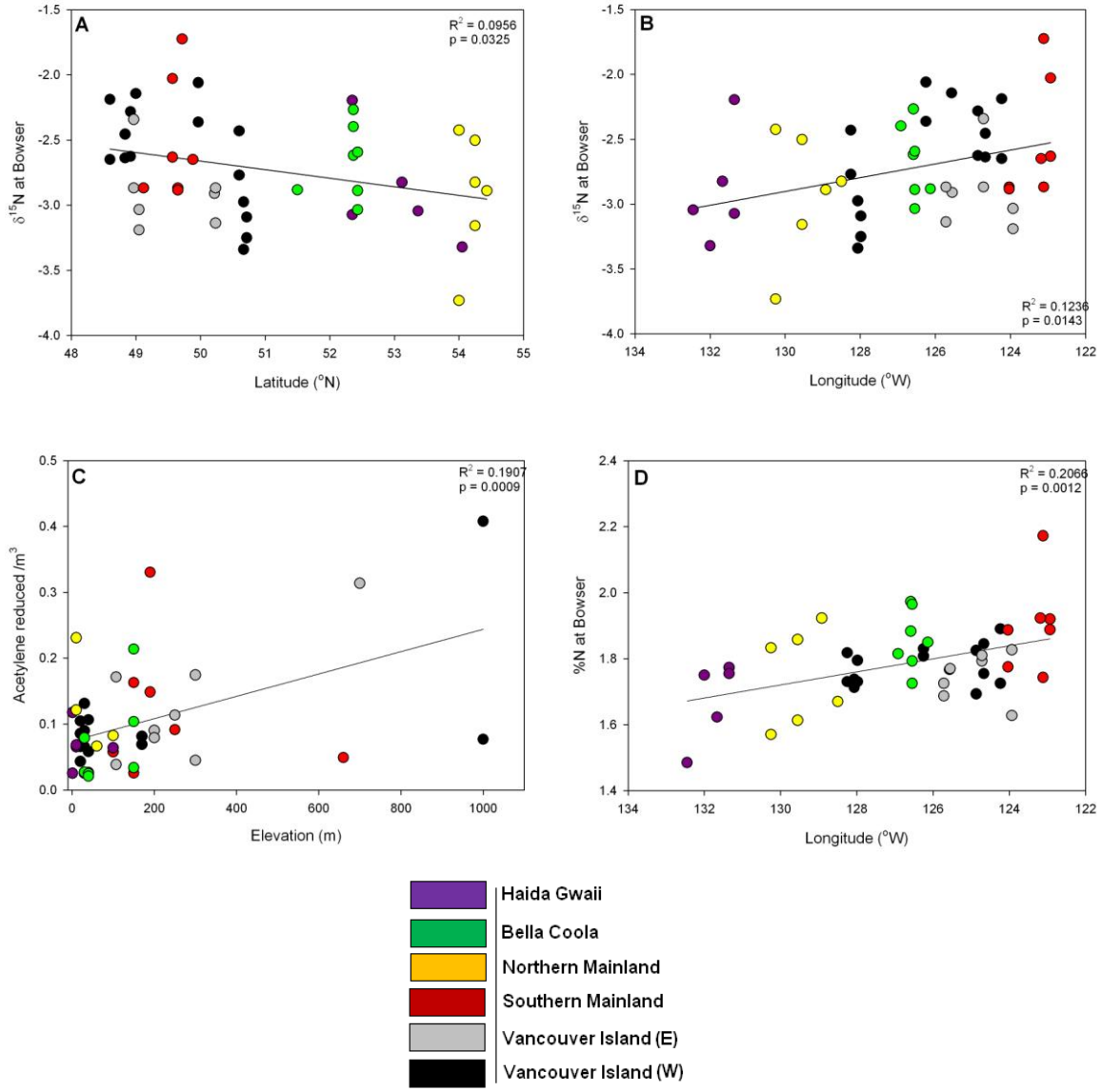

Figure 19: Regressions of location of family origin and nitrogen fixation variables for families grown at the Bowser test site. A: $\delta^{15}N$ with latitude of family origin, B: $\delta^{15}N$ with longitude of family origin, C: acetylene reduced per m^3 of soil with elevation of family origin, D: percent nitrogen content (by mass) in bud tissues with longitude of family origin. Family means are coloured to reflect region of family origin.

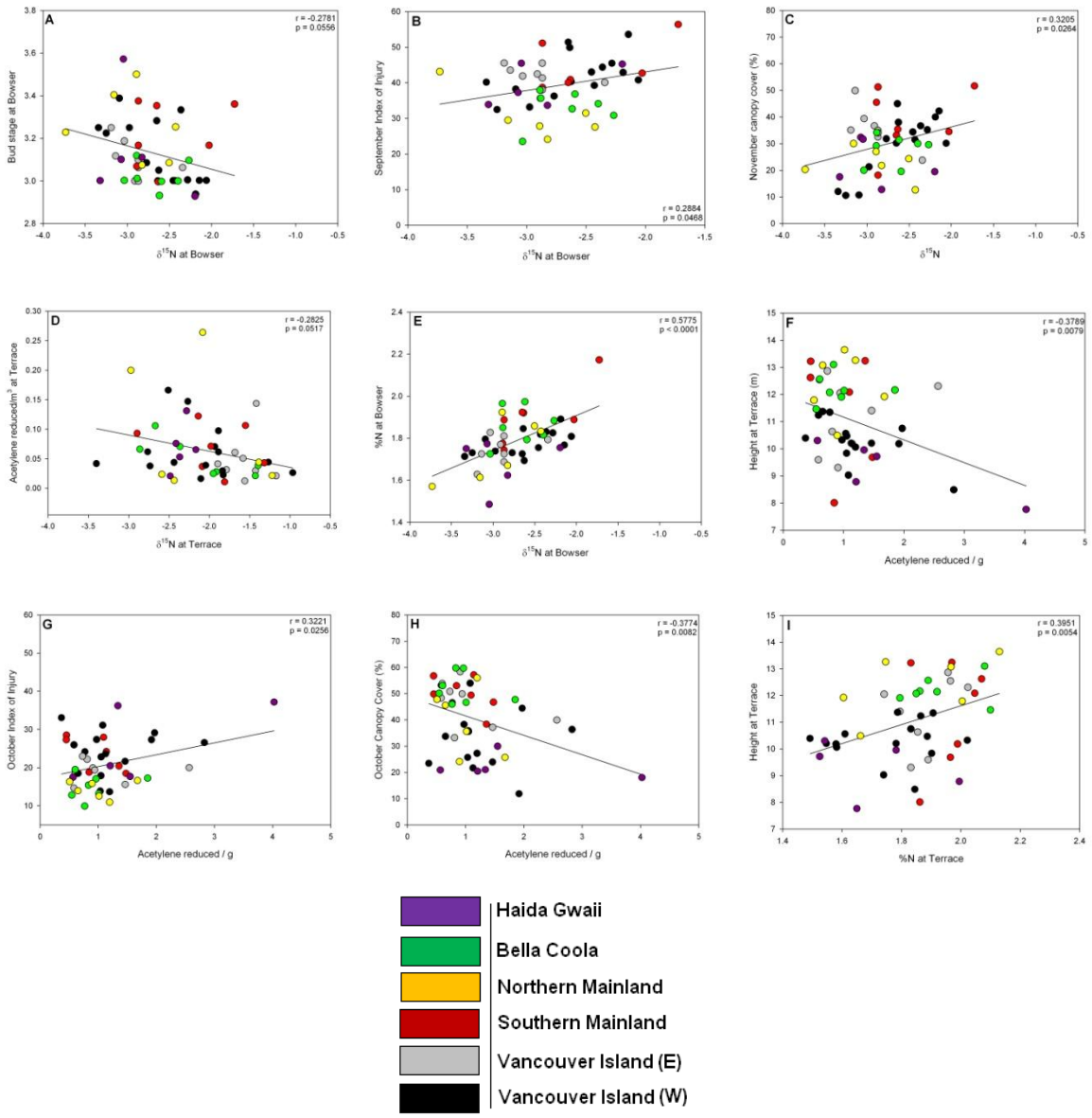

Figure 20: Correlations among nitrogen fixation and other physiological or phenotypic variables. Mean family $\delta^{15}N$ at Bowser with (A), mean family bud stage at Bowser (B), mean family September index of injury and (C), November mean family canopy cover; (D) mean family $\delta^{15}N$ at Terrace with acetylene reduced per m^3 of soil at Terrace; (E) mean family $\delta^{15}N$ at Bowser with mean family percent nitrogen (by mass) in bud tissue collected at Bowser; mean family acetylene reduction per gram of nodule at Terrace with (F) mean family height measured at Terrace, (G) October index of injury, and (H) October mean family canopy cover; and (I) mean family percent nitrogen (by mass) in bud tissue collected at Terrace with height measured at Terrace. Family means are coloured to indicate region of family origin.

Discussion

Significant differences were identified among families for growth (height and diameter), bud burst, leaf senescence, cold hardiness, and bud nitrogen content. No significant differences among families were identified for water use efficiency as measured by $\delta^{13}C$, or for rates of nitrogen fixation as measured by either acetylene reduction or natural abundance $\delta^{15}N$. In some cases, such as bud burst or water use efficiency, my results are similar to those of experiments done previously on red alder seedlings (Cannell *et al.* 1987, Dang *et al.* 1994). My data compliment these previous studies by extending the work done on red alder to adult trees.

My study identified possible adaptive differences among genotypes, especially in traits such as bud flush timing, cold hardiness, or nitrogen content and their respective contributions to growth. These often reflected a tradeoff between growth and the ability to tolerate an extreme environment. The data presented here include important considerations and possible cautions for red alder breeding programs or potential assisted migration programs in terms of increased risks in a latitudinal movement of genotypes.

Bud Burst

Previous work has shown that northern families of deciduous trees tend to burst bud earlier in the spring than do southern families when planted at the same site (Jensen and Hansen 2008, Li *et al.* 2003). My results showed a less clear trend, with no straightforward significant correlation between latitude and bud burst. The northwest to southeast orientation of the coastline of British Columbia and the maritime influence on all coastal populations likely reduced the impact of simple latitudinal trends in bud burst timing, and trends were more easily explained by differences among regions of seed origin. While the northern mainland and Haida Gwaii regions showed an early resumption of growth in spring at Bowser, my data suggest that the southern mainland region may form a group together with these two more northerly regions (Fig. 1, Fig. 5). While it might have been expected, based on previous research (Jensen and Hansen 2008, Li *et al.* 2003), for the Vancouver Island families to show a more delayed emergence from winter dormancy, the grouping of these regions with the Bella Coola families is

surprising (Fig. 5). The reasons for a delayed emergence in the Bella Coola region are unclear. The pattern among regions was consistent throughout the duration of bud flush (Fig. 5A,B), between years (Fig. 5B,D), and between sites (Fig. 5B,C).

Previous work with red alder has found that seedlings in a common garden plot, whose origins spanned from Washington to Alaska, all burst bud within one week of each other (Cannell *et al.* 1987). From my data, it is not possible to comment directly on the duration of each stage of bud burst. However, despite statistically significant differences between regions and families, the assessments performed cannot refute the hypothesis that red alder bud flush is nearly synchronous (Cannell *et al.* 1987). Regional means were all within one-quarter point on the four-point scale used by Cannell *et al.* (1987) at each date of assessment (Fig. 5), while family means were nearly all within one-half point (Fig. 6). Also, the relatively strong correlation between two assessments carried out in the spring of 2010 at Bowser (Table 3) suggests that bud flush progresses at an approximately even pace in red alder, regardless of family climate of origin. Along with the previous work performed by Cannell *et al.* (1987), this suggests that the overall growing season length in red alder is not greatly determined by regional or family differences in bud flush timing (Vitasse *et al.* 2009). However, even small differences in bud flush timing between families may have a large impact. Those families that burst bud earlier did show increased height growth at Terrace, on average. Thus, while differences in the timing of foliage emergence appear to be slight, they may be sufficient to influence the overall growth and development of individual families.

Leaf Senescence

As with the beginning of the growing season, the timing of the end of the growing season is a trade off between increased opportunity for growth and increased risk of damage due to winter frost. It is generally the end of the growing season that determines the overall duration of growth (Vitasse *et al.* 2009), and it has been suggested that the delayed senescence of southern ecotypes of some woody plants is responsible for the increased growth of these ecotypes (Jensen and Hansen 2008, Li *et al.* 2003). In the current study, significant differences in canopy cover among regions and among families

were detected at all assessments, though the patterns were not consistent among assessments.

In September, at Bowser, the Bella Coola region showed significantly higher canopy cover compared to the other regions (Fig. 7A). As with bud flush, this is not easily explained by a north-south division of regions. Trees from the northern regions (Haida Gwaii and the northern mainland), as expected, had a lower percentage of canopy cover early in autumn than did those from Bella Coola. Unexpected was the similarity among the northern regions and the southern mainland and Vancouver Island regions (Fig. 7A). The eastern Vancouver Island region showed the expected trend of southern regions delaying leaf drop, but I believe that the weakness of these trends may be due to a complication in these data. The measure used for the end of the growing season was the percentage of canopy cover present, which includes canopy cover lost over the summer months due to drought-induced leaf abscission (Pezeshki and Hinckley 1988). The lack of a strong north-south trend in canopy cover early in the autumn may be due to the confounding influence of regional adaptations to summer drought, and early leaf drop because of drought stress. It might be expected that regional trends in leaf drop associated with the end of the growing season would become more prominent later in the autumn. Indeed, in November there was a strong negative correlation between latitude and canopy cover; those families originating from further north had less canopy cover than did those from the south (Fig. 7C, 8C).

Although I did not measure soil water status, it likely varied between the two test sites (Table 1). I observed that the Terrace site was usually wetter than the blocks measured at Bowser. Therefore, the October assessment of canopy cover at Terrace was not an ideal measure for distinguishing a possible point of transition from drought-influenced leaf loss to latitude-influenced autumn leaf loss. It may be noted, with this caution in mind, that comparing the September assessment at Bowser and the October assessment at Terrace the Haida Gwaii families showed the greatest difference, with a drastic decrease in canopy cover between the assessments on the two sites compared to other regions (Fig. 7A,B). It was expected that the Haida Gwaii families would show an earlier loss of foliage than southern families (Jensen and Hansen 2008, Li *et al.* 2003),

but the expected early foliage drop was not observed in northern mainland or Bella Coola families in either the September or the October assessment (Fig. 7A,B).

The October and November assessments showed positive correlations between canopy cover and growth measures of the respective sites. Both height and diameter were greater in those families having more foliage later in the year. A higher canopy cover later in the season might be expected to be associated with increased growth, though this trend has been shown to be reversed in some species (Vitasse *et al.* 2009, Jensen and Hansen 2008). Vitasse *et al.* (2009) found both positive and negative correlations between height growth and canopy cover, but the majority of species showed positive correlations. Only a single species showed a significant negative correlation, which was weak (r= -0.16) (Vitasse *et al.* 2009). Thus, the trend seems to be toward increased growth associated with more canopy cover later in the year for most species. Despite a possible confounding of drought and autumn senescence in my study, the trends emerging later in the autumn (November) confirm what was expected for the end of the growing season.

All three assessments showed significant negative correlations between longitude and canopy cover; those families originating from farther west had less canopy cover than eastern families on each assessment date. This may provide more indirect evidence for the influence of drought over canopy cover, as it is likely that western coastal families would be less drought-adapted than more inland eastern ones. This will be elaborated further in the discussion of water use efficiency.

Red alder's inability to tolerate drought, and the species' overall "water-spender" characteristics result in high rates of early leaf abscission when compared to other co-occurring trees such as *P. trichocarpa* (Pezeshki and Hinckley 1988). This impacted my measure of the end of the growing season, as the percentage of canopy present for each tree in autumn would not account for foliage lost due to drought earlier in the season. As discussed previously, despite this confounding of variables, it appears as though the earlier autumn canopy results may be dominated by drought processes while the late autumn assessment better reflected the timing of the end of the growing season.

While earlier bud burst may increase the duration of the growing season for some families, it was found that those families which burst bud earliest at Bowser showed the

least canopy cover in the autumn of that year. An early start to growth may represent not only a lengthened growing season, but also a strategy of maximizing growth during the period when water is readily available on the west coast. The strategy of early growth, followed by early leaf abscission to avoid drought stress later in summer would allow for more efficient seasonal use of water. Alternatively, an emphasis on springtime growth may come at the cost of lower summer drought hardiness, and so lower canopy cover later in the year.

$\delta^{13}C$

Differences of mean $\delta^{13}C$ values among families and among regions were not statistically significant, confirming results for two year old red alder seedlings (Dang *et al.* 1994). Mean $\delta^{13}C$ was significantly different between the Bowser and Terrace site; the slightly less negative value at Bowser suggests that this site was drier and trees on this site were more water use efficient. Alternatively, this increase in water use efficiency may have been due to higher rates of photosynthesis, rather than more time spent with stomata closed (Sun *et al.* 1996). The mean value for $\delta^{13}C$ at Bowser (-26.20‰) was slightly higher (less negative) than has been reported for drought-stressed *B. pendula* (ranging from -29.57‰ to -27.38‰) (Aspelmeier and Leuschner 2004). The mean value of $\delta^{13}C$ at Terrace was -29.09‰, falling within the range for well-watered *B. pendula* (30.92‰ to -28.85‰) (Aspelmeier and Leuschner 2004). However, the range of mean family values of $\delta^{13}C$ observed at Terrace (-33.10‰ to -25.22‰) indicate that some families were more water use efficient than drought stressed *B. pendula* (Aspelmeier and Leuschner 2004).

In Terrace, October mean family canopy cover was positively correlated with $\delta^{13}C$, indicating that canopy cover was higher in those trees showing a higher water use efficiency. This indicates that higher water use efficiency was achieved through greater stomatal closure. As both water use efficiency and autumn canopy cover were negatively correlated with longitude (Fig. 9,10), more eastern families of red alder in British Columbia may be adapted to a drier continental climate on average. While neither of these data sets present a direct line of evidence, I suggest that further study be conducted into possible clines in drought tolerance in red alder, using more direct methods such as

xylem water potential. Quantification of foliage loss due to drought over summer months would also strengthen the evidence for such a cline in drought tolerance.

The future climate of British Columbia is likely to be drier than current conditions in many areas (Hamann and Wang 2006). It is critical that any breeding program for red alder consider the ability of families to tolerate drought (Kumar *et al.* 2011). While my study did not detect any correlation between water use efficiency and growth characteristics, previous studies have identified *Alnus* as a genus generally unable to maintain high rates of growth under drought conditions (Schrader *et al.* 2005, Hibbs *et al.* 1995, Giordano and Hibbs 1993). One study identified different growth patterns among genotypes experiencing drought, and suggested that families maintaining favourable growth patterns under drought conditions, such as less branching or faster growth, could potentially be identified in the future (Hibbs *et al.* 1995). Based on my study, no families of significantly superior water use efficiency were identified. A trend for eastern families to show higher water use efficiency does provide a possible starting point for identifying families of higher water use efficiency in future investigations.

Cold Hardiness

Trees originating from farther north generally begin development of cold hardiness earlier in autumn than do southern trees when grown in a common garden (Friedman *et al.* 2008, Weng and Parker 2008, Jensen and Deans 2004, Li *et al.* 2003). Differences in autumn index of injury were significant among regions at both test sites (Fig. 11A,E) and generally followed the expected trend with latitude. Families from the two most northerly inland regions (northern mainland and Bella Coola) showed the greatest level of cold hardiness in autumn. I identified a significant negative correlation between family latitude of origin and autumn cold hardiness, confirming the expected trend of northern families showing a deeper cold hardiness earlier than southern families. Families from the southern mainland and both Vancouver Island regions were consistently more injured by the freezing treatment in autumn, which is to be expected for these more southern and coastal regions (Fig. 11A,E). Previous work has demonstrated that coastal provenances of temperate deciduous trees tend to show less tolerance of cold than do inland provenances, due to the temperature buffering of the

coastal environment by the ocean (Jensen and Deans 2004). The intermediate-to-high injury sustained by trees originating from the Haida Gwaii suggests that the influence of the coastal environment is stronger than the influence of latitude for this region in autumn (Fig. 11A,E). The significant family differences detected for autumn cold hardiness in mature trees support previous findings of variability in autumn cold hardiness among families of two year old red alder seedlings (Cannell *et al.* 1987).

There is no widely applicable pattern regarding differences in midwinter cold hardiness among geographically distant families of trees. Some studies have found that the autumn pattern of northern or inland families demonstrating a greater tolerance of cold continues through to midwinter (Friedman *et al.* 2008, Jensen and Deans 2004), while other studies have found no differences among families (Taulavuori *et al.* 2004, Li *et al.* 2003). Studies of trees in the Betulaceae have generally found no differences in midwinter cold tolerance among families originating from geographically distant provenances (Taulavuori *et al.* 2004, Li *et al.* 2003). My data did identify significant family differences in red alder cold hardiness at midwinter (Table 5). When examined at the regional level, the mean index of injury was found to follow a pattern similar to that observed in autumn (Fig. 11A,B,C). The northern inland regions continued to show the lowest index of injury at midwinter (Fig. 11B,C), though the differentiation among regions was not as strong as in the autumn. While the trend was not statistically significant, the southern mainland and Vancouver Island regions remained the most heavily damaged by freezing treatment (Fig. 11B,C). Trees originating from the Haida Gwaii were intermediate between the cold hardy northern inland regions and the more cold sensitive southern coastal regions, though again, the differences were not statistically significant (Fig. 11B,C). Overall, there was a significant negative correlation between family latitude of origin and index of injury; those families originating from farther north sustained less damage from the controlled freezing treatments at midwinter. These findings extend the work done by Cannell *et al.* (1987), where methodological limitations prevented assessment of midwinter cold hardiness of red alder seedlings. Much of the previous work on cold hardiness in the Betulaceae has focused on *Betula spp.* (e.g.: Taulavuori *et al.* 2004, Li *et al.* 2003, Li *et al.* 2002, Weih and Karlsson 1999). It appears that in *A. rubra*, significant differences in midwinter cold hardiness among

families were maintained, though these differences were less pronounced than in autumn or spring. Similar results have been observed in *A. sinuata* (Benowicz *et al.* 2000a), suggesting that these results may be more generally applicable within the genus *Alnus*. These differences among genera in the Betulaceae demonstrate the importance of investigating individual species before making ecological or silvicultural decisions.

Differences in spring cold hardiness were statistically significant among regions and among families of red alder (Fig. 11D, 12B). The regional pattern in March was similar to that in the autumn and midwinter assessments (Fig. 11B,C,D), with one notable exception. The dehardening of families from the northern mainland region occurred earlier in spring than those from the Bella Coola (Fig. 11D). This distinction between the two northern continental regions emerged only in the spring assessment, and may be due to the inland origin of the individual families of the Bella Coola region (Fig. 2). Families from the southern coastal regions (the southern mainland and Vancouver Island) were again less cold hardy than those from the northern regions, and this difference appears to be more significant in spring than midwinter (Fig. 11D). Latitude of family origin and index of injury were negatively correlated in March, once again suggesting that northern-sourced genotypes sustained less damage during the controlled freezing treatments.

The negative correlations between longitude and mean family indices of injury in September and March are surprising, given that the regional pattern suggests that families from coastal regions were more heavily damaged by the freezing treatment than were families from inland regions (Fig. 11A,D). These correlations may be in part due to the angled coastline of British Columbia placing more cold hardy northern families farther to the west than less cold hardy families from the southern mainland region (Fig. 2).

Mean family index of injury at each of the autumn, midwinter, and spring assessments were all correlated with each of the other assessments (Fig. 13, Table 6). Thus, families that were cold hardy earlier in autumn tended to remain among the most cold hardy throughout the winter, and remain cold hardy later in spring. In contrast, no correlation between autumn and spring assessments was found for the co-occurring Douglas-fir (Hawkins and Stoehr 2009).

The adaptive significance of an increased duration and depth of cold hardiness varied depending on the test site. Height and cold hardiness showed opposite correlations

between Bowser and Terrace (Fig. 14). This may represent a tradeoff between height growth and cold hardiness for red alder.

Families showing an earlier bud burst in spring also experienced more damage due to freezing treatments in March. This highlights the risk of emerging from winter dormancy too early (Beaubien and Hamann 2011). Those families more able to take advantage of a longer growing season through earlier bud flush are more likely to suffer damage due to late spring frosts, especially on colder sites. As mentioned, an increased canopy cover later in the year was associated with increased growth in both height and diameter on both test sites; however, no significant correlation between canopy cover in autumn and cold hardiness was detected. These data demonstrate the importance of local adaptation of phenology and cold hardiness to climate.

Families from the south of the province showed greater growth at Bowser than did northern families. This higher growth rate may be an adaptation to inter- and intraspecific competition, which can be very strong in high density stands arising early in stand development (Ager *et al.* 1993, Giordano and Hibbs 1993, Burns and Honkala 1990). At Terrace, the fast-growing southern families likely experienced increased damage from spring or autumn frosts, reducing their height growth in the following season. Correlations were not significant between latitude and height or diameter at Terrace, though the signs of the correlations are opposite to those at Bowser (Appendix B). As has previously been discussed, northern families showed a greater tolerance of the freezing treatments than did southern families throughout the entire winter, so it is not clear from these data when during the winter growth-retarding damage occurs. Based on previous research, it seems likely that spring and autumn cold hardiness play a major role, as these are the periods in which trees are generally most vulnerable to damage (Beaubien and Hamann 2011). However, red alder is not a very cold hardy species (Tremblay and Lalonde 1987), so it is possible that southern families planted at the northern test site experienced some level of damage from extreme midwinter cold.

Nitrogen Fixation

Neither the instantaneous nor the season-integrated measures of nitrogen fixation were found to vary significantly among families at either site. The ARA results did not

show significant differences among families, while $\delta^{15}N$ at both sites was found to vary among families only weakly. The wide variation in $\delta^{15}N$ (from -5.58‰ to -0.39‰) may therefore have been due to factors other than alder family, or my sample sizes may not have been sufficiently large. Heterogeneous distribution of nitrogen in the soil could lead to uneven rates of nitrogen fixation among individual trees or among families, as all individuals from a single family were placed in a single row in each block. The process of nitrogen fixation is energetically expensive, and so in the presence of abundant soil nitrogen, alder will reduce nodulation or fixation rates (Burris 1991, Binkley 1983). Alternatively, the heterogeneous makeup of natural soils may provide an uneven distribution of *Frankia* strains. Both the nodulation and fixation abilities of the bacteria vary among strains (Carpenter and Robertson 1984), and once an individual tree has initiated a symbiotic relationship with one strain of *Frankia*, the receptivity of the plant host to further symbioses is greatly reduced (Berry and Sunell 1990). As nodulation densities were found to vary significantly among families, but with no significant regional pattern (Fig. 15), it seems likely that one (or both) of the symbionts varies in its ability to form nodules among genotypes. Future studies identifying the diversity and natural distribution of *Frankia* strains within British Columbia would shed light on this issue.

Neither the acetylene reduction assay nor the measurement of natural abundance $\delta^{15}N$ was able to identify significant differences in nitrogen fixation among regions. $\delta^{15}N$ identified significant differences among families only weakly at both sites. Both acetylene reduction and natural abundance nitrogen isotope analysis have limitations, as discussed in the Introduction.

The percentage of nitrogen by mass in collected bud tissues was found to be slightly lower, but overlapping with values reported previously for red alder seedlings under one year old (Hibbs *et al.* 1995). On both sites, families from the southern mainland and Bella Coola regions had higher bud nitrogen content while Haida Gwaii families had lower in mean bud nitrogen content, on average (Figs 17, 18) These observed differences may be in part due to differing glacial histories among the regions. Allozyme work has suggested that the Haida Gwaii may have acted as a refuge for red alder during the last North American glaciation (Hamann *et al.* 1998). This period of

genetic isolation may have caused a specialization of alder family symbioses with then-local strains of *Frankia*. As the distribution of strains of *Frankia* in British Columbia is, as of yet, unmapped, it may be that the planting of families from the Haida Gwaii without providing the specific strains of bacteria to which they are adapted led to reduced rates of nitrogen fixation in those families. Though no significant regional differences were detected, the pattern of mean $\delta^{15}N$ among families shows that families from the Haida Gwaii are consistently among the lower half of families in terms of proportion of nitrogen from fixation in the nodules (Fig. 16). Again, further information regarding the diversity and distribution of *Frankia* in the soils of British Columbia is required.

Correlations between latitude and $\delta^{15}N$ at Bowser, and longitude and $\delta^{15}N$ at both sites indicate that northern families obtained more of their nitrogen from fixation than did southern families, and western families obtained more of their nitrogen from fixation than did eastern families. The east-west division may again be because of the angled British Columbia coastline placing the northern families to the west of the southern families used in this study. Another, non-exclusive explanation is that selection pressures in the western coastal area may be more driven by competitive interactions than are adaptations in the more climatically severe eastern inland area. A more extreme environment may select less for rapid uptake of soil nitrogen, and more for those genotypes capable of exploiting the largest range of habitat conditions or tolerating climatic extremes. The increased rate of nitrogen fixation observed in northern families would broaden the acceptable habitat conditions for those genotypes, and may reflect the genetic history of those families recolonizing the northern region following the last glaciation (Hamann *et al.* 1998).

At Bowser, the rate of acetylene reduction was positively correlated with elevation of family origin, though the result is driven by the few families originating from high elevation (Fig 19C). While no significant correlation was found between elevation and percentage of bud nitrogen in my study, previous work has found that tree genotypes originating from higher elevations tend to have a higher nitrogen content in leaves than do low elevation genotypes, even when only separated by a few hundred metres (Weih and Karlsson 1999, Körner 1989). Higher elevation genotypes also demonstrate increased rates of photosynthesis when compared to low elevation genotypes (Körner 1989). A high rate of photosynthesis may enable more rapid fixation of nitrogen in red alder, due

to the increased abundance of photosynthate. As there was no significant trend between the total ratio of fixed nitrogen to soil nitrogen and the elevation of origin or between bud nitrogen and elevation of origin, it is likely that the observed high instantaneous rate of acetylene reduction in some high elevation families is not sustained throughout the growing season.

Inland families experience a longer winter than coastal families, and so may have adapted for rapid growth during the short growing season. The prediction of high nitrogen as an adaptation to a shorter growing season (Körner 1989) is opposite to what was observed, and it is unclear why the observed percent nitrogen in bud tissue was lower for western families than eastern families on both sites.

Mean acetylene reduction rates were lower among the tallest and largest diameter families at Terrace. As the soil at the Terrace site was richer in nitrogen, this is likely related to differences in energy expenditure among families. Those families expending energy on the expensive process of fixation likely had less photosynthate to allocate to growth, as has been found previously for *A. hirsuta* (Son *et al.* 2007). High rates of acetylene reduction at Terrace were correlated with a high index of injury in autumn. It is unclear how the differences in N fixation might directly impact cold hardiness, but it is possible that the increased availability of photosynthate allowed greater cold hardening in those families exhibiting a slower rate of acetylene reduction.

Studies of conifers have found that higher nitrogen content in evergreen needles was associated with increased fall cold hardiness (Hawkins and Stoehr 2009, Dalen and Johnsen 2004). At Bowser, those families with more nitrogen sourced from nodules (more negative $\delta^{15}N$) sustained less damage due to freezing treatment in September and December. Families with more nitrogen sourced from nodules had a lower total bud percent nitrogen in autumn, but no direct association was found between bud nitrogen content and cold hardiness.

Increased bud nitrogen content was associated with increased growth at both sites. A higher percentage of nitrogen in bud tissues was correlated with taller trees at Terrace, and with larger diameter trees at Bowser. Higher rates of growth have been reported for conifers fertilized with nitrogen, despite synchronous bud burst (Bigras *et al.* 1996). As the relationship between the proportion of nitrogen from fixation and the total nitrogen

content of buds was negative, it seems as though nitrogen fixation was not a significant factor in the growth rate of individual red alder families. Rather, the ability to either retranslocate more nitrogen in autumn or acquire more nitrogen from the soil is likely to be associated with increased growth in red alder. In this view, nitrogen fixation is an adaptive mechanism allowing an expanded range of habitat for red alder, but of limited competitive value on higher nitrogen soils.

A higher proportion of nitrogen from fixation was found to be associated with earlier bud development in spring. It may be that an earlier start to the growing season enables an earlier start to fixation, during a period when soils contain more moisture. While differences in bud stage among families were small, they were significant, and appear to be correlated with increased nitrogen fixation over the course of the season, as well as increased growth. In contrast, those families with more canopy cover in autumn had a lower proportion of nitrogen from fixation in the nodules than did families with less canopy cover. The duration of the growing season may be less important than the timing of the growing season when discussing nitrogen fixation, as the nodules require specific soil moisture conditions to function optimally (Shipton and Burggraaf 1982). An earlier start to the growing season may be more advantageous because of increased soil moisture in the coastal British Columbian climate as compared to the drier summer and autumn months.

While seasonal moisture differences may have had significant impact on the total nitrogen fixed, the instantaneous rates of acetylene reduction were not found to vary significantly with rainfall. The number of days since the last rainfall event was not significantly correlated with acetylene reduction rates. As with Ekblad *et al.*'s (1994) study, the number of rainfall events during the assessment period was limited, and so it is impossible to rule out rainfall as a significant factor in the general case.

Acetylene reduction rates were not correlated with the temperature of the incubation pit. Nitrogenase activity is negatively impacted by sudden changes in temperature or other sudden disturbance (Winship and Tjepkema 1985, Sundström, and Huss-Danell 1987 Wheeler *et al.* 1978). While pit temperatures ranged from 13.3 - 24°C, the approximation of ambient soil temperature appears to have limited the damage to the nodules in my study. The lack of correlation with rates of acetylene reduction suggests

that the buffering effect of incubating in a covered pit reduced any change in temperature caused by removing the nodules from the soil.

Contrary to expectations, canopy cover at both sites was positively correlated with $\delta^{15}N$, indicating that those trees with more canopy later in the year obtained less of their nitrogen from fixation by the nodules. This suggests that photosynthate availability in autumn is not the dominant factor in determining nitrogen fixation over the entire growing season, as may have been expected from a simple interpretation of Sundström and Huss-Danell's (1987) theory. A higher water use efficiency, which was correlated with high canopy cover, implies that less time was spent with open stomata and so less photosynthate may have been available. It may also be that in red alder, fixation is constrained by factors other than the availability of photosynthetic area late in the growing season, such as physical damage to the nodules due to drought or other stresses over the growing season (Wheeler *et al.* 1978). However, the negative correlation between canopy cover in autumn and acetylene reduction rates in summer at Terrace does not support this hypothesis. Those families with less canopy cover late in the year had a higher instantaneous rate of nitrogen fixation at Terrace and so, as has been suggested previously, drought may not have had a direct physical effect on nitrogen fixation rates (Lee *et al.* 2005). It is more likely then, that those families with a shorter growing season likely exhibit higher rates of photosynthesis, as has been found previously for high altitude trees (Körner 1989). An increased rate of photosynthesis earlier in the year would increase photosynthate availability during a period when nodules are less likely to be stressed by drought, satisfying both Sundström and Huss-Danell's (1987), and Wheeler *et al.*'s (1978) theories.

Canopy cover and bud nitrogen concentration were positively correlated, thus those families with more foliage later in the season also had a higher concentration of nitrogen in the buds. Bud nitrogen concentration at the end of the growing season may not be reflective of the nitrogen content of the leaves during the growing season; the proportion of bud N observed was lower than has previously been observed for red alder foliage in seedlings (1-2.5% in buds vs. 2.5-3.1% in foliage) (Tripp *et al.* 1979). Even so, this trend was somewhat unexpected in light of previous work that has found that those trees with higher foliar nitrogen content may be adapted to a shorter growing season

(Körner 1989). As the rates of photosynthesis of red alder were not measured in the current study, it is impossible to say for certain whether or not an increased rate of photosynthesis was associated with higher nitrogen fixation or canopy cover. As the best explanation for the trends observed for nitrogen fixation relies on the rate of photosynthesis, further research is clearly necessary before the (not necessarily contradictory) theories may be fully evaluated.

Implications of a Changing Climate

Correlations among Bowser and Terrace indices of injury suggest that red alder families are tightly adapted to their region of origin. Fast-growing families originating from the south of the province appear to have been damaged by frost events at the Terrace site, due to an inability to significantly acclimate to the local conditions. Similarly, the regional differences in canopy cover, specifically involving families from the Haida Gwaii, were maintained regardless of site. Tight adaptations may pose a problem for assisted migration programs, as more care must be exercised to properly match genotypes to planting sites than if the species showed a greater ability to acclimate.

While climates are generally expected to warm in the coming decades, possibly decreasing the risk of midwinter cold damage, extreme early fall and late spring frost events are expected to become more common (Beaubien and Hamann 2011). This increased risk of frost damage must be considered in any context where alder families are being planted outside of the range to which they have adapted. In this study, I found that at the warmer southern site those families showing high growth were also those that were least cold tolerant throughout the entire period from autumn to spring. Thus, while a south to north movement of families may be tempting to take advantage of increased growth rates in a warmer climate, this increased growth may be negated or even reversed due to increased susceptibility to early or late cold. This concern is illustrated clearly by the data for the Terrace site, where the tallest families were also the most cold hardy, while those less cold hardy families were notably smaller.

No significant differences in water use efficiency as measured by $\delta^{13}C$ were identified among red alder families or regions. A clinal trend was identified, with more western families of alder showing decreased water use efficiency and increased foliage

loss. While other studies have also failed to find significant differences among families in water use efficiency (Dang *et al.* 1994), other drought-related characteristics have been found to vary among families of red alder (Hibbs *et al.* 1995, Dang *et al.* 1994). While drought hardiness may not be an immediate priority in tree breeding programs seeking to improve yield, a drying climate necessitates vigilance in maintaining drought hardy stock for planting. One positive finding in light of a drying climate is that as atmospheric CO_2 increases, red alder is likely to show an increased tolerance to drought (Hibbs *et al.* 1995).

A final consideration in light of climate change is the reduction of carbon emissions afforded by a silvicultural system utilizing red alder in lieu of chemically synthesized fertilizers. Man-made fertilizers require large amounts of energy to fuel their production, the primary source of which is petrochemical combustion (Woods *et al.* 2010). By more closely replicating a natural system, with red alder adding biologically fixed nitrogen to soils, it may be possible to reduce the carbon emission cost of silviculture, while simultaneously taking advantage of larger yields associated with fertilization (Binkley 1984).

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Appendix A: ANOVA Tables

Summary of results from outputs of Proc MIXED analyses (REML estimation method). Covariance parameter estimates for random effects (Estimate), Z value for random effects or F value for fixed effects (Family, Region or Temperature) (Z or F), and significance of covariance parameter estimate tests (Pr Z) or tests of fixed effects (p > F)

Table 7: Proc MIXED analysis of family differences in height at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		1.77	0.0206
Block	1.4332	0.69	0.2454
Family*Block	1.5019	3.86	<0.0001
Residual	1.7450	12.15	<0.0001

Table 8: Proc MIXED analysis for regional differences in height at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		11.53	0.0090
Block	1.4743	0.70	0.2434
Family*Block	0.02072	0.29	0.3876
Residual	3.5336	13.97	<0.0001

Table 9: Proc MIXED analysis for family differences in height at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		3.89	<0.0001
Residual	2.028	8.54	<0.0001

Table 10: Proc MIXED analysis for regional differences in height at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		12.36	<0.0001
Block	0	.	.
Family*Block	214463	9.85	<0.0001
Residual	2.7903	9.85	<0.0001

Table 11: Proc MIXED analysis for family differences in DBH at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		2.53	0.0003
Block	0.1504	0.56	0.2881
Family*Block	0.5011	1.18	0.1186
Residual	6.1103	12.55	<0.0001

Table 12: Proc MIXED analysis for regional differences in DBH at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		15.00	0.0082
Block	0.2146	0.61	0.2709
Family*Block	0	.	.
Residual	7.3265	0.5080	14.42

Table 13: Proc MIXED analysis for family differences in DBH at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		1.74	0.0086
Block	0.1844	0.70	0.2423
Family*Block	1.8571	3.04	0.0012
Residual	7.92898	14.27	<0.0001

Table 14: Proc MIXED analysis for regional differences in DBH at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		10.07	0.0150
Block	0.1795	0.65	0.2567
Family*Block	0.2308	0.87	0.1924
Residual	10.0540	16.43	<0.0001

Table 15: Proc MIXED analysis for family differences in bud burst at Bowser in March 2010

	Estimate	Z or F	Pr Z or p > F
Family		1.48	0.0707
Block	$2.29e^{-4}$	0.26	0.3957
Family*Block	$5.230e^{-3}$	1.24	0.1072
Residual	0.0604	12.79	<0.0001

Table 16: Proc MIXED analysis for regional differences in bud burst at Bowser in March 2010

	Estimate	Z or F	Pr Z or p > F
Region		5.46	0.0430
Block	$2.36e^{-4}$	0.31	0.3798
Family*Block	$1.4e^{-5}$	0.01	0.4952
Residual	0.0673	14.65	<0.0001

Table 17: Proc MIXED analysis for family differences in bud burst at Bowser in April 2010

	Estimate	Z or F	Pr Z or p > F
Family		3.52	<0.0001
Block	$4.48e^{-4}$	0.44	0.3297
Family*Block	$2.037e^{-3}$	0.74	0.2309
Residual	0.0492	12.94	<0.0001

Table 18: Proc MIXED analysis for regional differences in bud burst at Bowser in April 2010

	Estimate	Z or F	Pr Z or p > F
Region		8.01	0.0197
Block	$5.83e^{-4}$	0.47	0.3203
Family*Block	0	.	.
Residual	0.0652	14.68	<0.0001

Table 19: Proc MIXED analysis for family differences in bud burst at Terrace in April 2010

	Estimate	Z or F	Pr Z or p > F
Family		1.46	0.0518
Block	3.12e-4	0.48	0.3153
Family*Block	5.046e-3	2.14	0.0163
Residual	0.03732	14.12	<0.0001

Table 20: Proc MIXED analysis for regional differences in bud burst at Terrace in April 2010

	Estimate	Z	Pr Z or p > F
Region		5.50	0.0109
Block	$1.10e^{-4}$	0.29	0.3852
Family*Block	$8.4e^{-5}$	0.11	0.4576
Residual	0.0433	16.15	<0.0001

Table 21: Proc MIXED analysis for family differences in bud burst at Bowser in April 2011

	Estimate	Z or F	Pr Z or p > F
Family		1.91	0.0078
Block	0	.	.
Family*Block	5.111e-3	1.25	0.1061
Residual	0.06498	12.83	<0.0001

Table 22: Proc MIXED analysis for regional differences in bud burst at Bowser in April 2011

	Estimate	Z or F	Pr Z or p > F
Region		2.68	0.1513
Block	$1.7e^{-5}$	0.02	0.4925
Family*Block	$1.319e^{-3}$	0.52	0.2999
Residual	0.07679	14.49	<0.0001

Table 23: Proc MIXED analysis for family differences in canopy cover at Bowser in September 2010

	Estimate	Z or F	Pr Z or p > F
Family		2.15	0.0023
Block	1.2924	0.33	0.3694
Family*Block	45.1348	3.00	0.0014
Residual	132.09	12.68	<0.0001

Table 24: Proc MIXED analysis for regional differences in canopy cover at Bowser in September 2010

	Estimate	Z or F	Pr Z or p > F
Region		6.65	0.0290
Block	1.1136	0.29	0.3851
Family*Block	3.1467	0.51	0.3044
Residual	202.17	14.48	<0.0001

Table 25: Proc MIXED analysis for family differences in canopy cover at Terrace in October 2010

	Estimate	Z or F	Pr Z or p > F
Family		7.64	<0.0001
Block	1.0678	0.48	0.3173
Family*Block	10.4262	1.09	0.1385
Residual	171.29	13.94	<0.0001

Table 26: Proc MIXED analysis for regional differences in canopy cover at Terrace in October 2010

	Estimate	Z or F	Pr Z or p > F
Region		40.36	<0.0001
Block	1.3758	0.52	0.3020
Family*Block	0	.	.
Residual	240.21	16.37	<0.0001

Table 27: Proc MIXED analysis for family differences in canopy cover at Bowser in November 2010

	Estimate	Z or F	Pr Z or p > F
Family		4.53	<0.0001
Block	2.038	0.51	0.3065
Family*Block	11.07	1.28	0.0998
Residual	130.41	12.79	<0.0001

Table 28: Proc MIXED analysis for regional differences in canopy cover at Bowser in November 2010

	Estimate	Z or F	Pr Z or p > F
Region		10.77	0.0104
Block	0.7903	0.33	0.3721
Family*Block	0	.	.
Residual	200.97	14.68	<0.0001

Table 29: Proc MIXED analysis for family differences in $\delta^{13}C$ at Bowser

	Estimate	Z or F	Pr Z or p > F
		1.34	0.1583
Block	0.2947	0.66	0.2559
Family*Block	0.4409	1.85	0.0319
Residual	1.1871	6.77	<0.0001

Table 30: Proc MIXED analysis for regional differences in $\delta^{13}C$ at Bowser

	Estimate	Z or F	Pr Z or p > F
		1.89	0.2506
Block	0.2278	0.64	0.2606
Family*Block	0.01659	0.18	0.4276
Residual	1.7503	9.35	<0.0001

Table 31: Proc MIXED analysis for family differences in $\delta^{13}C$ at Terrace

	Estimate	Z or F	Pr Z or p > F
		1.27	0.2029
Block	2.3651	0.70	0.2417
Family*Block	0.1934	0.78	0.2179
Residual	1.6311	6.84	<0.0001

Table 32: Proc MIXED analysis for regional differences in $\delta^{13}C$ at Terrace

	Estimate	Z or F	Pr Z or p > F
		2.10	0.2175
Block	2.3701	0.70	0.2417
Family*Block	$5.421e^{-3}$	0.07	0.4720
Residual	1.8989	9.57	<0.0001

Table 33: Proc MIXED analysis for family differences in index of injury at Bowser in September 2010

	Estimate	Z or F	Pr Z or p > F
Family		1.80	0.0219
Temperature		1308.75	0.0008
Family*Temperature		0.97	0.5684
Block	0.4507	0.18	0.4268
Family*Block	45.7789	3.58	0.0002
Block*Temperature	0	.	.
Residual	100.01	14.05	<0.0001

Table 34: Proc MIXED analysis for regional differences in index of injury at Bowser in September 2010

	Estimate	Z or F	Pr Z or p > F
Region		4.38	0.0655
Temperature		795.60	0.0013
Family*Temperature		1.62	0.0983
Block	0	.	.
Family*Block	9.829	1.28	0.1005
Block*Temperature	0	.	.
Residual	142.99	16.88	<0.0001

Table 35: Proc MIXED analysis for family differences in index of injury at Terrace in October 2010

	Estimate	Z or F	Pr Z or p > F
Family		1.93	0.0119
Temperature		77.64	0.0127
Family*Temperature		1.06	0.3366
Block	4.7411	0.50	0.3097
Family*Block	31.3947	3.56	0.0002
Block*Temperature	3.2171	0.82	0.2074
Residual	71.5500	14.00	<0.0001

Table 36: Proc MIXED analysis for regional differences in index of injury at Terrace in October 2010

	Estimate	Z or F	Pr Z or p > F
Region		5.43	0.0434
Temperature		71.83	0.0137
Family*Temperature		2.45	0.0072
Block	2.2924	0.34	0.3685
Family*Block	5.9827	1.23	0.1093
Block*Temperature	3.0149	0.75	0.2274
Residual	100.75	16.86	<0.0001

Table 37: Proc MIXED analysis for family differences in index of injury at Bowser in December 2010

	Estimate	Z or F	Pr Z or p > F
Family		2.52	0.0008
Temperature		820.27	0.0012
Family*Temperature		1.04	0.3840
Block	0.3614	0.50	0.3092
Family*Block	3.8582	2.53	0.0057
Block*Temperature	0	.	.
Residual	20.0511	14.07	<0.0001

Table 38: Proc MIXED analysis for regional differences in index of injury at Bowser in December 2010

	Estimate	Z or F	Pr Z or p > F
Region		6.87	0.0271
Temperature		576.57	0.0017
Family*Temperature		2.87	0.0016
Block	0.3763	0.49	0.3120
Family*Block	0.3985	0.65	0.2565
Block*Temperature	0	.	.
Residual	26.2834	17.02	<0.0001

Table 39: Proc MIXED analysis for family differences in index of injury at Bowser in January 2011

	Estimate	Z or F	Pr Z or p > F
Family		1.99	0.0092
Temperature		136.59	0.0073
Family*Temperature		0.27	1.000
Block	0.8216	0.32	0.3738
Family*Block	30.0753	3.03	0.0012
Block*Temperature	0	.	.
Residual	108.24	14.06	<0.0001

Table 40: Proc MIXED analysis for regional differences in index of injury at Bowser in January 2011

	Estimate	Z or F	Pr Z or p > F
Region		5.78	0.0384
Temperature		96.02	0.0103
Family*Temperature		0.72	0.7082
Block	0.1381	0.08	0.4693
Family*Block	3.1898	0.95	0.1722
Block*Temperature	0	.	.
Residual	133.29	16.91	<0.0001

Table 41: Proc MIXED analysis for family differences in index of injury at Bowser in March 2011

	Estimate	Z or F	Pr Z or p > F
Family		1.84	0.0184
Temperature		635.87	0.0252
Family*Temperature		1.07	0.3609
Block	3.2169	0.49	0.3118
Family*Block	34.5226	3.41	0.0003
Block*Temperature	0.7569	0.37	0.3544
Residual	85.6709	14.79	<0.0001

Table 42: Proc MIXED analysis for regional differences in index of injury at Bowser in March 2011

	Estimate	Z or F	Pr Z or p > F
Region		9.99	0.0123
Temperature		715.49	0.0238
Family*Temperature		4.11	0.0011
Block	3.0840	0.50	0.3068
Family*Block	3.7284	0.79	0.2148
Block*Temperature	0.1850	0.12	0.4528
Residual	117.98	16.82	<0.0001

Table 43: Proc MIXED analysis for family differences in $\delta^{15}N$ at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		0.93	0.5935
Block	0.5647	0.70	0.2423
Family*Block	0.0635	0.84	0.1999
Residual	0.4583	6.67	<0.0001

Table 44: Proc MIXED analysis for regional differences in $\delta^{15}N$ at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		1.26	0.4030
Block	0.6043	0.70	0.2416
Family*Block	0	.	.
Residual	0.5050	9.49	<0.0001

Table 45: Proc MIXED analysis for family differences in $\delta^{15}N$ at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		1.60	0.0510
Block	0	.	.
Family*Block	$7.41e^{-3}$	.	0.4656
Residual	0.6806	6.96	<0.0001

Table 46: Proc MIXED analysis for regional differences in $\delta^{15}N$ at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		2.50	0.1689
Block	0	.	.
Family*Block	0	.	.
Residual	0.7643	9.72	<0.0001

Table 47: Proc MIXED analysis for family differences in acetylene reduced per metre at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		1.30	0.1809
Block	0	.	.
Family*Block	0	.	.
Residual	0.0249	8.72	<0.0001

Table 48: Proc MIXED analysis for regional differences in acetylene reduced per metre at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		1.91	0.2476
Block	0	.	.
Family*Block	0	.	.
Residual	0.02547	10.61	<0.0001

Table 49: Proc MIXED analysis for family differences in acetylene reduced per metre at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		1.24	0.2242
Block	0	.	.
Family*Block	0	.	.
Residual	$8.49e^{-3}$	8.51	<0.0001

Table 50: Proc MIXED analysis for regional differences in acetylene reduced per metre at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		0.45	0.7987
Block	0	.	.
Family*Block	0	.	.
Residual	$9.20e^{-3}$	9.72	<0.0001

Table 51: Proc MIXED analysis for family differences in acetylene reduced per gram nodule tissue at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		0.85	0.7223
Block	0	.	.
Family*Block	0.0504	0.14	0.4436
Residual	2.8597	7.14	<0.0001

Table 52: Proc MIXED analysis for regional differences in acetylene reduced per gram nodule tissue at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		0.43	0.8131
Block	0.0242	0.36	0.3585
Family*Block	0	.	.
Residual	2.6789	10.61	<0.0001

Table 53: Proc MIXED analysis for family differences in acetylene reduced per gram nodule tissue at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		0.99	0.5193
Block	0	.	.
Family*Block	0	.	.
Residual	1.5576	8.51	<0.0001

Table 54: Proc MIXED analysis for regional differences in acetylene reduced per gram nodule tissue at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		0.83	0.5762
Block	0	.	.
Family*Block	0.0330	0.34	0.3679
Residual	1.5322	9.47	<0.0001

Table 55: Proc MIXED analysis for family differences in percent nitrogen in bud tissues at Bowser

	Estimate	Z or F	Pr Z or p > F
Family		1.53	0.0727
Block	0	.	.
Family*Block	0	.	.
Residual	$3.164e^{-6}$	.	.

Table 56: Proc MIXED analysis for regional differences in percent nitrogen in bud tissues at Bowser

	Estimate	Z or F	Pr Z or p > F
Region		4.99	0.0512
Block	0	.	.
Family*Block	0	.	.
Residual	$3.26e^{-6}$	.	.

Table 57: Proc MIXED analysis for family differences in percent nitrogen in bud tissues at Terrace

	Estimate	Z or F	Pr Z or p > F
Family		1.64	0.0433
Block	$1.971e^{-7}$	.	.
Family*Block	$1.478e^{-6}$	.	.
Residual	$3.386e^{-6}$	.	.

Table 58: Proc MIXED analysis for regional differences in percent nitrogen in bud tissues at Terrace

	Estimate	Z or F	Pr Z or p > F
Region		5.69	0.0397
Block	$1.947e^{-7}$	.	.
Family*Block	0	.	.
Residual	$5.226e^{-6}$	.	.

Appendix B: Correlation Table

Table 59: Correlation table showing Pearson correlation coefficients for geographic origin and physiological data. Correlations with p-values from 0.025-0.05 are indicated with a single asterisk (*), from 0.0001-0.025 are indicated by two (**), and under 0.0001 are indicated by three (***). Variable abbreviations: latitude (Lat), longitude (Long), elevation (Elev), mean family height at Bowser (hBW) and at Terrace (hTR), mean family diameter at breast height at Bowser (dbhBW) and at Terrace (dbhTR), mean family acetylene reduced per cubic metre of soil at Bowser (ARA/mBW) and Terrace (ARA/mTR), mean family acetylene reduced per gram of nodule tissue at Bowser (ARA/gBW) and Terrace (ARA/gTR), mean family total mass of nodules excavated at Bowser (mpBW) and Terrace (mpTR), mean family index of injury in September (iiS), October (iiO), December (iiD), January (iiJ), and March (iiM), mean family bud burst stage at Bowser in March, 2010 (bbM10BW), April, 2010 (bbA10BW), and April, 2011 (bbA11BW), as well as in April, 2010 at Terrace (bbA10TR), mean family canopy cover at Bowser in September (ccSBW) and November (ccNBW), as well as in October at Terrace (ccOTR), mean family $\delta^{13}C$ for both sites ($\delta^{13}C$), for Bowser ($\delta^{13}CBW$) and for Terrace ($\delta^{13}CTR$), mean family $\delta^{15}N$ for both sites ($\delta^{15}N$), for Bowser ($\delta^{15}NBW$) and for Terrace ($\delta^{15}NTR$), and mean family percent nitrogen by mass in bud tissue at Bowser (%NBW) and Terrace (%NTR).

	Lat	Long	Elev	hBW	hTR	dbhBW	dbhTR	ARA/mBW
Lat	-	.812***	-.250	-.390***	.220	-.377***	.167	-.003
Long		-	-.262	-.376***	-.143	-.540***	-.144	-.093
Elev			-	-.150	.093	-.115	.043	.473***
hBW				-	-.004	.738***	.060	-.118
hTR					-	.003	.591***	-.064
dbhBW						-	.197	-.073
dbhTR							-	-.008
ARA/mBW								-
ARA/gBW								
mpBW								
ARA/mTR								
ARA/gTR								
mpTR								
iiS								
iiO								
iiD								
iiJ								
iiM								
bbM10BW								
bbA10BW								
bbA11BW								
bbA10TR								
ccSBW								
ccOTR								
ccNBW								
$\delta^{13}C$								
$\delta^{13}CBW$								
$\delta^{13}CTR$								

$\delta^{15}N$
$\delta^{15}NBW$
$\delta^{15}NTR$
%NBW
%NTR

	ARA/gBW	mpBW	ARA/mTR	ARA/gTR	mpTR	iiS	iiO	iiD
Lat	-.095	.052	.159	.020	.051	-.671***	-.380***	-.390***
Long	-.093	-.087	.191	.217	-.066	-.445***	-.101	-.228
Elev	.140	.302*	-.161	.001	-.177	.129	-.169	.064
hBW	.033	-.112	-.041	-.127	-.036	.373***	.349**	.340**
hTR	-.026	.107	.092	-.379***	.350*	-.332**	-.372***	-.195
dbhBW	.101	-.138	-.219	-.313*	-.093	.332**	.178	.204
dbhTR	.067	.002	-.136	-.450***	.170	-.374***	-.201	-.256
ARA/mBW	.234	.739***	.082	.069	.183	-.010	-.217	-.014
ARA/gBW	-	-.074	.113	.044	.013	.015	.018	-.066
mpBW		-	.131	.002	.252	-.127	-.258	-.100
ARA/mTR			-	.388***	.690***	.068	.019	.051
ARA/gTR				-	-.142	.271	.322**	.023
mpTR					-	-.086	.007	.046
iiS						-	.527***	.530***
iiO							-	.289*
iiD								-
iiJ								
iiM								
bbM10BW								
bbA10BW								
bbA11BW								
bbA10TR								
ccSBW								
ccOTR								
ccNBW								
$\delta^{13}C$								
$\delta^{13}C$BW								
$\delta^{13}C$TR								
$\delta^{15}N$								
$\delta^{15}N$BW								
$\delta^{15}N$TR								
%NBW								
%NTR								

	iiJ	iiM	bbM10BW	bbA10BW	bbA11BW	bbA10TR	ccSBW	ccOTR
Lat	-.363**	-.577***	.081	.177	.024	.361**	-.171	-.223
Long	-.102	-.378***	.075	.144	-.052	.028	-.460***	-.650***
Elev	-.038	.117	.016	.055	.057	.078	-.078	.003
hBW	.468***	.371***	.123	.202	.206	.029	.172	.111
hTR	-.230	-.171	-.230	.085	.042	.383***	.314*	.414***
dbhBW	.238	.190	.155	.171	.257	.181	.375***	.403***
dbhTR	-.102	-.497***	-.168	-.102	-.039	.187	.303	.333**
ARA/mBW	-.070	.060	-.047	-.010	.083	.056	.018	.093
ARA/gBW	.058	.023	-.173	-.036	-.020	-.064	.012	.089
mpBW	-.216	-.039	-.147	.016	.055	.121	.141	.216
ARA/mTR	-.023	-.009	.220	.120	.053	.021	-.265	-.059
ARA/gTR	-.046	-.028	.182	-.189	-.177	-.093	-.392***	-.377***
mpTR	.053	.067	.021	.091	.097	.059	.050	.227
iiS	.293*	.561***	.088	.067	.053	-.092	.115	.039
iiO	.526***	.349**	.034	-.072	-.125	-.221	-.103	-.214
iiD	.316*	.447***	.182	.100	.185	-.049	.043	-.092
iiJ	-	.478***	.211	.201	.118	-.187	-.218	-.269
iiM		-	.122	.297*	.220	-.105	.018	.011
bbM10BW			-	.613***	.586***	.333**	-.316*	-.231
bbA10BW				-	.629***	.488***	-.053	-.103
bbA11BW					-	.386***	-.202	-.107
bbA10TR						-	.119	.197
ccSBW							-	.778***
ccOTR								-
ccNBW								
$\delta^{13}C$								
$\delta^{13}CBW$								
$\delta^{13}CTR$								
$\delta^{15}N$								
$\delta^{15}NBW$								
$\delta^{15}NTR$								
%NBW								
%NTR								

	ccNBW	$\delta^{13}C$	$\delta^{13}CBW$	$\delta^{13}CTR$	$\delta^{15}N$	$\delta^{15}NBW$	$\delta^{15}NTR$	%NBW	%NTR
Lat	-.515***	-.043	.059	-.086	-.352**	-.309*	-.262	-.200	-.105
Long	-.580***	-.281	-.126	-.262	-.469***	-.352**	-.403***	-.455***	-.467***
Elev	.076	.162	.164	.047	.157	.126	.118	.040	.173
hBW	.353**	-.296*	-.259	-.188	.041	.006	.041	.233	.113
hTR	.108	.209	.213	.108	-.045	-.178	.076	.152	.395***
dbhBW	.439***	-.113	-.128	-.025	.227	.169	.198	.369***	.376***
dbhTR	.059	.337**	.340**	.158	-.083	-.018	.087	.230	.216
ARA/mBW	.098	.046	.060	-.027	.107	.062	.097	.003	.040
ARA/gBW	.079	-.037	-.086	.030	-.072	.195	-.241	-.005	-.090
mpBW	.119	-.062	-.074	-.052	.199	-.037	.292*	.040	.072
ARA/mTR	.009	-.138	-.245	.071	-.305*	-.200	-.283	-.310*	-.203
ARA/gTR	-.210	-.198	-.179	-.132	-.100	.060	-.167	-.127	-.192
mpTR	.243	.066	.017	.094	-.268	-.204	-.234	-.148	-.078
iiS	.584***	-.095	-.123	-.029	.279	.288*	.186	.149	.154
iiO	.215	-.110	.009	-.188	-.053	.133	-.173	.088	-.146
iiD	.356**	.095	.078	.041	.225	.300*	.086	.271	.065
iiJ	.082	-.106	-.026	-.147	-.139	.026	-.217	-.021	-.328**
iiM	.372***	-.111	-.128	-.054	.163	.058	.158	.049	-.088
bbM10BW	-.278	.025	-.129	.187	-.184	-.163	-.136	-.159	.036
bbA10BW	-.059	-.151	-.261	.047	-.172	-.278	-.051	-.107	.028
bbA11BW	-.095	.040	-.070	.168	-.252	-.186	-.208	.024	-.002
bbA10TR	-.007	.061	-.118	.211	.139	.043	.172	.257	.445***
ccSBW	.661***	.184	.091	.140	.456***	.243	.452***	.385***	.579***
ccOTR	.569***	.294*	.121	.303*	.443***	.251	.438***	.413***	.594***
ccNBW	-	.137	.086	.089	.365**	.320*	.278	.342**	.389***
$\delta^{13}C$		-	.730***	.735***	.159	.258	.049	.342	.228
$\delta^{13}CBW$			-	.082	-.023	.243	-.173	.258	.144
$\delta^{13}CTR$				-	.209	.102	.208	.218	.174
$\delta^{15}N$					-	.704***	.869***	.474***	.535***
$\delta^{15}NBW$						-	.267	.577***	.285*
$\delta^{15}NTR$							-	.244	.556***
%NBW								-	.332**
%NTR									-