Cold hardiness and carotenoid variation in western redcedar (*Thuja plicata* Donn ex. D. Don.): Implications for assisted migration for future climates

by

Elizabeth van der Merwe
B.S.F., University of British Columbia, 2013

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Supervisory Committee

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Dr. Barbara Hawkins, Department of Biology
Supervisor

Dr. Jürgen Ehlting, Department of Biology
Departmental Member

Dr. Alvin Yanchuk, Department of Biology
Departmental Member

Abstract

Western redcedar (*Thuja plicata* Donn ex D. Don; redcedar), an indeterminate conifer in the Cupressaceae family, is vulnerable to maladaptation in the face of climate change. Assisted gene flow is one mitigation strategy and involves human-mediated migration of populations, where the projected climate of the area of deployment matches the source climate of the population. Despite the overall projections of warmer temperatures globally, in British Columbia (B.C.), the risk of seasonal frost events will remain and therefore the potential for cold damage and mortality of redcedar exists if the newly migrated populations cannot withstand these freezing events. Knowledge of redcedar's ability to withstand freezing temperatures (cold hardiness) is therefore crucial. Redcedar, like many Cupressaceae species, produces and accumulates the purple-coloured carotenoid rhodoxanthin during the winter. This was hypothesized to be correlated with cold hardiness.

Assessment of variation in overall, fall and spring cold hardiness and associated rhodoxanthin concentrations were done through repeated, seasonal freeze testing of clonal grafts originating from across the range of redcedar, and seedling progeny from a subset of these clones. Cold damage was quantified using electrolyte leakage and rhodoxanthin concentrations were quantified using high performance liquid chromatography. Cold hardiness and rhodoxanthin were individually modelled using univariate and bivariate mixed effect models with clone/family as a random effect. Model outputs were compared to climatic variables associated with clonal origin to test for climatic relationships.

This study found genetic variation in cold hardiness of redcedar with weak climatic clines. This indicates that assisted gene flow of redcedar should be done on a case-by-case basis, with no need for a climatic threshold. Overall heritability of cold hardiness was 0.17 ± 0.03. Novel findings included the positive genetic correlation between fall and spring cold hardiness (0.55 ± 0.33); lack of reciprocal or parental effect for overall cold hardiness; and weak climatic relationships between cold hardiness and predominantly temperature, with the strongest correlation between number of frost-free days in January (0.38, $p < 0.01$) in the location of origin and cold hardiness.

All findings related to rhodoxanthin were novel. Rhodoxanthin varied with family/provenance and season with heritabilities of 0.30 ± 0.09 in fall, 0.42 ± 0.09 in winter and 0.28 ± 0.09 in spring. Winter and spring rhodoxanthin concentrations were phenotypically correlated (0.50, $p < 0.01$) and genetically correlated (0.76 ± 0.14). Surprisingly, rhodoxanthin was not detected in clonal grafts of redcedar in any season. Results also indicate that rhodoxanthin cannot be used to estimate cold hardiness. The absence of rhodoxanthin in the clonal grafts compared to the seedlings suggests that plant age impacts rhodoxanthin accumulation.

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Dedication

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Chapter 1: Introduction

Western redcedar (*Thuja plicata* Donn ex. D. Don; redcedar), a conifer and member of the Cupressaceae family, is an important climax species in British Columbia's (B.C.) coastal and southern interior forests. Known as the 'Tree of Life', redcedar is culturally significant to First Nation's peoples (Zahn et al. 2018) and is the provincial tree of British Columbia. Redcedar wood and lumber products generate approximately CAN $1 billion in revenue annually and support an estimated 1900 jobs in B.C. (Gregory et al. 2018).

Projections of climate change for most tree species, redcedar included, suggest many populations are vulnerable. Because trees are long-lived, stationary organisms with lengthy reproductive cycles, they are typically well-adapted to their places of origin (Morgenstern 1996) but vulnerable to environmental change. Climate change over the last twenty-five years has already shifted climate conditions in B.C. an average of 130 km northward and 60 m upward in elevation (Gray & Hamman, 2013). This significant change over such a short period of time is too rapid for most tree species to be able to adapt (Aitken & Bemmels, 2016).

Assisted migration is one strategy that has been proposed to mitigate this shift in suitable climatic envelopes for tree species resulting from climate change. Assisted migration involves human-mediated movement of species or populations to regions that are projected to match their historical climate (Aitken & Whitlock, 2013). In the case of forest trees, this involves planting tree seedlings outside their locally adapted range into areas that are projected to match the historical climate associated with those seedlings (O'Neill et al. 2008). Not all tree populations are good candidates for assisted migration.

Species or populations that inhabit high elevations or northern latitudes will have limited new habitat to which they can migrate (Gray & Hamman, 2013). In the case of redcedar, its extensive range, from the coast of northern California to the Alaska panhandle and from central Idaho to central B.C. in the interior (Minore 1981) allows for assisted gene flow northward into B.C. Redcedar is therefore an appropriate candidate for assisted migration as populations from southern or low elevation regions can be migrated northward and upward in elevation (Gray & Hamman, 2013).

A major challenge with this movement of germplasm out of its native range is that, while global temperatures are predicted to increase on average, in B.C. it is still likely that freezing events will occur, particularly on the coast where changes in future temperatures are predicted to be less than in continental regions (Fettig et al. 2013). It is possible that populations of redcedar from southern, warmer climates migrated into B.C. may not be sufficiently cold hardy enough to survive extreme winter freezing events.

Assisted migration and deployment in B.C.'s forests

Canada has the largest percentage of publicly owned forestlands in the world with approximately 93.4% across the country (Luckert et al. 2011). B.C. has a similar percentage, with approximately 95% of B.C. forestland being publicly owned (Luckert et al. 2011). Under Crown ownership, companies that wish to harvest trees from B.C.'s public forestlands are subject to government regulation under the Forest and Range Practices Act (*Forest and Range Practices Act*, 2002). Reforestation is one of the legal obligations of harvesting forests in B.C. Under Section 31 and 169 of the *Forest and Ranges Practices Act* (2002), companies must reforest harvested cutblocks by planting trees grown from seed procured under the Chief Forester's Standards for Seed Use.

Historically in B.C., seed has been sourced from local wild stands or from tree seed orchards that have selected trees chosen for favourable traits, with geographic origins tightly controlled. Local seed is considered to have suitable adaptation for local sites (O'Neill et al. 2008).

Since 2018, an assisted migration strategy, Climate Based Seed Transfer (CBST), has been approved as an optional seed deployment strategy with full adoption estimated to occur after 2023 (British Columbia, Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2020). CBST essentially utilizes northward and upward migration for species based on projecting future climates and assessing species' genetic suitability. It is conservative in its approach, assessing projected climate change over a period of one quarter of a timber harvest rotation (O'Neill et al. 2017), approximately 20 years in the Interior of B.C. and 15 years on the Coast (O'Neill et al. 2017). For example, redcedar to be planted in the southern, central interior will no longer have an appropriate seed source after full implementation of CBST (British Columbia, Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2019). While redcedar does grow south of this region, these more southern populations have not been tested and subsequently approved for incorporation in B.C. tree seed orchards due to limited demand for seedlings (fewer than two million redcedar are planted in this interior region each year) and the uncertainty regarding the cold hardiness and drought resistance of these populations. Understanding the cold hardiness of redcedar populations would help fill these knowledge gaps when selecting seed sources for the future.

Cold hardiness

Cold hardiness has been studied extensively in plants, including conifers, and is considered to be one of the major factors determining the range of tree species (Parker, 1963). Cold hardening (the process of becoming cold hardy and eventually ceasing growth) followed by the return to active growth requires a series of coordinated physiological events (Howe et al. 2003). As a seasonal process, cold hardening is typically triggered in late summer and early autumn by decreasing photoperiod (Irving & Lanphear, 1967), decreasing temperature (Scarth 1944), or both (Sakai & Weiser, 1973). Conversely, spring de-hardening is typically triggered by increasing temperature and/or increasing photoperiod. In redcedar, temperature appears to be the main driver of induction and cessation of cold hardiness (Silim & Lavender, 1994).

As many conifers have vast geographic ranges, inter-population variation in cold hardiness has been well-studied. Geographic and climatic clines have been observed for cold hardiness in Sitka spruce (*Picea sitchensis* (Bong.) Carr.; Holliday et al. 2008; Mimura & Aitken, 2007), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.*;* Hannerz et al. 1999), Douglas-fir (*Pseduotsuga menziesii* var *menziesii* (Mirb.) Franco*;* O'Neill et al. 2001; Hawkins & Stoehr, 2009), and yellow cypress (*Callitropsis nootkatensis* (D. Don) Orsted (syn. *Chamaecyparis nootkatensis* (D. Don) Spach) (Russell 1993; Russell & Krakowski, 2012). For redcedar, differences in cold hardiness have been found between coastal and interior seedlots (Cherry, 1995; Hawkins et al. 2003). In contrast, no difference in cold hardiness was observed for redcedar populations that were close in latitude but differed in elevation (Grossnickle & Russell, 2006). Weak geographic clines in winter cold hardiness were found among redcedar populations in Idaho (Rehfeldt

1994). These studies in redcedar have been limited to examining small numbers of populations at once and have not encompassed the extremes of its range. Given the mixed results, it is uncertain whether redcedar will demonstrate the geographic clines in cold hardiness across the entire species' range, as observed in the Pinaceae.

Adaptive traits, including cold hardiness, tend to be quantitative in nature and largely driven by additive genetic variation (Alberto et al. 2013). Quantitative traits in trees tend to be polygenic, meaning they are controlled by multiple genes with each gene locus providing a small effect on the expression of the phenotype and causing variation in the phenotype (e.g., growth rate; White et al. 2007).

Under the quantitative genetic model, phenotypic variation is equal to the sum of the genetic variation and environmental variation. Genetic variation can be partitioned into additive and non-additive variation. Additive variation is the variance exclusively resulting from breeding values (Falconer & Mackay, 1996). When a diploid organism sexually reproduces, the alleles of the parent organism segregate to form haploid gametes that are transmitted to its offspring. The sum of the average effect of each of those alleles for a trait is the breeding value of that trait and is responsible for the resemblance between relatives (Falconer & Mackay, 1996). The non-additive variation can be further partitioned into dominance and interaction/epistatic variation (White et al. 2007). When examined from a single locus, dominance refers to the change of the heterozygote from the midpoint of the two homozygotes (Roff & Emerson, 2006) and thus is an interaction between alleles (Falconer & Mackay, 1996). Epistatic effects occur when different genes interact with each other and are interactions between loci. Together, both dominance and epistasis are considered the non-additive genetic component (Falconer & Mackay, 1996)

and because of the independent segregation of alleles, they are not necessarily transmitted to offspring (White et al. 2007; Hartl, 2011). As a result, breeding programs tend to focus on traits with larger additive variation as greater gains can be made to improve a trait. Non-additive effects and associated variation are often the drivers of fitness-related traits, however, and can play an important role in long-term response to artificial selection (Roff & Emerson, 2006).

The ratio of additive genetic variation to total phenotypic variation is the heritability (heritability in the narrow-sense) and expresses "the extent to which individuals' phenotypes are determined by the genes transmitted from their parents" (Falconer & Mackay 1996, p. 123). As a proportion, heritability is bound between 0 and 1, with higher heritability indicating greater additive genetic control and greater potential for improvement in tree breeding programs (White et al. 2007). For many stem and growth traits in commercial tree species, heritabilities range from 0.1 to 0.3, although wood property trait heritabilities tend to be higher, from 0.3 to 0.6 (White et al. 2007).

Heritability of fall cold hardiness has been found to be 0.09 in western hemlock (Hannerz et al. 1999), 0.37 in Douglas-fir (O'Neill et al. 2001) and 0.38 in yellow cypress (Russell 1993). Spring cold hardiness tends to be under greater additive genetic control with higher heritability in both western hemlock (0.17; Hannerz et al. 1999) and Douglas-fir (0.57; O'Neill et al. 2001). Spring cold hardiness heritability has not been reported for yellow cypress or redcedar.

Although additive variation is common for cold hardiness, on occasion significant dominance variation has been found. In pomegranate cultivars (*Punica granatum* L), winter cold hardiness was influenced by additive effects, but dominance effects were

greater (Soloklui et al. 2018). This has also been found for select F1 hybrid crosses of *Eucalyptus* (Tibbits et al. 1991). In the case of *Eucalyptus*, dominance effects have also been observed for growth traits in many species and hybrids (Bouvet et al. 2009). Redcedar is unusual among gymnosperms as it exhibits high levels of homozygosity arising from its propensity for inbreeding. El-Kassaby (1999) suggested that much of the variation in redcedar's growth patterns reflects phenotypic plasticity rather than genetic diversity. Considering this, it is possible that the quantitative genetics underpinning cold hardiness in redcedar may be different from those found in the more heterozygous species of the Pinaceae, and cold hardiness in redcedar could reflect a greater tendency towards plasticity or dominance effects.

For species in Pinaceae, spring cold hardiness is well correlated with bud burst, although the relationship between fall cold hardiness and bud set is somewhat more variable (Howe et al. 2003). Fall and spring cold hardiness have also been found to have negative genetic correlations in western hemlock (Hannerz et al. 1999) and Douglas-fir (O'Neill et al. 2001) although it is unclear what is driving these negative correlations. There is an absence of information in the literature on genetic correlations between fall and spring cold hardiness in indeterminate species in general, and redcedar in particular. Given that redcedar does not form a bud, the mechanisms underlying growth cessation and the subsequent resumption of growth may be more similar (i.e. downregulation of growth in fall and upregulation in spring) and thus positively correlated.

Rhodoxanthin

Carotenoids are organic pigments that are essential to the photosynthetic capability of plant cells as part of light-harvesting complexes and protection against damage from

reactive oxygen species (Pfündel & Bilger, 1994). Carotenoids are synthesized in the plastid and, when stored in chromoplasts, create the yellow, orange and red colours of the leaves, stems, flowers and fruits of many plants (Hirschberg 2001). Carotenoids are also precursors in the synthesis of the growth hormone abscisic acid (Rock & Zeevaart, 1991). Xanthophylls, a type of carotenoid, are oxygenated forms of carotenes that act as accessory pigments in the light harvesting complex of the chloroplasts and transfer energy to chlorophyll for photosynthesis (Hirschberg 2001). The other major role of the xanthophylls is in non-photochemical quenching (NPQ), the dissipation of excess light energy that exceeds the energy required for photosynthesis through the xanthophyll cycle, a cycle of epoxidation and de-epoxidation, from violaxanthin to antheraxanthin to zeaxanthin and back (Demmig et al. 1987).

Winter provides additional challenges to evergreen plants compared to their deciduous relatives. While cold stress and freezing injury are the main winter hazards for all plants, the chloroplasts of evergreen plants are also subject to light stress under cold temperatures. Photosynthesis is a temperature-dependent process and low temperatures inhibit the function of the Calvin cycle enzymes (Taiz & Zeiger 2002). If the Calvin cycle is fully inhibited or operating at reduced capacity, the light reactions of photosynthesis are inhibited, and light energy harvested by the chloroplasts may then create damaging reactive oxygen species. Under continued exposure to light but with reduced ability to photosynthesize due to low winter temperatures, NPQ is thought to be the main way evergreen plants protect their chloroplasts from light stress during the winter (Adams et al. 1994).

Rhodoxanthin, a reddish-purple coloured carotenoid is found across both animal, and plant kingdoms. Rhodoxanthin has been found in *Tilapia* fish (Katsuyama & Matsuno, 1987), songbirds (Hudon et al. 2007), honeysuckle (Royer et al. 2020) and many gymnosperm species (Ida 1981a; Czeczuga 1987), including redcedar (Weger et al. 1993). It is likely synthesized from zeaxanthin with three intermediary compounds yet to be characterized (Hudon et al. 2007; Royer et al. 2020). Although the exact function of rhodoxanthin has not been determined (Hughes 2011), evidence strongly suggests it functions as an additional photoprotective compound in winter, along with the compounds of the xanthophyll cycle (Ida 1981b; Weger et al. 1993; Han et al. 2003; Merzlyak et al. 2005; Sofronova et al. 2014).

Han et al. (2003) found evidence of a photoprotective role of rhodoxanthin that allowed for maintenance of high photosynthetic rates in *Cryptomeria japonica* (L.F.) D. Don in winter. They found wild-type saplings that produced rhodoxanthin had less reduction in photosynthetic rates than mutants that remained green all winter. Sofranova et al. (2014) found rhodoxanthin accumulated from September to October in the leaves of the gymnosperm shrub *Ephedra monosoperma* J.G. Gmel. ex C.A. Mey and dissipated upon the development of a snowpack when light levels were reduced. In redcedar, Weger et al. (1993) found that rhodoxanthin concentrations increased upon exposure to low light and low temperatures associated with increased cold hardiness.

Development of rhodoxanthin has also been found in conditions of warmer temperatures but during high light stress conditions in *C. japonica*. It was suggested that the combination of overheating and drought stress may have caused the accumulation (Ida, 1981b). Merzlyak et al. (2005) reported high rhodoxanthin levels in *Aloe*

arborescens Mill during the winter in the deserts of Israel. This may suggest that light stress is more important than temperature in inducing rhodoxanthin development and accumulation.

While the presence of rhodoxanthin has been documented in several plant species, little work has compared variation within species in rhodoxanthin development, loss and maximum concentration. Comparisons have been few and limited to differences between sun and shade leaves. Ida (1981a) found differences in rhodoxanthin concentration between sun and shade leaves of *C. japonica.* She also found differences between juvenile seedlings (two years old) and thirty-year-old trees, where the thirty-year-old trees had significantly less rhodoxanthin, particularly in the shade leaves, which had only a trace. In *Thuja occidentalis* L., a close relative of redcedar, Czeczuga (1987) found sun leaves had 1.2 times or approximately 20% greater rhodoxanthin content than shade leaves. To the best of my knowledge, no studies comparing provenance or family variability in rhodoxanthin concentration have been completed for any rhodoxanthin-producing plant.

Anecdotally, redcedar seedlings from different geographic provenances observed at Cowichan Lake Research Station on Vancouver Island, B.C., have shown visible differences both in magnitude and timing of winter reddening. Interior B.C. provenances appear to develop purple colouration earlier and to a deeper shade than coastal provenances, but no systematic experiments or analysis has been performed.

Objectives

In order to successfully incorporate redcedar genotypes from the southern regions of the species' range into B.C.'s tree seed orchards, an understanding of the cold hardiness

of redcedar is crucial. There are gaps in the literature with regards to geographic clines in cold hardiness for redcedar and the quantitative genetics of the cold hardiness of redcedar, including heritability and inter-seasonal genetic correlations.

Given that interior provenances of redcedar have been found to be more cold hardy than coastal provenances (Cherry 1995) and my observations of earlier development of winter reddening in interior provenances, there is potential for redcedar cold hardiness to be correlated with rhodoxanthin content.

This research therefore aims to answer three questions:

1. Is there genetic variation in the cold hardiness phenotypes of redcedar across its range and are there geographic/climatic clines evident in the development of cold hardiness?

2. To what extent is cold hardiness in redcedar driven by additive genetic effects? Are there genetic correlations between fall and spring cold hardiness?

3. Does production of the carotenoid rhodoxanthin correlate with development, maintenance and loss of cold hardiness and can rhodoxanthin content be used as a proxy measure for cold hardiness?

Questions one and two regarding cold hardiness genetics and geographic/climatic clines will be largely discussed in Chapter two of this thesis. Chapter three will discuss questions related to rhodoxanthin. Chapter four will present conclusions and suggested avenues for future research.

Chapter 2: Cold Hardiness[1]

Introduction

As long-lived, stationary species with slow reproductive cycles, trees are at considerable risk for maladaptation due to climate change (Morgenstern 1996), including western redcedar (*Thuja plicata* Donn ex D. Don; redcedar). While B.C. has adopted guidelines for assisted migration as a climate change mitigation strategy, questions remain regarding the implementation, specifically on incorporating redcedar trees originating from the Pacific North West of the USA (Washington, Oregon and California) into B.C.'s coastal seed orchards for seed deployment in coastal B.C. Although climate change is expected to result in warmer temperatures, on average, the likelihood of cold events, particularly outside of historical seasonal cycles is expected to increase (Fettig et al. 2013). Coastal redcedar families located in field trials at high elevations in coastal B.C. exhibited significant stem damage due to snow, with no clear family differentiation on sites with high snow loads (Degner 2019, unpublished). If trees from warmer climates are not able to tolerate these events, the risk of redcedar plantation mortality could increase.

Quantitative genetics of cold hardiness

The ability of a plant to withstand freezing temperatures is referred to as cold hardiness or cold tolerance (Sakai & Weiser, 1973). The process of becoming cold hardy requires physiological changes to induce dormancy and associated cold hardiness, followed

[1] Manuscript intended for publication in Canadian Journal of Forest Research

eventually by the loss of cold hardiness and return to active growth. This process requires a series of coordinated processes triggered by the environmental cues of photoperiod and temperature and is controlled by many genes throughout the plant (Howe et al. 2003).

Cold hardiness has been well studied in plants including conifers native to B.C., such as Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*; Aitken & Adams 1997; O'Neill et al. 2001; Hawkins & Stoehr, 2009), Sitka spruce (*Picea sitechensis* (Bong.) Carr.; Mimura & Aiken, 2007), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.; Hannerz et al. 1999), and whitebark pine (*Pinus albicaulis* Engelm.; Bower & Aitken, 2006). Studies have typically focused on seasonal cold hardiness and linkages to bud set and bud flush. Species within Cupressaceae, redcedar included, have indeterminate growth strategies and do not produce vegetative or reproductive buds (Owens & Pharis, 1971; Zobel, 1983). As a result, studies on cold hardiness of bud tissues from determinate conifers may not apply to redcedar.

As with many other adaptive traits, cold hardiness is largely driven by additive genetic effects (Howe et al. 2003, Alberto et al. 2013). Heritabilities for overall and seasonal cold hardiness vary from nil to 0.78, although spring heritability is generally higher than fall (Howe et al. 2003). Heritability of cold hardiness has not been frequently been reported in Cupressaceae but work in Patagonian cypress (*Austrocedrus chilensis* (D. Don). Pic. Ser. Et. Bizzarri) (Aparicio et al. 2012) suggests it is moderately heritable, averaging 0.28. Similar heritability (0.38) was found for heritability of fall cold hardiness in yellow cypress (*Callitropsis nootkatensis* (D. Don) Orsted (syn. *Chamaecyparis nootkatensis* (D. Don) Spach) (Russell, 1993). Heritability for cold hardiness in redcedar has not been reported.

While cold hardiness typically behaves as an additive trait, there are exceptions. In pomegranate (*Punica granatum* L.), genetic dominance effects were found to be greater than additive effects for cold hardiness (Solokui et al. 2018). The *Eucalyptus* genus is also notable for dominance effects in growth traits of both individual species and hybrids (Bouvet et al. 2009) as well as in cold hardiness (Tibbits et al. 1991; He et al. 2012).

Within the seasonal rhythms of cold hardening and de-hardening, fall hardening and spring de-hardening can be considered as two distinct traits. Findings in Pinaceae species suggest weak genetic overlap between the two. In western hemlock, Hannerz et al. (1999) found a very weak genetic correlation between fall and spring cold hardiness (-0.05) whereas O'Neill et al. (2001) found no genetic correlation in Douglas-fir. Bower and Aitken (2006) found a weak but positive genetic correlation (0.18) between fall and spring cold hardiness in whitebark pine, concluding this was evidence of only a weak genetic relationship between hardening and de-hardening. No work has been reported to date on fall and spring cold hardiness genetic correlations for any indeterminate or Cupressaceae species, including redcedar.

Forest tree species populations are also often well differentiated in cold hardiness, with strong geographic clines (e.g. Sitka spruce) (Mimura & Aitken 2007) although exceptions do exist, such as in western white pine (*Pinus monticola* Dougl.) (Rehfeldt et al. 1984). Rehfeldt (1997) found differences in the presence of geographic clines for cold injury in *Cupressus arizonica* var. *arizonica* Greene (hereafter *Cupressus arizonica*) and *Cupressus arizonica* var *glabra* (Sudw.) Little (hereafter *Cupressus glabra*)[2] where

[2] Rehfeldt (1997) reports findings separately for *Cupressus arizonica* and *Cupressus glabra*, referring to the taxonomy designated by Wolf (1948). Taxonomy for both "species" remains unsettled. Both have been

Cupressus arizonica showed minimal evidence of a relationship between elevation of origin and cold damage, compared to *Cupressus glabra* where the correlation was moderate (0.49). In Patagonian cypress, geographic clines for latitude and elevation were not significantly correlated with survival after cold exposure (Aparicio et al. 2012).

Cold hardiness in redcedar

Relatively little research on cold hardiness of redcedar has been conducted. Development of cold hardiness in redcedar has been found to be induced entirely by temperature with photoperiod not appearing to play a role (Silim & Lavender, 1994). This contrasts with determinate conifers in which photoperiod has an important influence on dormancy induction and acclimation (Howe et al. 2003). Differences in the degree of cold hardiness have been found between coastal and interior seedlots of redcedar (Cherry, 1995; Hawkins et al. 2003). Rehfeldt (1994) compared cold hardiness in redcedar provenances from the south of its interior range (Okanagan B.C. south to Idaho) and found weak geographic clines. These comparisons have been limited to small groups of populations and have not been made across the species' range. Considering that the range of redcedar is vast, it is possible that there will be significant variation in cold hardiness and evidence of geographic clines.

A complicating factor in physiological and genetic studies of redcedar is that it is a highly inbred species with reportedly low genetic diversity (Yeh, 1988). It is also prone to selfing or self-fertilization (El-Kassaby 1999) with approximately 30% of the trees in

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considered one species but different subspecies (Farjon & Filer, 2013) and debate remains as to their genus (e.g., references to *Hesperocyparis arizonica* (Greene) Bartel by Wolf (2017) whereas Farjon & Filer (2013) use *Cupressus*). For the purposes of this study, I report per Rehfeldt (1997) and define the two as *Cupressus arizonica* and *Cupressus glabra*.

wild stands arising from self-fertilized seed, on average (O'Connell et al. 2004). Many conifers have the capacity to self-fertilize, but this typically results in seed abortion (Owens et al. 1990) or significant inbreeding depression later in life. For redcedar, however, inbreeding depression is minimal and causes less than 10% reduction in growth in the first generation of selfing (Russell & Ferguson, 2008). Genetic diversity appears greatest in the southern, interior range of the species and decreases as the ranges moves northward and latitude increases (O'Connell et al. 2008). Due to its highly homozygous nature, it is possible that redcedar cold hardiness is driven more by environmental factors and plasticity than genetic factors, even at the extreme northern edge of its range.

Objectives

This study was primarily undertaken to characterize cold hardiness across the range of redcedar. In addition, the study also aimed to:

1) Compare fall and spring cold hardiness to assess genetic correlations between these traits;
2) Examine seedling families from controlled crosses to determine parental effects related to cold hardiness; and,
3) Assess geographic and climatic clines for cold hardiness.

Ultimately, the goal of this study is to determine any specific geographic or climatic limits for incorporating redcedar genotypes from the Pacific Northwest into B.C.'s seed orchards for deployment consistent with climate-based seed transfer.

Methods

Experimental Design

All tested material was grown at Cowichan Lake Research Station (coordinates: 48° 49' 24.78" N, 124° 8' 7.86" W, 180 m elevation) and tested over a four-year period (2014/15, 2017/18, 2018/19 and 2019/20) using controlled freezing tests. Two groups of germplasm were studied, seedlings from controlled crosses and older, clonal grafts. Seed from controlled crosses (families) were sown in the January preceding their first test date (e.g., sown January 2014 and tested first in November 2014) and grown in a container system using 412A styroblocks (Beaver Plastics, Edmonton, AB). Two sets of families were tested. The first set of families (tested in 2014/15) were second generation progeny produced from controlled crosses of a subset of the clonal grafts, specifically focused on USA origin crossed with B.C. origin redcedar (Figure 1 1). The second set of seedling families (tested in 2019/20) were second generation progeny produced from controlled crosses of coastal B.C. clonal grafts (Figure 1).

A common garden of range wide collections of redcedar scions clonally propagated by grafting (hereafter, clones) and grown at Cowichan Lake Research Station for approximately 20 years was used in this study. Clones were selected because they were parents of the seedlings described previously and/or based on their collection record origin coordinates as recorded in the B.C. Seed Planning and Registry System (SPAR; British Columbia. Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2020) to cover the range of redcedar (Figure 2).

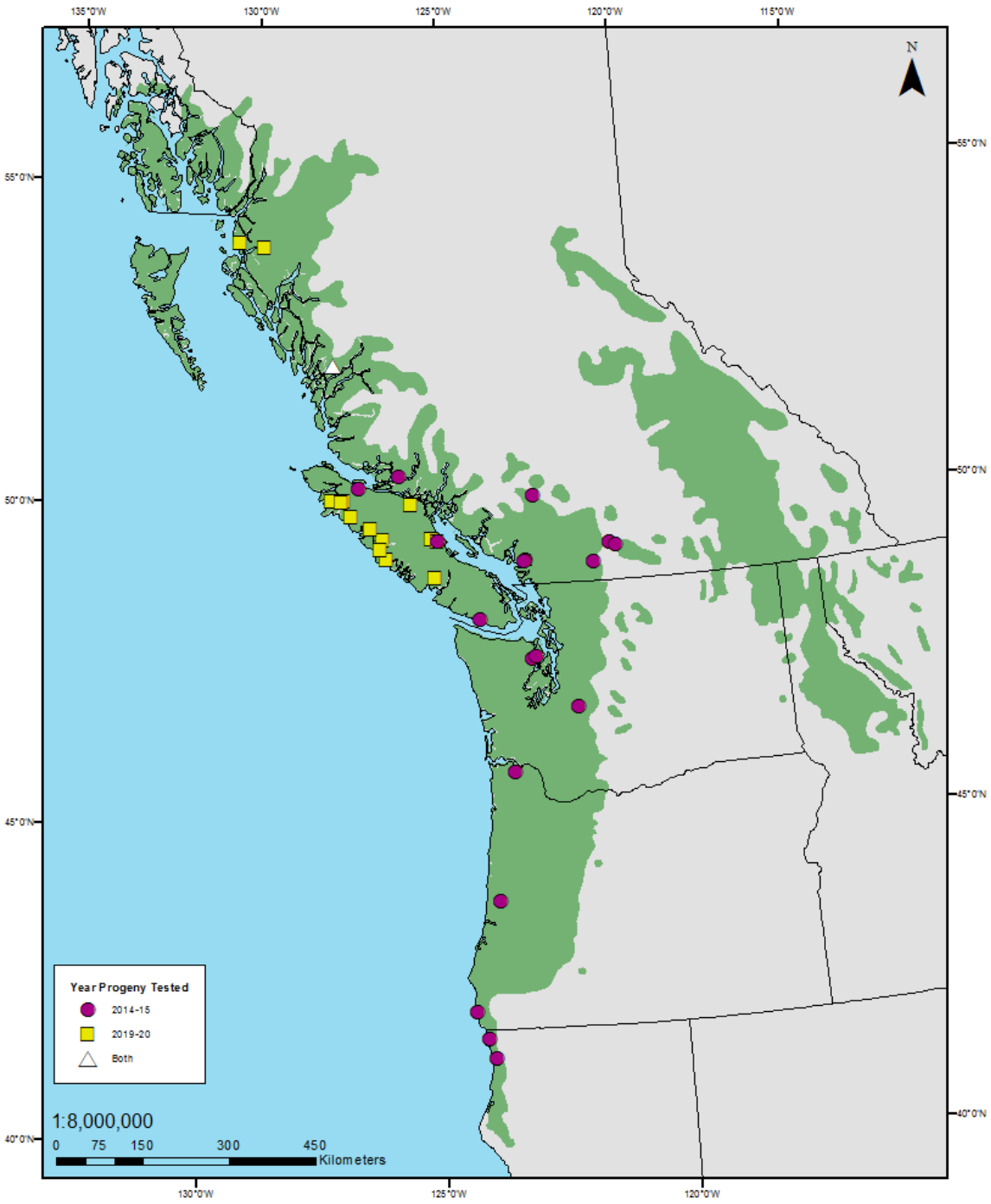

Figure 1: Map of the origin of the parents of the controlled cross families (seedlings) sampled by corresponding year. Parents of seedlings tested in 2014/15 are coloured with burgundy dots, 2018/19 are coloured with yellow squares and the single parent with progeny tested in both years is coloured with a white triangle. Parents locations are overlaid with the natural range of redcedar (dark green).

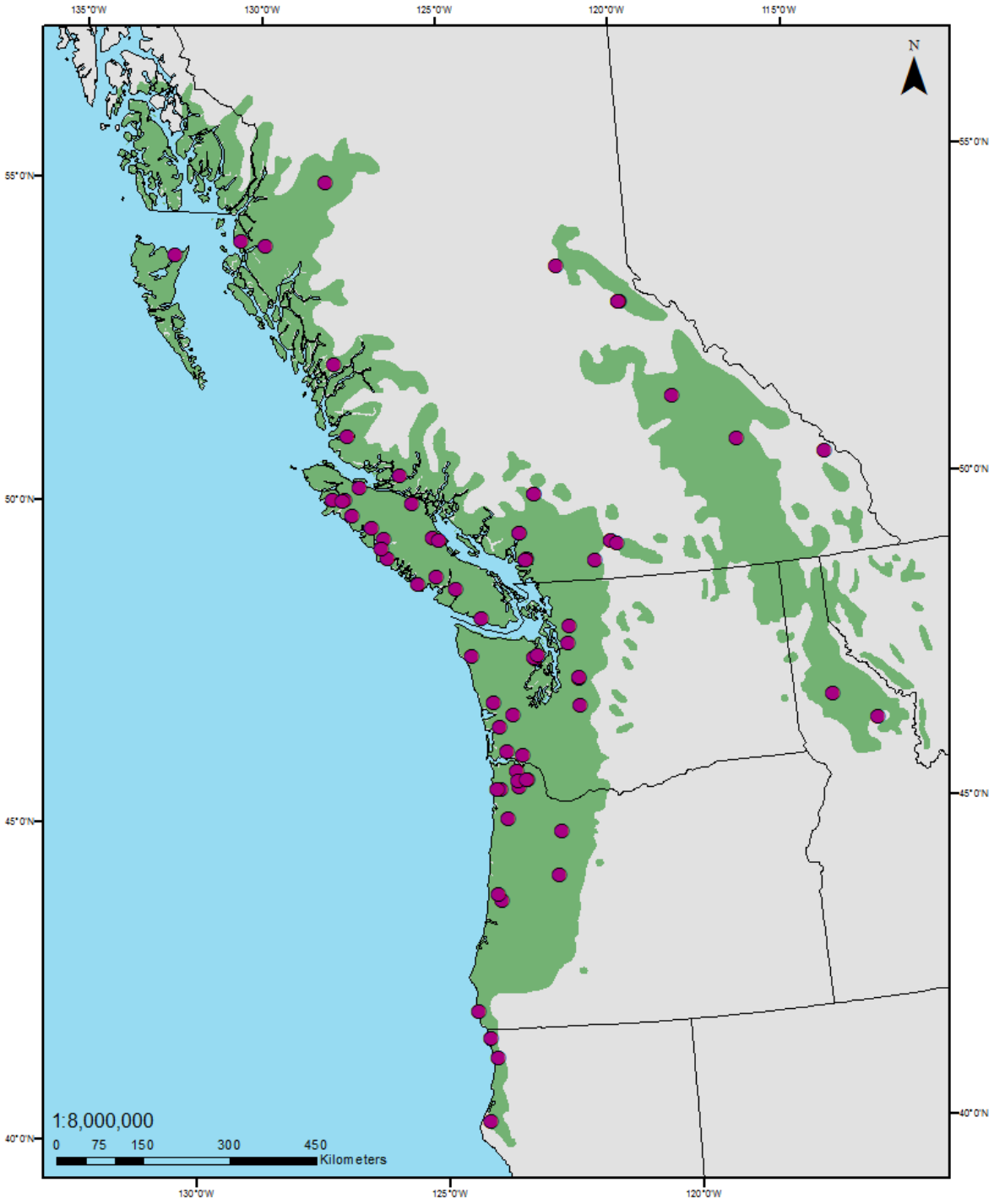

Figure 2: Map of the origin of redcedar clonal grafts sampled (burgundy dots) overlaid with the natural range of redcedar (dark green).

Data from four years of freezing tests were collected for this study and are summarized in Table 1. Appendix A: List of material tested by year, summarizes each clone and family tested by type and dataset.

Table 1: Summary of the freezing tests conducted over four years.

Year:	2014/15			2017/18			2018/19			2019/20	
Primary germplasm tested:	Seedlings			Clones			Clones (subset seedlings)			Seedlings (subset clones)	
Description of seedlings tested:	20 families from controlled crosses of 19 parents (B.C. by U.S.A. and vice versa)						6 families repeated from 2014/15 tests			16 families from reciprocal crosses from 16 parents (coastal B.C.)	
Description of clones tested:				<u>Fall:</u> 61 clones including 16 parents from 2014/15 families <u>Winter, Spring:</u> 21 clones including 7 parents from 2014/15			21 clones across the range including 17 that were parents in 2014/15			6 clones that were parents in 2014/15 and tested in 2017/18 & 2018/19	
Germplasm testing and replications:	<u>Seedlings:</u> 5 individual seedings per family. The same individual was tested in all seasons. Each individual was tested once in each temperature treatment (four treatments)			<u>Clones:</u> <u>Fall:</u> 1 to 2 ramets per clone and same number per treatment <u>Winter, Spring</u>: 1 ramet per clone sampled 4 replicates of each ramet per clone			<u>Clones:</u> 1 ramet per clone. Ramet subdivided into four replicates per season and freeze treatment <u>Seedlings</u>: Five individual seedlings per family each season (15 seedlings total). Each seedling was subdivided into four replicates per freezing treatment (four treatments)			<u>Clones:</u> 1 ramet per clone. Ramet subdivided into four replicates per season and freeze treatment <u>Seedlings</u>: Five individual seedlings per family each season (10 seedlings total). Each seedling was subdivided into four replicates per freezing treatment (four treatments)	
Fall test date:	Nov. 14, 2014			Nov. 20, 2017			Dec. 4, 2018			Nov. 18, 2019	
Winter test date:	Jan. 27, 2015			Jan. 22, 2018			Jan. 21, 2019			Jan. 27, 2020	
Spring test date:	Mar. 23, 2015			Mar. 12, 2018			Mar. 24, 2019			Cancelled	
Seasons tested	Fall	Win	Spr	Fall	Win	Spr	Fall	Win	Spr	Fall	Win
Freeze treatments by season (°C)	Ctrl -8 -13 -18	Ctrl -18 -23 -28	Ctrl -8 -13 -18	Ctrl -8 -13 -18	Ctrl -18 -23 -28	Ctrl -8 -13 -18	Ctrl -13 -18 -23	Ctrl -23 -28 -33	Ctrl -8 -13 -18	Ctrl -8 -13 -18	Ctrl -23 -28 -33

2014/15 dataset

The first experiments conducted in 2014/15 (2014/15 dataset) tested individual seedlings from 20 controlled cross families (see Appendix B for pedigrees). These families were produced from parents primarily originating from coastal USA (Washington, Oregon and California) and crosses were made among these parents as well as with parents from coastal B.C. (Vancouver Island). Each family was tested in fall, winter, and spring. This dataset was incorporated into the overall model and used to support analysis of paternal effects.

2017/18 dataset

The second set of experiments was conducted in 2017/18 and tested the grafts of clones of redcedar (see Appendix C for geographic origin of clones). Initially for the fall season, 64 clones with one to two ramets per clone were sampled. Each ramet was used once per treatment. During winter and spring, 21 clones were sampled. Four replicates of each clone were tested in each of the three freeze treatments. The 21 clones tested contained seven clones that were parents of the seedlings' families in 2014/15. The 14 other clones were selected to provide coverage across the range. This dataset was incorporated into the overall model and used to support analysis of geographic clines.

2018/19 dataset

The third set of experiments was conducted in 2018/19 and tested primarily clones (24 clones) of redcedar and seedlings from six families (see Appendix C for geographic origin of clones). The six seedling families were from the same families tested in 2014/15, with seedlings re-sown in 2017 (one year of age at measure). These were

included to provide linkages to the 2014/15 dataset. Of the 24 clones tested, 17 were parents of the seedlings' families in 2014/15. The remaining seven were clones selected to provide coverage across the range. Experiments were performed in fall 2018, winter 2019 and spring 2019 (see Table 1 for sampling dates). This dataset was incorporated into the overall model and used to support analysis of geographic clines.

2019/20 dataset

The fourth set of experiments was conducted in 2019/20 and tested individual seedlings from 16 families of eight reciprocal controlled crosses (Appendix D lists families and associated reciprocal group). A reciprocal cross is a combination where both parents have been tested as both maternal and paternal parent. A family with parent A as mother and B as father and the family with parent B as mother and A as father are two reciprocal families. Together, the two families are considered a reciprocal group. Six clones were also tested that were parents from 2014/15 and repeated in 2017/18 and 2018/19 to provide linkage to the previous datasets. Experiments were performed in fall 2019 and winter 2020. Due to the covid-19 pandemic, testing in spring 2020 was cancelled.

Freeze-induced electrolyte leakage

Cold hardiness was quantified using freeze-induced electrolyte leakage (EL) which is a well-studied method of characterizing cold hardiness in plants and has been found to produce results similar to whole-plant freezing (Burr et al.1990). Seedling shoots or branches from older trees were sampled to conduct cold hardiness tests. During sampling of clones (older trees), three-meter pruning poles were used to cut branch tips from the

south side of the tree and as high in the tree crown as possible to obtain the most juvenile tissue available.

Samples from the various experiments described above were transported to the lab at the University of Victoria. For all material, side branches and leaves were removed, leaving the stem (seedlings) or primary branch (clones) intact. The lower part of the stem or branch, if lignified was removed. The remainder of the stem or branch was chopped transversely into 5 mm pieces and five pieces were randomly allocated to each scintillation vial. 8 µL of water was added to each vial to ensure consistent freezing. Vials were then randomly assigned to four freezing treatments (a tray of sample vials was prepared for each freezing treatment): a control that remained in the refrigerator, and three freezing temperatures that depended on season/year (Table 1). After treatment assignment, all samples were placed in the refrigerator at 4°C overnight. The control treatment remained in the refrigerator while the other treatments were frozen the next day.

The following morning, the three freeze treatment trays were removed from the fridge and placed into a pre-programmed Caltec Scientific temperature controller and Forma Scientific BioFreezer freezer which was set at 0°C and programmed to cool at a rate of -5°C h^{-1}. Once each treatment temperature threshold had been reached, the freezer was held at the freeze treatment temperature for one hour. The respective tray was then removed and placed back into the fridge to thaw overnight.

The following day, 10 mL of distilled water was added to each vial and trays were affixed to a gyrometer at low speed, providing gentle mixing. Trays were left on the gyrometer for the remainder of the day and overnight. The following morning, electrical

conductivity was measured for each vial using a Jenway 4020 conductivity meter. Post-measurement, the trays were placed into an oven at 97°C and heated for two hours. Trays were removed from the oven and temperature was measured for a middle vial to ensure heat-kill temperatures (minimum 90°C) had been reached. Trays were again placed on the gyrometer overnight for mixing.

The following day, the final electrical conductivity measurement was taken for each vial. Index of injury, as described by Burr et al. (1990) was calculated using equations 1 and 2 below where R_T refers to the ratio of conductivity after freezing at the respective freeze temperature divided by the conductivity of those samples after heat-kill (e.g. R_{-13} is the ratio of the conductivity after one hour in freezer at -13°C divided by the conductivity of the same samples after two hours in the oven) and R_0 is the same ratio for the respective control samples. Index of injury scores should range between 0 and 100%, with lower scores indicating less freezing injury.

$$R_T = \frac{Conductivity\ after\ freezing}{Conductivity\ after\ heat-kill} \quad [1]$$

$$Index\ of\ Injury = \frac{R_T - R_0}{1 - R_0} \times 100 \quad [2]$$

Data and statistical analysis

3,203 observations in total resulted from the four years of experiments. Preliminary examination of the data found seven observations with index of injury less than -20%. These seven observations were removed as they were considered as likely outliers given index of injury cannot be less than 0% and a negative index of injury is likely due to lack of precision with conductivity measurements at lower electrolyte concentrations resulting

from minimal freeze damage. Eighty percent of the remaining 106 negative index of injury observations were associated with the -8°C treatment. The -8°C freeze treatment group was therefore removed from analysis. After removal of the outliers and the -8°C treatment group, 2,603 observations were used for analysis.

Statistical analysis of the data was completed using R version 3.6.3 (R Core Team, 2020), ASReml-R version 4.1.0.110 for linear mixed effect modelling (Butler, 2019) and the R package psych version 2.0.9 which includes correction factors when conducting multiple correlation tests (Revelle, 2020). Data visualization was performed using the ggplot2 R package (Wickham, 2016). All inferential tests were considered significant when $p < 0.05$.

Data from this study was correlated through the inclusion of related germplasm (i.e. parents and progeny) and temporal correlation of repeated measures over multiple seasons and years. Observations therefore could not be considered independent. Linear mixed effects models (LMMs) with random effects were used to accommodate the violation of the assumption of independence of observations, required for traditional analysis of variance (Faraway, 2005; Crawley, 2015). LMMs are also more robust for unbalanced designs as the Restricted Maximum Likelihood (REML) method can be used to estimate parameters with less bias than ordinary least squares (Faraway, 2005). Furthermore, the LMM can also incorporate the pedigree information to both account for the correlation between relatives, as well as predict the effects associated with parents not tested based on their progeny's performance (Isik et al. 2017).

Random effects in an LMM represent an unknown variable whereas fixed effects represent an unknown constant (Faraway, 2005). The LMM instead estimates parameters

of the unknown variable (i.e., variance) with an assumed multivariate normal distribution (MVN) from which inferences about the population underlying that unknown variable can be made (Faraway, 2005). Partitioning the variance into components produced by the clones/families and those from the residual (environment) allows estimates of heritability (Isik, 2017).

In this study, narrow-sense heritability (h^2) (Falconer & Mackay, 1996) was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{a\times e}^2 + \sigma_e^2}$$

where σ^2_a is the additive genetic variance component (clone/family effect with relationship matrix incorporated), $\sigma^2_{a\,x\,e}$ is the interaction between the additive genetic component and environment and σ^2_e is the environmental (residual) variance component. Standard errors were estimated using the Taylor series approximation (Butler, 2018).

As a rule of thumb, variance components are likely significant when the Z ratio is greater than 2.0 (Isik et al. 2017). The significance of random effects and their associated interactions was formally tested using a nested log-likelihood test (Self & Liang, 1987) where the likelihood ratio statistic was compared to the associated null χ^2 distribution for χ^2_{n+1} and χ^2_n where n is the number of random effects and $n+1$ is for the additional random effect or interaction.

Best linear unbiased predictors (BLUPs) were predicted for each clone/family tested and for associated, untested parents as described in the pedigree/relationship matrix. BLUPs represent an individual's scaled and weighted deviance from the overall population mean (μ).

Overall model

For an overall measurement and determination of heritability of cold hardiness, a REML method was used to estimate variance components from a LMM described as:

$$\boldsymbol{y} = \mu + \boldsymbol{Z}\mathbf{p} + \boldsymbol{\varepsilon}$$

where,

$$\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{\varepsilon} \end{bmatrix} \sim MVN\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \boldsymbol{G} & 0 \\ 0 & \boldsymbol{\Sigma} \end{bmatrix}\right)$$

and,

$$\boldsymbol{G} = \boldsymbol{A}\sigma_a^2$$

Where **y** is the vector of 2,603 observations (index of injury) after outlier removal, μ is the only fixed effect and is the constant, overall mean, $\boldsymbol{Z}$ is the design incidence matrix of random effects, $\boldsymbol{p}$ is the vector of all random effects (e.g., year of measurement, freeze treatment nested within season of measurement, and additive random effects described by the clone/family code), and $\boldsymbol{\varepsilon}$ is the vector of residual deviations. Vectors $\boldsymbol{p}$ and $\boldsymbol{\varepsilon}$ are assumed to be multivariate normal (MVN) with a mean of 0 and variance-covariance described by matrices $\boldsymbol{G}$ and $\boldsymbol{\Sigma}$. $\boldsymbol{G}$ is described by matrix $\boldsymbol{A}$ which represents the genetic relationship matrix between families and clones, thus accounting for the estimated correlation between relatives (i.e. the offspring of a parent has an estimated coancestry of 0.5).

Bivariate model

In order to compare the relationship between fall and spring index of injury, a subset of the overall dataset was used. This subset was clones (grafts) and families (seedlings)

where the same individual was tested in both fall and spring. Pilot studies comparing older grafts and younger grafts found no significant difference in index of injury. It was not possible to test seedlings and grafts because the same genotype was not available in both germplasm types. This meant the complete data from 2014/15 and 2018/19 were used for this analysis and only partial data from 2017/18; only those clones who had been tested in both fall and spring 2017/18. Data from 2019/20 was excluded due to the lack of a spring measurement. In total, 20 families and 36 clones were used for this analysis. Fall index of injury was considered trait 1 and spring index of injury was considered trait 2. A bivariate LMM approach (Holland, 2006) was used where:

$$\begin{bmatrix} \boldsymbol{y}_{fall} \\ \boldsymbol{y}_{spring} \end{bmatrix} = \begin{bmatrix} \mu_{fall} \\ \mu_{spring} \end{bmatrix} + \begin{bmatrix} \boldsymbol{T}_{fall} & 0 \\ 0 & \boldsymbol{T}_{spring} \end{bmatrix} \begin{bmatrix} \boldsymbol{e}_{fall} \\ \boldsymbol{e}_{spring} \end{bmatrix} + \begin{bmatrix} \boldsymbol{X}_{fall} & 0 \\ 0 & \boldsymbol{X}_{spring} \end{bmatrix} \begin{bmatrix} \boldsymbol{g}_{fall} \\ \boldsymbol{g}_{spring} \end{bmatrix}$$
$$+ \begin{bmatrix} \boldsymbol{Z}_{fall} & 0 \\ 0 & \boldsymbol{Z}_{spring} \end{bmatrix} \begin{bmatrix} \boldsymbol{ge}_{fall} \\ \boldsymbol{ge}_{spring} \end{bmatrix} + \begin{bmatrix} \boldsymbol{\varepsilon}_{fall} \\ \boldsymbol{\varepsilon}_{spring} \end{bmatrix}$$

where $\boldsymbol{y}_{fall}$ and $\boldsymbol{y}_{spring}$ are the vectors of index of injury observations of the fall and spring season, μ_{fall} and μ_{spring} are the constant, overall means for each season; $\boldsymbol{e}_{fall}$ and $\boldsymbol{e}_{spring}$ are the vectors of the year and treatment random effects; $\boldsymbol{g}_{fall}$ and $\boldsymbol{g}_{spring}$ are the vectors of clone/family effects; $\boldsymbol{ge}_{fall}$ and $\boldsymbol{ge}_{spring}$ are the vectors of clone/family by associated season effect and $\boldsymbol{\varepsilon}_{fall}$ and $\boldsymbol{\varepsilon}_{spring}$ are the vectors of the residual effects by associated season. $\boldsymbol{T}_{fall}$, $\boldsymbol{T}_{spring}$, $\boldsymbol{X}_{fall}$, $\boldsymbol{X}_{spring}$, $\boldsymbol{Z}_{fall}$, and $\boldsymbol{Z}_{spring}$ are the corresponding incidence matrices. As the data was selected to be balanced, $\boldsymbol{T}_{fall} = \boldsymbol{T}_{spring}$, $\boldsymbol{X}_{fall} = \boldsymbol{X}_{spring}$, and $\boldsymbol{Z}_{fall} = \boldsymbol{Z}_{spring}$.

Variance covariance matrices on the clone/family and treatment/year interaction were modelled using a correlated heterogeneous structure where:

$$V\begin{bmatrix} \boldsymbol{e}_{fall} \\ \boldsymbol{e}_{spring} \end{bmatrix} = \begin{bmatrix} \sigma^2_{fall} & 0 \\ 0 & \sigma^2_{spring} \end{bmatrix}$$

$$V\begin{bmatrix} \boldsymbol{g}_{fall} \\ \boldsymbol{g}_{spring} \end{bmatrix} = \begin{bmatrix} \sigma^2_{fall} & \rho\sigma_{fall}\sigma_{spring} \\ \rho\sigma_{fall}\sigma_{spring} & \sigma^2_{spring} \end{bmatrix}$$

$$V\begin{bmatrix} \boldsymbol{ge}_{fall} \\ \boldsymbol{ge}_{spring} \end{bmatrix} = \begin{bmatrix} \sigma^2_{fall} & \rho\sigma_{fall}\sigma_{spring} \\ \rho\sigma_{fall}\sigma_{spring} & \sigma^2_{spring} \end{bmatrix}$$

$$V\begin{bmatrix} \boldsymbol{\varepsilon}_{fall} \\ \boldsymbol{\varepsilon}_{spring} \end{bmatrix} = \begin{bmatrix} \sigma^2_{fall} & \rho\sigma_{fall}\sigma_{spring} \\ \rho\sigma_{fall}\sigma_{spring} & \sigma^2_{spring} \end{bmatrix}$$

Genetic (Type A, ρ_A) correlations (Falconer & Mackay, 1996) between fall and spring index of injury were calculated as:

$$\rho_A = \frac{Cov_{g_{fall,spring}}}{\sqrt{\sigma^2_{g_{fall}} \times \sigma^2_{g_{spring}}}}$$

Where $Cov_{g_{fall,spring}}$ is the estimated clone/family covariance between fall and spring index of injury, and

$$Cov_{g_{fall,spring}} = \frac{\sigma^2_{g_{fall,spring}} - \sigma^2_{g_{fall}} - \sigma^2_{g_{spring}}}{2}$$

Reciprocal and paternal effects

For analysis of reciprocal effects on the 2019/20 dataset, a reciprocal coding approach was used as described in Isik et al. (2017). The eight reciprocal groups, with two families per group were coded as either 1 or -1 with the female parent of the lower digit assigned as 1 i.e. family 170 x 220 was coded as 1 and family 220 x 170 was coded as -1. This reciprocal coding was incorporated into the model as an additional random effect (see Appendix D for assigned code by family).

For analysis of paternal effects, in the 2014/15 data, the male parent and the associated pedigree $\boldsymbol{A}$ variance matrix was incorporated as an additional random effect using the method described in Thomson et al. (2018). This allows for the variance to be partitioned onto the male parent while accounting for the correlations associated with the male parent's pedigree.

Determination of geographic clines

Climate data

All of the clones tested, as part of the B.C. provincial western redcedar breeding program, had origin coordinates listed in B.C. Seed Planning and Registry System (SPAR). Furthermore, because of the pedigree relationships included into the overall model, BLUPs were also predicted for 11 clones, which also had origin coordinates, based on their seedling progeny's performance. In total, 78 clones were assessed for geographic/climatic clines. These origin coordinates were used to map the location of these clones coloured by the BLUPs calculated from the overall index of injury model. The presence/absence of geographic trends was visually assessed using this map.

Climate data for each of the 78 clones were acquired from ClimateNA (Wang et al. 2016). ClimateNA provides downscaled Parameter-elevation Regressions on Independent Slopes Model (PRISM) data (Daly et al. 2018) for western North America. Variables were selected based on 1961 to 1990 climate normals as spatial coverage is best for this time period (Wang et al. 2016). Although older climate data would have been preferred, as redcedar can live over one thousand years (Minore, 1981) and may be adapted to

historical climates, I considered the greater spatial accuracy to be more important, particularly given the mountainous location of origin for several provenances tested.

Annual climate variables were extracted from ClimateNA (23 total; Table 2) and included both precipitation, temperature, radiation and temperature:precipitation interaction variables (e.g., Annual heat-moisture index). Fourteen of the 23 variables (Hargreaves moisture deficit, degree days below -18°C, degree days below 0°C, degree days below 5°C, degree days above 18°C, Hargreaves Reference Evaporation, number of frost-free days, precipitation as snow (mm), radiation, relative humidity, mean average temperature, mean minimum temperature and mean maximum temperature) also had monthly data (168 variables total) and seasonal data (56 seasonal variables total) available. In total, the combined annual, monthly and seasonal variables with data available was 250 and data was extracted for all 250 variables (Appendix F).

Pearson correlation coefficients were calculated for 241 variables. Nine variables were excluded from calculation as their variance was zero (Table 3). Because of the large number of climate variables compared, the Benjamini & Hochberg (1995) false discovery rate test was used to account for the error associated with multiple comparisons as it has greater power than the Bonferroni false discovery method when applied to a larger number of tests (Benjamini & Hochberg, 1995). All climate variables, correlations with BLUPs and associated p values of the correlations are detailed in Appendix F.

Table 2: List of annual climate variables extracted using ClimateNA, indicating whether they measured temperature, radiation, precipitation or the interaction between temperature and precipitation (temperature:precipitation). Variables in red have associated monthly and seasonal measurements, also (Appendix F).

Variable acronym	**Climate variable**	**Type**	**Determination**
MAT	Mean Annual Temperature (°C)	Temperature	Directly calculated variable
MWMT	Mean warmest month temperature (°C)	Temperature	
MCMT	Mean coldest month temperature (°C)	Temperature	
TD	Continentality (difference between MWMT and MCMT (°C)	Temperature	
MAP	Mean annual precipitation (mm)	Precipitation	
MSP	Mean annual summer (May to September precipitation) (mm)	Precipitation	
AHM	Annual heat-moisture index (MAT+10)/(MAP/1000)	Temperature: Precipitation	
SHM	Summer heat-moisture index (MWMT)/(MSP/1000)	Temperature: Precipitation	
DD<0	Degree-days below 0°C – chilling degree days	Temperature	Derived variable
DD>5	Degree-days above 5°C – growing degree days	Temperature	
DD<18	Degree-days below 18°C – heating degree days	Temperature	
DD>18	Degree-days above 18°C – cooling degree-days	Temperature	
NFFD	Number of frost-free days	Temperature	
FFP	Frost-free period	Temperature	
bFFP	Day of year which FFP begins (beginning frost-free period)	Temperature	
eFFP	Day of year which FFP ends (end frost-free period)	Temperature	
PAS	Precipitation as snow (mm)		
EMT	Extreme minimum temperature over 30 years	Temperature	
EXT	Extreme maximum temperature over 30 years	Temperature	
Eref	Hargreaves reference evaporating (mm)	Precipitation	
CMD	Hargreaves moisture deficit (mm)	Precipitation	
MAR	Mean annual solar radiation (MJ m^{-2} d^{-1})	Radiation	
RH	Mean annual relative humidity (%)	Temperature: Precipitation	

Table 3: Climatic variables excluded from correlation comparison with BLUPs due to lack of variation.

Variable	Description	Type	Time period
DD_0_06	Degree days less than 0°C in June	Temperature	Monthly
DD_0_07	Degree days less than 0°C in July	Temperature	Monthly
DD_0_08	Degree days less than 0°C in August	Temperature	Monthly
CMD01	Hargreaves Moisture Deficit in January	Precipitation	Monthly
CMD02	Hagreaves Moisture Deficit in February	Precipitation	Monthly
CMD11	Hargreaves Moisture Deficit in November	Precipitation	Monthly
CMD12	Hargreaves Moisture Deficit in December	Precipitation	Monthly
DD_0_sm	Degree days less than 0°C in summer	Temperature	Seasonal
CMD_wt	Hargreaves Moisture Deficit in winter	Precipitation	Seasonal

Principal component analysis

Considering that climatic clines are often multidimensional with more than one variable of influence, a Principal Component Analysis (PCA) was conducted to assess relationships between multiple climate variables and BLUPs. As a dimension reducing technique, PCA calculates principal components that are vectors of the linear combinations of the inputted variables designed to maximize the variance explained (Zuur et al. 2007). With 241 variables and high levels of collinearity between variables (e.g. Mean Annual Winter Temperature and Mean temperature in November, Mean temperature in December and Mean temperature in January), I restricted the PCA to only the annual climate variables and further restricted to twelve variables, total (MAT, MCMT, MWMT, PAS, TD, MAP, MSP, DD5, NFFD, bFFP, eFFP, Eref, CMD), as per the method used in Porter et al. (2014).

PCA was performed using the R function prcomp which uses the singular value decomposition approach to calculate the PCs and associated loadings and scores (R Core Team, 2020). The PCs reported were those for which the cumulative variation explained was greater than 80% (Zuur et al. 2007). A distance biplot of the clones was plotted and then overlaid with the respective individual's BLUP in order to determine if clusters of individuals could be identified, suggesting climatic clines incorporating the multiple climate variables.

Results

Overall index of injury

Analysis of electrolyte leakage data from the four years of artificial freeze test experiments showed that within the season of measurement, as freezing treatment temperature decreased, index of injury always increased (i.e. colder temperatures resulted in greater cold damage, regardless of season) supporting a lack of interaction effect between season and freeze treatment (Figure 3). Index of injury varied most in the spring season. The 2014/15 year had the highest mean index of injury (mean = 73.9 ± 0.9%) compared to the 2017/18 (mean = 29.1 ± 0.9%) and 2018/19 years (mean = 30.8 ± 1.7%). The differences in response among years, particularly winter 2019/20 were presumed to be due largely to different germplasm tested in different years (2019/20 sampled predominantly families from coastal parents) as evidenced by the relatively low Z ratio (1.2) for the year variance component compared to the clone/family component (Z ratio of 5.2) (Table 4). The likelihood ratio test still found year to be significant ($p < 0.01$) and thus year was included in the overall model.

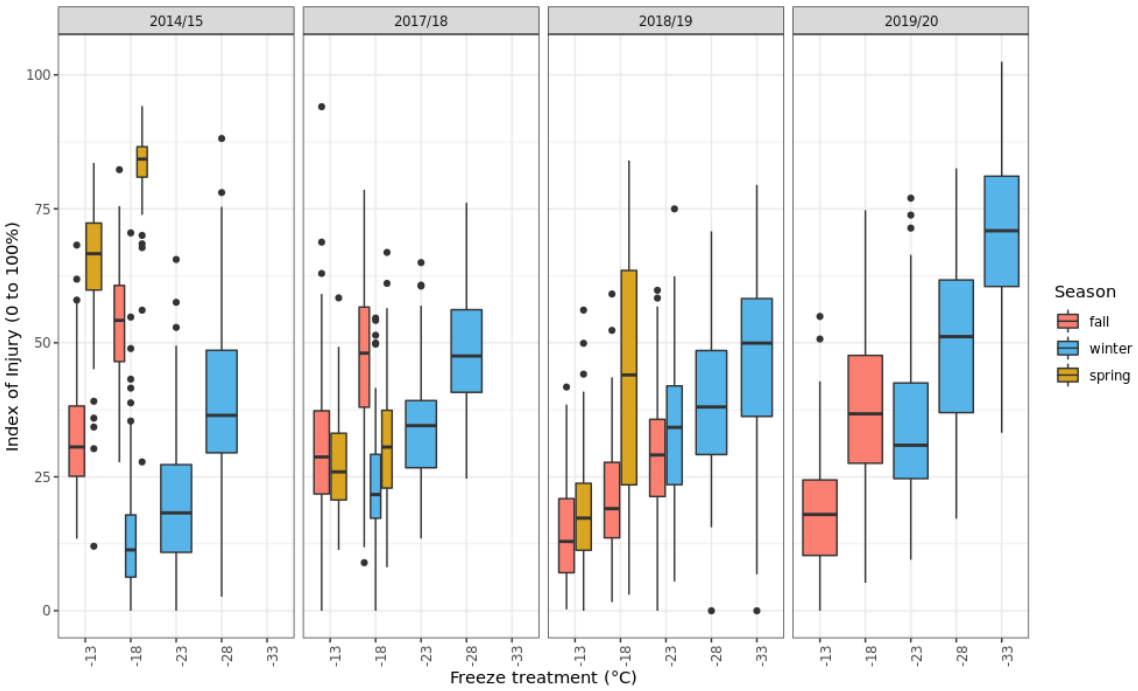

Figure 3: Boxplot of index of injury for each measurement year (panel) and freeze treatment (x axis), colored by season. Box midlines indicate median index of injury, box dimensions are 25th percentile and 75th percentile. Whiskers indicate minimum and maximum values, excluding suspected outliers. Black dots indicate potential outlier values.

Heritability, the proportion of index of injury explained by additive, genetic variation was calculated from the variance components from the overall model (Table 4) and was 0.17 ± 0.03. BLUPs, the individual clone/family scaled and weighted deviation from the mean, were predicted for each clone/family random effect (Figure 4, Appendix E). Negative BLUPs indicate lower index of injury values i.e. greater cold hardiness. Positive BLUPs indicate higher index of injury values i.e. greater cold susceptibility. BLUPs ranged from -17.0% to 16.2% with the lowest BLUPs for clone P1368 (originating near Prince Rupert B.C.) and the highest BLUP for clone P570 (originating from southern Oregon). Overall standard deviation of BLUPs was 5.2. A BLUP of -17.0% means that if

all the clones/families tested in a given treatment had a mean index of injury of 30%, for example, P1368 would be 17% less damaged by freezing, having a predicted index of injury of 13%. It would also contribute this freezing resistance to its progeny.

Table 4: Variance components from linear mixed effect model of all electrolyte leakage datasets for index of injury.

Random effect	**Component**	**Standard error**	**Z ratio**
Year	63.2	52.2	1.2
Season:treatment	228.5	114.5	2.0
Clone/family (Pedigree)	47.7	9.2	5.2
Residual	226.7	6.5	35.3

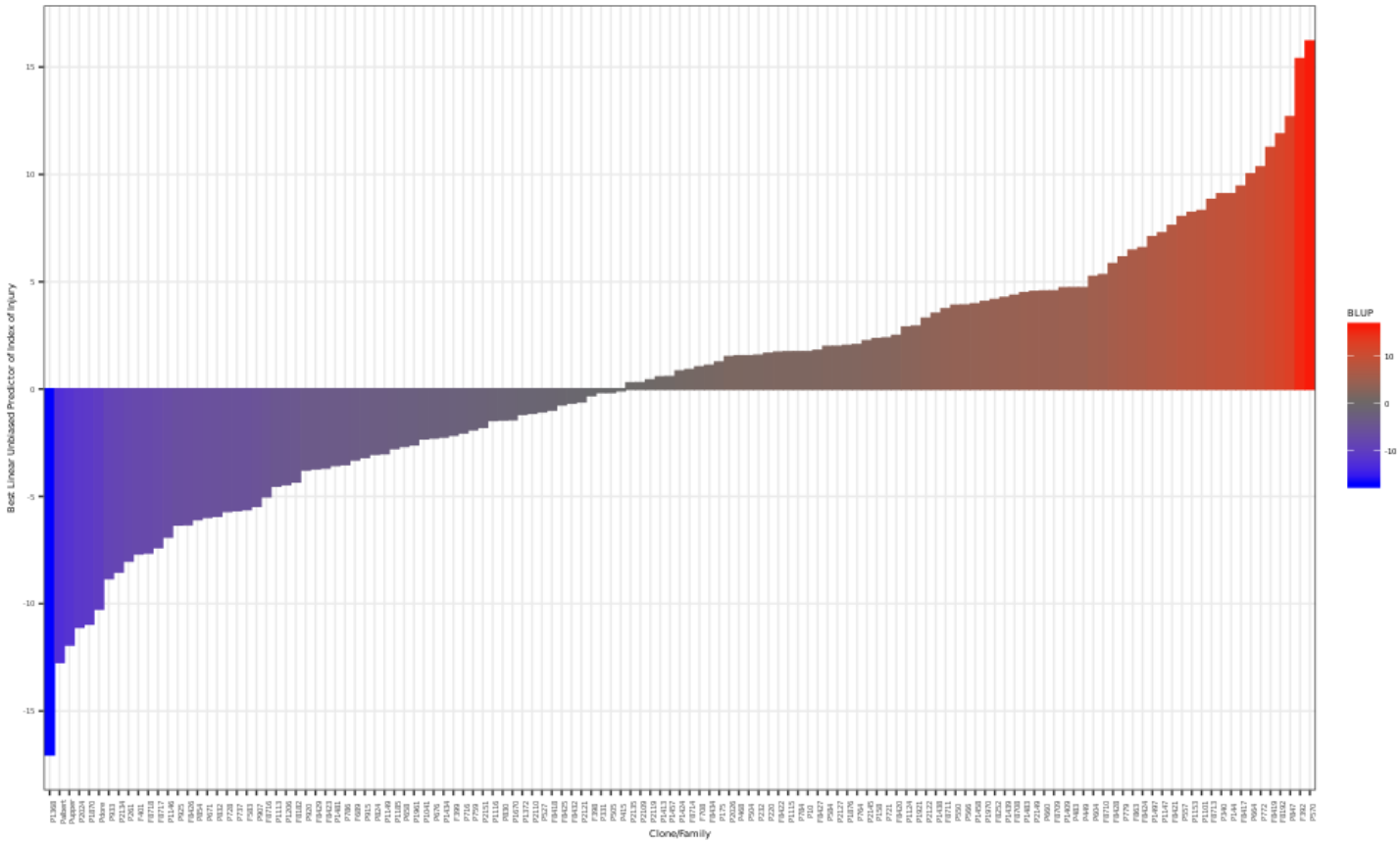

Figure 4: Best linear unbiased predictors (BLUPs) for index of injury from linear mixed effects model of all electrolyte leakage datasets (2014/15, 2017/18, 2018/19 and 2019/20). Positive BLUPs indicate greater index of injury (i.e. less cold hardiness) and negative BLUPs indicate less index of injury (i.e. greater cold hardiness). Clone P570 had the highest BLUP (16.2) and clone P1368 had the lowest BLUP (-17.0). BLUPs and ranks for all clones/families are listed in Appendix E.

Fall and spring correlations

Fall index of injury (FII) and spring index of injury (SII) were significantly phenotypically correlated (0.54, $p < 0.01$) although different years showed different strength of this relationship with all correlations significant ($p < 0.05$) and ranging from 0.27 to 0.74 (Figure 5). Individual phenotypic correlations within year by treatment (e.g., -13°C in 2014/15 year) were not significant for all years and treatments.

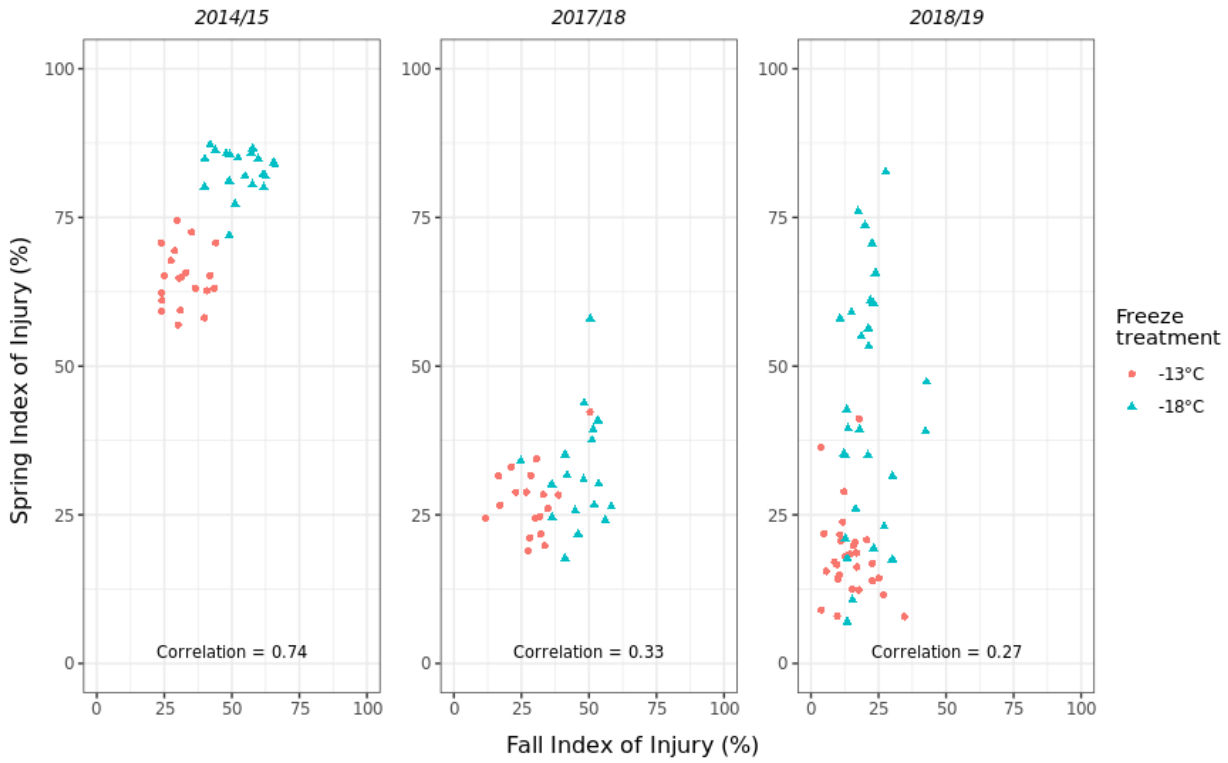

Figure 5: Correlations of mean index of injury by clone/family, pooled by by treatment (shape and colour) for fall index of injury (x axis) and spring index of injury (y axis). Points correspond to clone/family mean.

A bivariate linear mixed effect model was applied to model FII and SII together on the same family/clone while accounting for treatment and year effects. The interaction of year and clone/family was not significant ($p = 0.6$) despite the variance in phenotypic

correlations (Figure 5). This means that despite the observed variance in year, after accounting for treatment effects, families/clones behaved consistently (ranks did not change between the years). The overall Type A genetic correlation between these two traits was 0.55 with an estimated standard error of 0.33. Narrow sense heritability in the FII was 0.31 with an estimated standard error of 0.10 and in the SII was 0.26 with an estimated standard error of 0.11. Variance components for the model are presented in Table 5.

Table 5: Variance components from bivariate model of fall and spring cold hardiness with correlated heterogenous correlation structure (variance on diagonal; covariance on off-diagonal). Clone/family – season indicates the interaction effect between the additive genetic component and the corresponding season.

Random effect	**Component**	**Standard error**	**Z ratio**
Treatment - fall	95.7	136.4	0.70
Treatment - spring	152.8	218.3	0.70
Year - fall	167.3	166.0	1.0
Year – spring	647.9	651.8	1.0
Clone/family (Pedigree) – fall	23.4	9.4	2.5
Clone/family (Pedigree) – spring	37.0	17.1	2.2
Residual - fall	49.8	8.1	6.2
Residual – spring	107.1	17.2	6.2

Reciprocal and paternal effects

2014/15 families

Initial modelling of the 20 seedling families tested in 2014/15 showed differences in the variance components between the male and female parents (Table 6). The value of the variance component associated with the male parent was approximately four times the

variance component associated with the female parent (21.9 vs 5.4) with only a fraction of the variance on the interaction between the male and female parents (0.000001). This suggests that the male parent is influencing the offspring's variation in index of injury more than the female parent, a deviation from the typical additive genetic model implying a possible paternal effect. A likelihood ratio test comparing a modified version of the model incorporating a specific, additional male parent effect was not significant ($p = 0.16$) (Appendix G). The likelihood ratio test was repeated, instead substituting an additional female parent effect and it was also not significant ($p = 0.50$) (Appendix G). This confirms that the unequal variance component on the male parent from the initial model (Table 6) is not a real paternal effect.

Table 6: Variance components from 2014/15 cold hardiness data with male, female and female:male interaction included with season:treatment interaction. The male component has four times the variance of the female component.

Random effect	**Component**	**Standard error**	**Z ratio**
Season:treatment	583.8	292.4	2.0
Male parent	21.9	12.8	2.1
Female parent	5.4	NA	NA
Female:Male interaction	0.0000001	NA	NA
Residual	123.5	6.7	19.0

2019/20 reciprocal crosses

Sixteen seedling families representing eight reciprocal groups showed no obvious difference in mean index of injury between the two reciprocal crosses when compared visually (Figure 6).

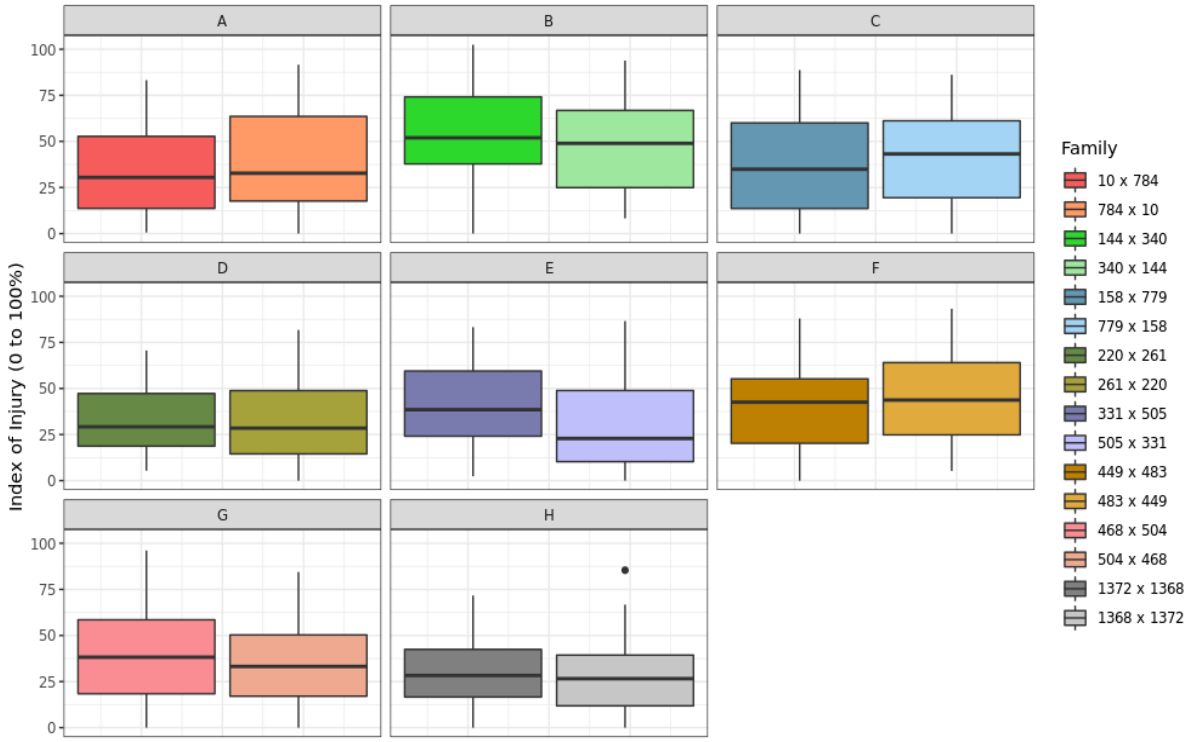

Figure 6: Boxplot of 2019/20 reciprocal cross seedling families' index of injury for combined fall and winter measurements. Plots A-H are grouped by reciprocal group and coloured by family cross. Bolded, black midlines within boxes are median index of injury (%), box dimensions are the 25th and 75th percentile, and whiskers indicate minimum and maximum values.

A linear mixed effect model including the additional reciprocal effect (Table 7) was not found to be significant when compared using a likelihood ratio effect against the model without the reciprocal effect ($p = 0.5$). The variance component for the reciprocal effect was too small for the standard error and Z ratio to be estimated. This indicates that the parental role (female versus male) does not significantly affect progeny index of injury.

Table 7: Variance components for 2019/20 reciprocal cross seedlings cold hardiness data with reciprocal effect included with season:treatment interaction, family (linked with pedigree relationship matrix) and residual.

Random effect	**Component**	**Standard error**	**Z ratio**
Season: treatment	469.0	332.3	1.4
Family (pedigree)	43.3	17.6	2.5
Reciprocal code	0.00001	NA	NA
Residual	100.3	7.3	13.8

Geographic and climatic clines

Although the map of the 78 clones coloured by BLUP from the overall model generally showed interior trees had lower BLUPs (were more cold hardy) than coastal trees, there were notable exceptions with clones originating from around McBride and Northern Idaho coloured reddish and pale pink, respectively (higher BLUPs; corresponding to pink/red colouration) and a clone around Prince Rupert coloured dark blue (lowest BLUP; Figure 7).

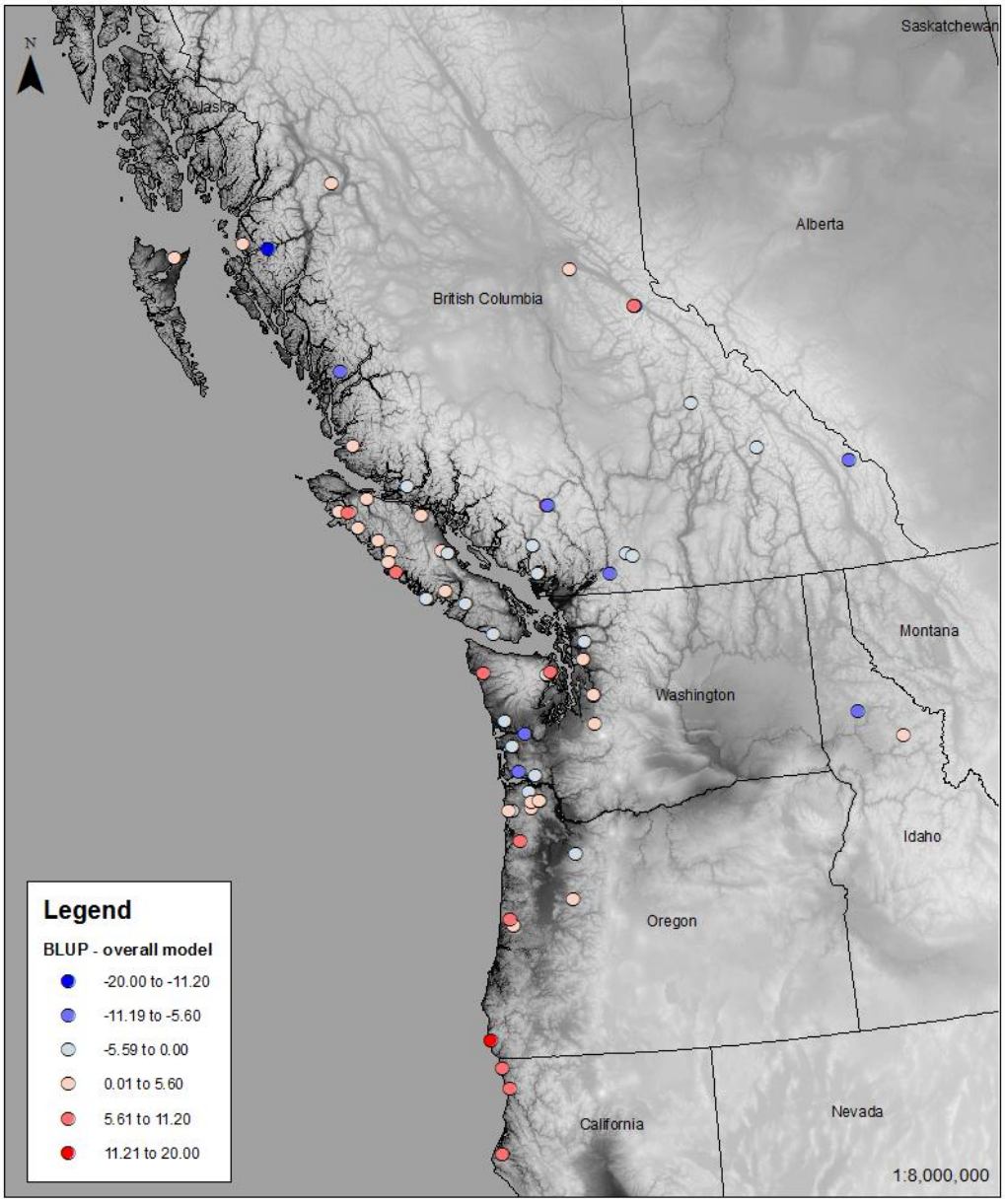

Figure 7: Map of 78 clones coloured by their respective BLUPs from the overall model. BLUP color gradient corresponds to a respective increase/decrease of one standard deviation (5.6%) with the mean of 0%. BLUPs are overlaid on 1 km resolution U.S. Geological Survey topographic map (2011); darker grey colouring indicates lower elevation.

Of the 241 annual, monthly and seasonal climate variables extracted from ClimateNA for the origin coordinates of 78 clones, 123 had significant correlations with cold

hardiness BLUPs ($p < 0.05$; Appendix F). 109 of these variables were directly related to temperature (e.g. Mean annual temperature in January), eight were directly related to precipitation (e.g., precipitation as snow in January) and five were directly related to radiation (e.g. Radiation in December). Of the 123 significantly correlated variables, 68 had an absolute correlation coefficient value greater than 0.3 although none exceeded 0.4, suggesting weak relationships between individual climate variables and BLUPs. The most highly correlated variable ($\rho = 0.38$. $p = 0.01$, Figure 8) was the number of frost-free days in January.

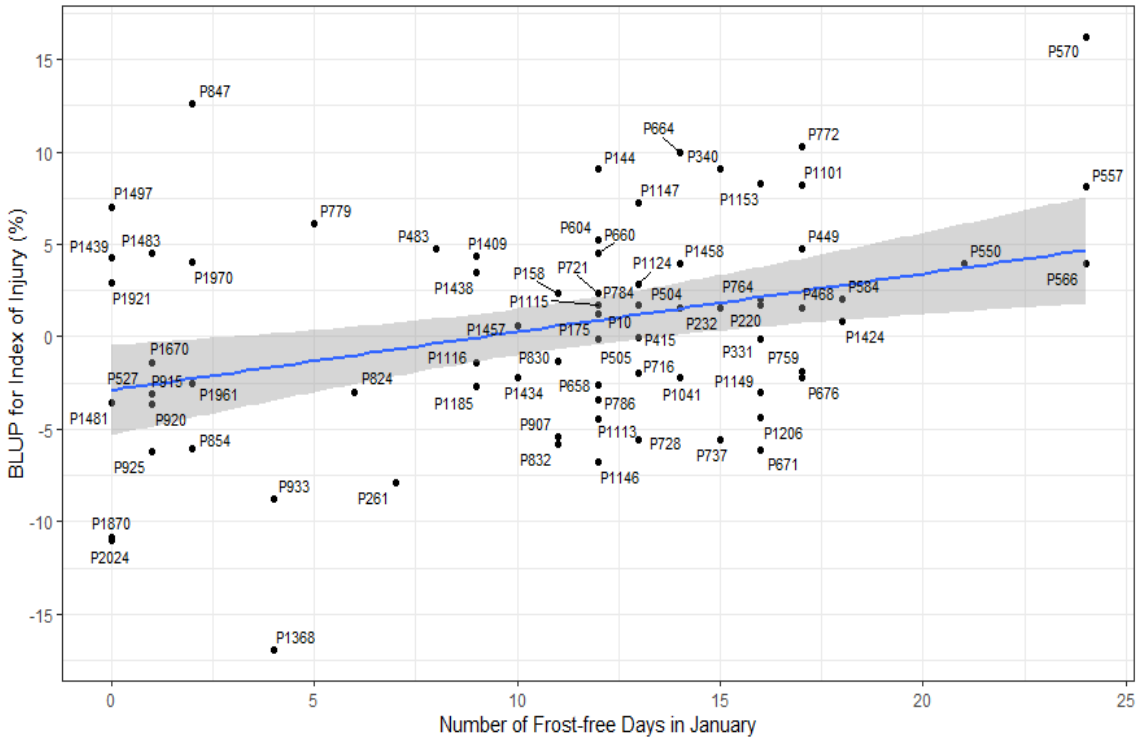

Figure 8: BLUPs from the overall model for 78 clones (points) with origin coordinates versus number of frost-free days in January. Error cloud surrounding the blue regression line is the 0.95 percent confidence interval. The correlation coefficient is 0.38, significant at $p = 0.01$.

PCA of the annual climate variables MAT, MCMT, MWMT, PAS, TD, MAP, MSP, DD5, NFFD, bFFP, eFFP, Eref, CMD (see Table 2 for abbreviations) found PC1 and PC2 explained 88.3% of the climate variation. In general, temperature related variables loaded onto PC1 (62.3%) and precipitation variables loaded onto PC2 (25.9%) with the exception of PAS which loaded onto PC1 (Table 8).

Table 8: Climate variables and loadings on PC1 and PC2. Red highlights correspond to the PC with the greatest absolute value of the loading.

Climate Variable	**PC1 score (62.3%)**	**PC2 score (25.9%)**
MAT	0.349	-0.031
NFFD	0.338	-0.134
MCMT	0.338	-0.122
DD5	0.338	0.11
eFFP	-0.331	-0.154
bFFP	-0.321	0.114
TD	-0.302	0.217
MWMT	0.273	0.188
PAS	-0.236	-0.215
Eref	0.268	0.278
CMD	0.135	0.469
MAP	0.101	-0.479
MSP	-0.042	-0.512

The 78 clones were plotted by their PC1 score on the x axis and PC2 score on the y axis. Initially, to confirm the biplot aligned with geography, clones were colored by seed planning unit for B.C. clones and American State for U.S. clones. Interior clones clustered in the upper left quadrant; coastal B.C. clones clustered in the lower two quadrants with northern coastal B.C. clones in lower left. All the American clones, excluding those from Idaho, clustered in the right quadrants and predominantly in the upper right quadrant. California origin clones clustered on the far right (Figure 9).

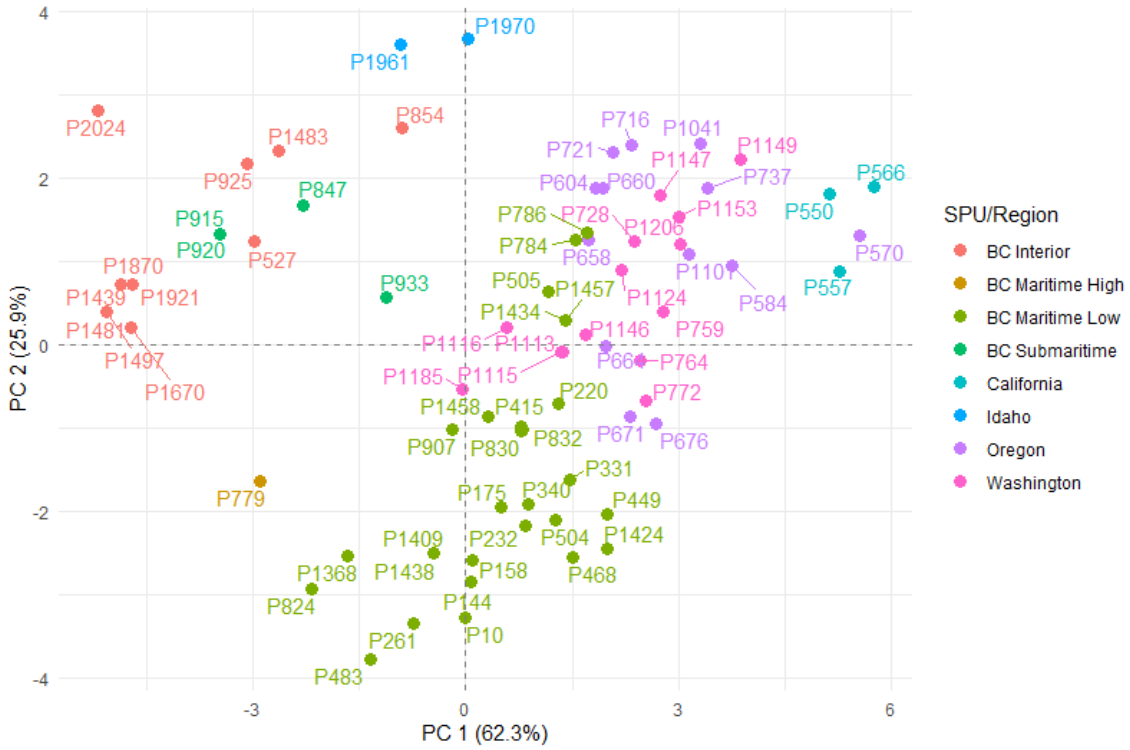

Figure 9: Biplot of individual clones' origins (points) by PC1 and PC2 coordinates (climatic variables) coloured by seed planning unit (SPU) for B.C. origin clones and by state for U.S.A. origin clones. Variables which loaded more onto PC1 include MAT, NFFD, MCMT, DD5, eFFP, bFFP, TD, MWMT, and PAS (62.3% of climatic variation explained). Variables which loaded more onto PC2 include Eref, CMD, MAP and MSP (25.9% of variation explained).

The individual biplot was then coloured by the value of the 78 clones' BLUPs for cold tolerance from the overall model (Figure 10; BLUPs in Appendix E). Generally, clones with lower BLUPs (more cold resistant; lower index of injury) clustered in the left quadrants (Figure 10, blue and purplish points) but there were clones with higher BLUPs (reddish coloured points) in every quadrant. Clone P1368 with the lowest BLUP placed in the lower left quadrant, although it was roughly halfway along the PC1 axis in the quadrant. Clone P570 with the highest BLUP was positioned in the upper right quadrant, far along the PC1 axis.

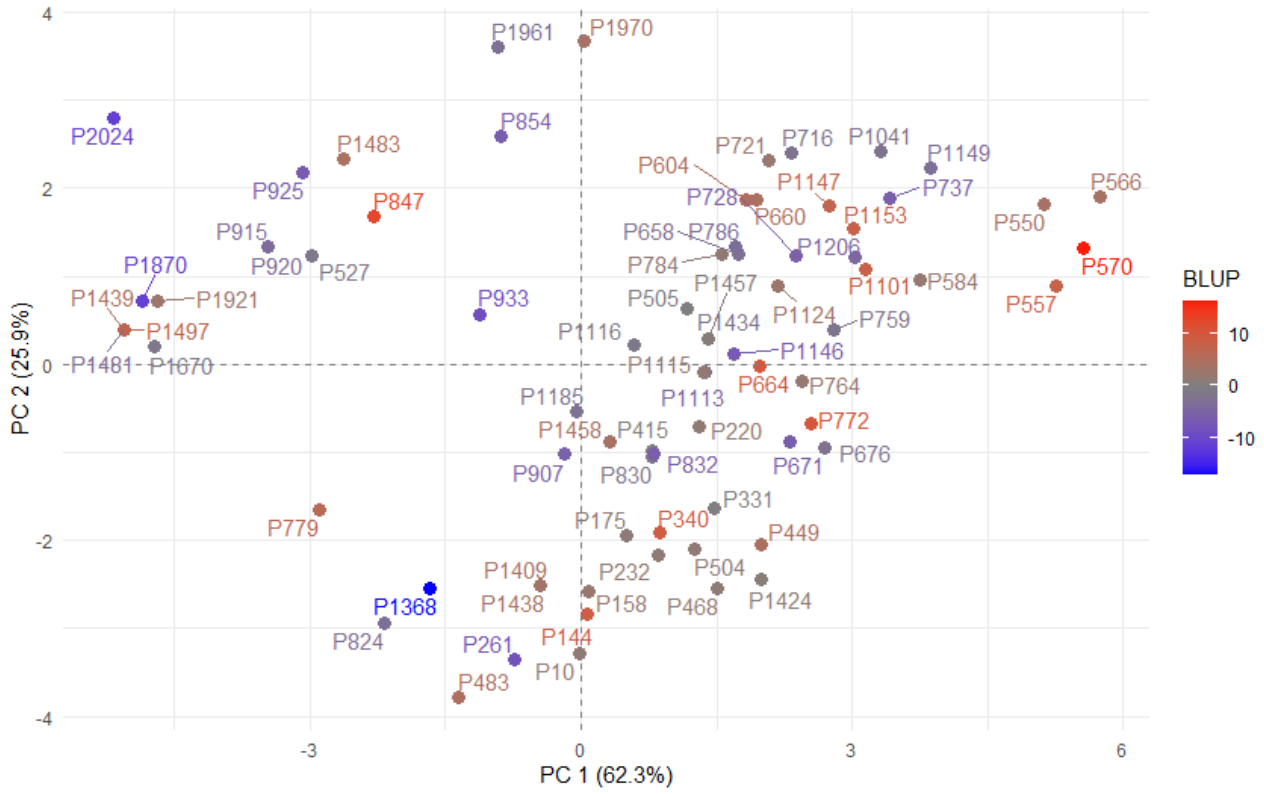

Figure 10: Biplot of individual clones (points) by PC1 and PC2 coordinates coloured by BLUP for overall cold hardiness. Variables which loaded more onto PC1 include MAT, NFFD, MCMT, DD5, eFFP, bFFP, TD, MWMT and PAS (62.3% of climatic variation explained). Variables which loaded more onto PC2 include Eref, CMD, MAP and MSP. Blue coloured points indication lower index of injury (lower BLUPs, more cold hardy) whereas red indicates higher index of injury (higher index of injury, more cold susceptible). Clones with BLUPs closer to zero are coloured in grey and tinted towards their respective positive (reddish tint) or negative (bluish tint) BLUP.

Discussion

As a climate change mitigation strategy, assisted gene flow of populations to novel locations has been proposed and is currently being implemented for reforestation in B.C. Understanding cold hardiness at the southern range of western redcedar, relative to cold hardiness within B.C., has implications for the success of assisted gene flow. Specifically, this study aimed to determine if genotypes from the Pacific Northwest of the USA (Washington, Oregon and California) could be incorporated into B.C. seed orchards without compromising cold tolerance of future planting stock. Given the weak clines observed in the BLUPs, the evidence from this study suggests that incorporation of redcedar clones from outside the range should be considered on a case-by-case parental basis and not with a climatic or geographic threshold.

Based on the heritability (0.17 ± 0.03) determined from the overall model for index of injury, cold hardiness in redcedar appears to be relatively low but driven by additive genetic effects, with no reciprocal effects. Although overall heritability is less frequently reported in the literature than fall and spring heritability, this result is consistent with the overall heritability for cold hardiness of 0.16 found in Douglas-fir (Hawkins & Stoehr, 2009) but lower than the heritability of 0.28 reported in Patagonian cypress (Aparicio et al. 2012).

Bivariate model

Bivariate analysis of fall and spring response to cold hardiness of a subset of 36 clones and 20 families found a moderately strong positive genetic correlation between fall and spring ($\rho_g = 0.54$), suggesting that fall and spring cold hardiness are partially controlled

by the same genes. Phenotypic correlations between fall and spring varied by year with 2014/15 highly correlated (correlation of 0.74) compared to the 2017/18 and 2018/19 years (correlation of 0.33 and 0.27, respectively). Correlations within year and treatment were not significant. This suggests the strength of these phenotypic correlations could relate to the difference in index of injury at different freeze treatments. Slopes for all years (treatments combined) also varied. This is likely explained by the slightly different temperature treatments and timing of freeze tests, suggesting that phenotypic correlations are sensitive to experimental factors. Testing a larger number of clones/families with consistent dates and one temperature treatment would provide greater clarity of the strength of the phenotypic relationship between fall and spring.

For 2014/15, fall was measured on Nov. 14, 2014 and spring was measured on Mar. 23, 2015. The range of damage was broad for fall (23 to 66%) and for spring, although spring was more heavily damaged (56 to 90%). In 2017/18, fall was measured on Nov. 20, 2017 and spring was measured on Mar. 12, 2018. The fall measurements produced a wide range of index of injury (12% to 58%) despite the slightly later measurement date, but the spring measurements had a smaller range (20 to 42% with one exception) suggesting the trees were still cold hardy, likely due to the earlier measurement date. In 2018/19, fall measurements were two weeks later than in 2014 (Dec. 4, 2018) and the trees appeared much more cold hardy, with damage ranging from 3% to 42%. Spring measurements occurred on roughly the same date as 2015 (Mar. 24, 2019) and the range of damage was high (8 to 83%). This suggests if the fall and spring measurements for 2017/18 and 2018/19 had occurred closer to the same dates as 2014/15, then the relationships between fall and spring may have been clearer.

Heritability was marginally higher in fall (0.29 ± 0.09) compared to spring (0.23 ± 0.09) suggesting fall cold hardiness is under slightly stronger genetic control. Both fall and spring had higher heritabilities than the overall model (0.17 ± 0.03). After accounting for the standard errors, both fall and spring heritability fall within a similar confidence bound as the overall heritability and thus may not differ significantly, although there are limitations to these estimates. Firstly, although the data was selected to be balanced to increase power of the estimation of genetic correlations, the 56 clones/families tested is less than the suggested minimum threshold of approximately 75 and may overinflate the estimate (Holland, 2006). Secondly, the later sampling date and different temperatures in fall 2018/19 may underinflate the genetic correlation as the phenotypic correlation was lower; however, this should be accounted for in the season:treatment interaction and year effects incorporated into the model. Nevertheless, once the standard errors are considered, it is likely that the correlation would remain positive and would fall between the wide range of 0.2 and 0.7. Given the lack of precision around this correlation, future studies incorporating larger numbers of clones/families would help solidify the confidence around this estimate.

A positive (non-adverse) genetic correlation between fall and spring cold hardiness is unique compared to the negative (adverse) genetic correlation in Douglas-fir (O'Neill et al. 2001) and the lack of genetic correlation in western hemlock (Hannerz et al. 1999). Neither of those studies suggested reasons for the weak and negative correlations. While fall cold hardiness has been found to have a heritability of 0.38 for yellow cypress (Russell, 1993), spring cold hardiness has not been reported. Heritability for cold hardiness in Cupressaceae overall and seasonally has also not been reported in the

literature, with yellow cypress as the exception. As such, this is the first time fall and spring cold hardiness heritabilities and genetic correlation have been reported for a member of Cupressaceae. Having a positive genetic correlation provides a benefit for the redcedar breeding program as it implies selection for increased fall cold hardiness in redcedar would provide increased spring cold hardiness and vice versa (Falconer & Mackay, 1996).

Cold hardiness measured in this study specifically tests cellular membrane damage upon exposure to freezing temperatures. There is a gap in the literature regarding the exact mechanisms underpinning the development and cessation of cellular membrane 'toughening' in redcedar specifically and other Cupressaceae species in general. As redcedar is indeterminate, it does not rely on bud burst to resume active growth and can be difficult to ascertain when it has stopped growing. Grossnickle and Russell (2006) analyzed mitotic activity in shoot tips to determine when active growth ceased. They found air temperatures below four degrees Celsius completely inhibited active shoot mitosis/growth, with mitotic activity only resuming when temperatures increased above six degrees Celsius, and cold hardiness rapidly dissipating upon onset of active growth. As such, I theorise that the cessation and resumption of mitotic activity is a simpler mechanism than budset or bud burst, as it only requires downregulation and subsequent upregulation of mitosis-related genes upon entrance into dormancy and the return to active growth, rather than development of a bud.

Genes contributing to cold hardiness in the Cupressaceae family have not been well studied. Previous gene expression work in Italian cypress (*Cupressus sempervirens* L.) found upon multi-day exposure to near-freezing temperatures, putative genes associated

with cellular membrane protection and water transport were upregulated while putative genes associated with photosynthesis were downregulated (Pedron et al. 2009). Additional work on Italian cypress also examined differences between cold hardy and cold susceptible genotypes and found putative genes for chaperonins (associated with protein folding) and heat-shock proteins differed between susceptible and cold hardy genotypes with the chaperonins more upregulated in the cold tolerant genotype and the heat-shock proteins more downregulated in the cold sensitive genotype (Balti et al. 2011). It is possible, given the taxonomic relationship between Italian cypress and redcedar, that similar genes would be responsible for cold hardening in redcedar and could explain part of the positive genetic correlation between fall cold hardiness and spring cold hardiness.

Reciprocal, paternal effects

Despite initial findings of unbalanced variance components between male and female parents for the 2014/15 dataset, no evidence of a true paternal or reciprocal effect on cold hardiness was found. Generally, cold hardiness is an additive trait (Howe et al. 2003) but non-additive effects (which would include paternal/reciprocal) have been observed, notably dominance effects in *Eucalyptus* (Tibbits et al. 1991; He et al. 2012). In conifers, Lehtinen and Pulkkinen (2017) found a weak effect of paternal genotype in budset and autumn hardening of Scot's pine, and Hawkins and Stoehr (2009) found an association between the maternal grandparent elevation of origin and cold hardiness in second generation progeny of Douglas-fir.

As reciprocal crosses could only be obtained for parents originating from similar coastal B.C. origins, it is possible reciprocal crosses of divergent geographic locations (e.g. California with McBride and McBride with California) may produce a stronger

paternal or maternal effect. Although all reciprocal groups originated from similar locations in coastal B.C., BLUPs were not always consistent between parents. Specifically, for reciprocal group H (P1368, P1372, and F8717, F8718), parental BLUPs were different, despite similar location of origin (both near Prince Rupert, B.C.), with P1368 (BLUP -17.0) nearly 17 times more cold hardy than P1372 (BLUP -1.2), yet no parental or reciprocal effect was found for their progeny. Although this is only one set of parents, considering the strong difference in their BLUPs and absence of significant reciprocal effect, it seems unlikely that any reciprocal effect for cold hardiness in redcedar exists.

Geographic and climatic clines

Climatic clines of the BLUPs for the 78 clones with origin coordinates were generally weak but appear largely driven by winter temperature and frost-free period. Despite being highly significant, the highest correlation with frost-free period in January and overall BLUPs was low ($\rho = 0.38$). This indicates the relationship exists but is weak, as significance shows that the correlation differs from 0 but the coefficient indicates the strength of the relationship is low. Examining the map of clones coloured by BLUP, certain clones from Vancouver Island had similar BLUPs to those in the northern California (e.g., P220 from Port McNeill had a BLUP of 1.7 and P584 from central Oregon had a BLUP of 2.1) suggesting similar levels of cold hardiness despite their widely differing latitudes of origin and associated seasonal temperature differences.

Clustering on the PCA was largely divided by PC1 (temperature) axis with some separation by PC2 (moisture). Colouration by BLUP generally showed higher BLUPs (less cold hardy) in the upper right quadrant of Figure 10, but there were exceptions.

Although the study was not designed to test provenances but rather individuals, several clones from similar geographic areas were tested. P847 and P854 reportedly originate from within 500 m in the same mountain location in submaritime B.C. yet had BLUPs almost three standard deviations apart (BLUPs 12.57 and -6.03 respectively, standard deviation overall 5.2). Another unusual pattern was with three clones (P1439, P1481 and P1497) originating from near McBride, B.C. in the interior, northern range of redcedar which had very divergent BLUPs of 4.24, -3.55 and 7.04 respectively.

There are several possible explanations for these inconsistent individuals. Firstly, standard errors were high for BLUPs from some of these clones because they were tested once in fall 2017/18 and then abandoned in subsequent testing. As a result, their associated BLUPs have greater uncertainty. These clones may have been more similar had they been measured repeatedly. Secondly, while origin coordinates for all clones have been recorded in SPAR, many of the original scion collections for the coastal B.C. and coastal U.S.A origin clones occurred prior to release of GPS technology and the clonal origin coordinates were assigned based on hand-drawn maps up to twenty years later (Russell, J.H., personal comm.). This may have caused inaccurate origin assignments. This is less likely for the interior B.C. and interior U.S.A. clones as these grafts came from more recent scion collections (post-2000) and geographic assignments were done with GPS technology. The maritime influence of coastal climates also provides a moderating effect, meaning regions 500 m apart would likely be climatically similar, suggesting if origins are not perfect, then the error in climatic variables should be limited. Finally, although all efforts have been made over the years at CLRS for accurate tagging and clone management, errors in clone identities in other redcedar clones (not

tested in this study) have been detected using genomic pedigree construction (Gamal El-Dien, O., personal comm.). Few errors of this type have been found but it is possible that the identities assigned to some of these clones may be incorrect

On the other hand, despite these inconsistent individuals, standard errors of the BLUPs for most of the clones tested (58 out of 76) were less than one standard deviation of 5.6%, suggesting there is good accuracy for these individuals. Furthermore, individuals who were repeated in multiple experiment years and seasons performed consistently. It therefore seems reasonable to conclude the weakness of climatic clines observed in this study is legitimate. The lack of strong climatic trends contrasts with the findings of Rehfeldt (1994) who found latitudinal clines with winter frost damage in provenances of redcedar in Idaho. They suggested differences in cold hardiness among populations would only occur with greater than two degrees difference in latitude. Cherry (1995) also found differences between the six provenances of redcedar they tested but noted that variation was greater within provenances than between provenances. Grossnickle and Russell (2006) did not find significant population differences in the lethal freezing temperature, although they attributed their finding to testing few populations that originated within a narrow elevational band of 300 to 500 m.

Latitude (which is correlated with temperature) clines for yellow cypress have also been found to be relatively flat, with the exception of the southernmost, Oregon population (Russell & Krakowski, 2012). More broadly in the Cupressaceae family, population differences for *Cupressaceae arizonica* along an elevational gradient found no significant population effects but a significant family effect for frost injury (Rehfeldt, 1997). Significant population and family effects for frost injury in *Cupressaceae glabra*

were found, with the population variance roughly four times the family variation Rehfeldt, 1997). This suggests that climatic clines in cold hardiness are not guaranteed in Cupressaceae.

The lack of strong climatic clines in this study, in combination with previous work in redcedar, suggests that there may not be strong hierarchical population structure for redcedar populations. O'Connell et al. (2008) examined genetic diversity of redcedar across its range using microsatellites to explore patterns of post-glacial colonization. They found evidence to suggest relatively recent colonization from a single coastal refugia and noted three population geographic clusters - central (eastern Vancouver Island into submaritime B.C.), northern/coastal (western Vancouver Island north to Alaska) and southern/interior (Washington, Oregon Idaho and all interior B.C. populations). They also found 93% of the genetic variance occurred within populations. The BLUPs from this cold hardiness study do not appear to align with their three geographic clusters but this may be a result of my experimental design, focusing on individuals rather than provenances. Examining cold hardiness at the provenance level with multiple provenances from across the range may find more evidence of these geographic clusters for cold hardiness as well as add to our understanding of population structure in redcedar.

Conclusions

Given the weak clines observed in redcedar cold hardiness, this study suggests that incorporation of redcedar clones from outside the province into B.C. seed orchards should be considered for individual clones and not with a climatic or geographic threshold.

A major limitation to this study is the phenotyping of cold hardiness using electrolyte leakage which focuses on cellular membrane damage due to low temperatures. Winter conditions may also come with increased snow load in certain areas which, while not necessarily causing tree death, can cause form abnormalities (e.g., broken branches, bent form) (Degner, 2019, unpublished). As a result, although cold damage alone can be considered on a case-by-case basis, I recommend that specific southern redcedar clones only be incorporated into B.C. seed orchards after successful testing in B.C. progeny field tests.

The positive genetic correlation between fall and spring cold hardiness in redcedar identified in this study is a novel finding and suggests overlap between genes regulating the entry and release from cold hardiness. As a complex trait, the identities of genes underlying cold hardiness are poorly understood and unknown for redcedar. Future work examining the gene expression associated with fall, winter and spring cold hardiness could help elucidate which genes are supporting this positive genetic correlation. It would also expand knowledge of genes involved in cold hardiness in Cupressaceae and other indeterminant conifers, in general.

The lack of obvious geographic and climatic clines suggests that there is limited population structure in redcedar. Future work examining cold hardiness in multiple provenances across the range could support or negate this finding, which is relatively weak given the design of this study. Beyond population structure, a robust comparison of regional differences, such as interior versus coastal may be beneficial.

Chapter 3: Rhodoxanthin[3]

Introduction

Although seasonal color changes in trees are most commonly associated with deciduous leaf senescence, many conifers and other gymnosperms also undergo foliage color changes throughout the year. A common transition is a change to a reddish hue during the winter (Hughes, 2011). For many conifers, this color change has been ascribed to an accumulation of the carotenoid rhodoxanthin and has been observed in many conifer families including Cupressaceae and Taxaceae (Ida, 1981a). Western redcedar (*Thuja plicata* Donn ex D.Don; redcedar), a species in the Cupressaceae, exhibits this winter reddening phenomenon.

Carotenoids are biological hydrocarbons that play a multitude of roles in plants, often giving flowers or leaves characteristic bright yellow or orange colours. Carotenoids are characterized into two subclasses, the carotenes that consist of hydrogen and carbon exclusively and the xanthophylls, which also contain oxygen. Xanthophylls are synthesized from the carotenes, specifically β-carotene (Hirschberg, 2001), and function within the thylakoid membrane of the chloroplast to dissipate excess energy that the plant cannot utilize in photosynthesis. This is done by pigments in the xanthophyll cycle, a cycling of epoxidation and de- epoxidation between zeaxanthin, antheraxanthin and violaxanthin (Demmig et al. 1987).

The onset of winter provides challenges to plants, as to survive colder temperatures, they must undergo numerous physiological changes to mitigate the damaging effects of

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[3] Manuscript intended for publication in Canadian Journal of Forest Research

cold temperatures on tissues. The processes of growth cessation and dormancy induction are components of cold hardiness, and these processes must be followed by the resumption of active growth in the spring (Sakai & Weiser, 1973). These processes require a complex suite of metabolic changes, all controlled by various genes (Howe et al. 2003).

Evergreen plants, which do not shed their leaves in autumn, have the added challenge of tolerating both low temperatures and bright sunlight in winter. Photosynthesis is a temperature-dependent process and low temperatures reduce or suspend the function of the Calvin cycle enzymes (Taiz & Zeiger, 2002). Despite the cold temperatures, plants below the Arctic circle are still exposed to the radiation from winter sunlight which can cause stress to the light harvesting reactions of photosynthesis due to absorption of excess excitation energy. As a result, exposure to normal sunlight in conjunction with cold temperatures can cause irreparable damage to the photosystems in the plant's chloroplasts. Nonphotochemical quenching (NPQ) is a mechanism plants have evolved to mitigate this light stress and is chiefly performed by the carotenoid xanthophyll cycle which remains active during the winter and is thought to be the primary photoprotective mechanism during this season (Adams et al. 1994).

Rhodoxanthin, a carotenoid pigment, is likely synthesized from the xanthophyll zeaxanthin with three intermediate compounds (Hudon et al. 2007; Royer et al. 2020). Rhodoxanthin has been found in many members of the Cupressaceae during winter, including redcedar, *Cryptomeria japonica* D. Don, *Thuja occidentalis* L. and others (Ida, 1981a; Czeguza, 1987; Han et al. 2003; and Weger et al. 1993). It has also been observed under conditions of warmer temperatures and high light stress in *C. japonica*. (Ida,

1981b) and in *Aloe arborescens* Mill. (Merzlyak et al. 2005). Weger et al. (1993) found increasing concentration of rhodoxanthin corresponded with increasing cold hardiness in redcedar seedlings originating from southern Vancouver Island, B.C. and a sharp decrease in accumulation of rhodoxanthin upon exposure to longer daylengths and warmer temperatures. They hypothesized that rhodoxanthin played a photoprotective role. Han et al. (2003) found evidence of a photoprotective role for rhodoxanthin in *C. japonica*, possibly allowing plants to maintain higher rates of net photosynthesis. They found wild-type saplings that produced rhodoxanthin maintained higher net photosynthetic rates than mutants that remained green all winter. This has also been found in another gymnosperm, the Siberian shrub *Ephedra monosoperma* J.G. Gmel. ex C.A. Mey. (Sofranova et al. 2014).

Little work has been done to compare variation within species in both the timing and degree of rhodoxanthin accumulation. Ida (1981a) found differences in rhodoxanthin concentration between sun and shade leaves of *C. japonica* but also between juvenile seedlings (two years old) and thirty-year-old trees, where the older trees had significantly less rhodoxanthin, particularly in shade leaves where only trace amounts were found. To the best of my knowledge, no studies comparing provenance or family variability in rhodoxanthin concentration have been completed for any rhodoxanthin-producing plant.

Observations of redcedar seedlings grown at the Cowichan Lake Research Station (CLRS) on southern Vancouver Island, B.C. found visible color differences between different provenances of redcedar in winter (Figure 11). The observed trends generally found deeper colouration in interior provenances than coastal provenances.

Figure 11: An example of colour differences in redcedar seedlings from different provenances. Left: provenance 106 from Port Renfrew on the west coast of Vancouver Island. Middle: provenance 55 from Upper Incompleaux River located southeast of Revelstoke, B.C. Right: provenance 91 from Albert River located east of Invermere, B.C. Colour changes are hypothesized to be a result of variation in rhodoxanthin concentrations, with green having the least rhodoxanthin. Photo taken at Cowichan Lake Research Station on November 19, 2018.

Objectives

Considering the observed variation in redcedar seedling winter colouration and given that rhodoxanthin production correlates with development of cold hardiness, it was hypothesized that rhodoxanthin accumulation is correlated with depth of cold hardiness. If this hypothesis is true, phenotyping of rhodoxanthin levels using a color scale as a proxy for cold hardiness could be more efficient than phenotyping for cold hardiness itself. The goal of this research was to determine if and how rhodoxanthin concentration varies in redcedar over a winter season, if rhodoxanthin production varies between juvenile seedlings and older saplings (clonal grafts), if rhodoxanthin concentration is related to the degree of cold hardiness, and if geographic clines are associated with this variation in rhodoxanthin concentration in redcedar.

Methods

Material and experimental design

All material tested was grown at CLRS on southern Vancouver Island, B.C. (coordinates: 48° 49’ N, 124° 8’ W 185 m elevation). Experiments were conducted on seedlings in 2015/16 and cloned grafts in 2017/18 and 2018/19.

2015/16

The first experiment occurred in 2015/16 with redcedar seedlings (referred to hereafter as family/provenance) from eight provenance collections and 23 controlled cross seedling families (31 families/provenances total; see Table 9 and 10 for description of families and provenance origins). Families were selected from controlled crosses of B.C. and U.S.A. origin parents. Provenances, grown from seed collected from old growth trees from specific regions, were selected to provide germplasm originating from submaritime, interior B.C. and Idaho, and northern coastal B.C. Seeds for both families and provenances were sown in January 2015 and grown for six months in styroblock containers (size 412A, Beaver Plastics, Edmonton, AB). In June 2015, five seedlings from each family/provenance were transplanted into an irrigated nursery bed outdoors where they remained for the duration of the experiment.

Seedlings were sampled once in fall (November 23, 2015), winter (January 25, 2016) and spring (March 18, 2016) for rhodoxanthin content and electrolyte leakage. When sampled, lateral branches from the seedling were cut, placed into a plastic zipper-sealed bag and placed on ice in a polystyrene cooler before transport to the respective lab for rhodoxanthin or cold hardiness testing.

A subset of these families/provenances were tested for cold hardiness. Eight of the 31 families/provenances were tested individually and 14 were combined into groups of two (seven groups total) and tested in these groups (Table 11). Cold hardiness test data were included in the analyses in Chapter 2, except for the tests conducted in 2015/16. Cold hardiness data from 2015/16 could not be used in Chapter 2 analyses because pairs of families/provenances were combined in one vial.

Table 9: Seedling F1 controlled cross families tested in 2015/16 experiments.

F1 controlled cross families tested

ID Code	Female parent	Female parent information				Male Parent	Male parent information			
		Female region	Latitude (DM)	Longitude (DM)	Elevation (m)		Male region	Latitude (DM)	Longitude (DM)	Elevation (m)
F398	P220	North Vancouver Island	50° 31'	127° 0'	120	P220	North Vancouver Island	50° 31'	127° 0'	120
F406	P372	South Vancouver Island	48° 35'	124° 1'	180	P175	South Vancouver Island	48° 56'	124° 42'	503
F583	P925	Submaritime BC	49° 33'	120° 48'	1100	P920	Submaritime BC	49° 34'	120° 58'	1150
F685	P220	North Vancouver Island	50° 31'	127° 0'	120	P232	Central Coast BC	51° 18'	127° 20'	110
F8417	P550	Northern California	41° 32'	124° 0'	60	P570	Southern Oregon	42° 16'	124° 22'	50
F8418	P550	Northern California	41° 32'	124° 0'	60	P854	Submaritime BC	50° 23'	122° 44'	570
F8419	P557	Northern California	41° 50'	124° 22'	20	P550	Northern California	41° 32'	124° 0'	60
F8420	P557	Northern California	41° 50'	124° 22'	20	P830	Lower Mainland	49° 21'	123° 1'	480
F8421	P584	Central Oregon	44° 1'	123° 49'	100	P557	Northern California	41° 50'	124° 22'	20
F8422	P584	Central Oregon	44° 1'	123° 49'	100	P658	Northern Oregon	46° 3'	123° 25'	476
F8423	P658	Northern Oregon	46° 3'	123° 25'	476	P786	East Vancouver Island	49° 42'	125° 5'	80
F8424	P786	East Vancouver Island	49° 42'	125° 5'	80	P550	Northern California	41° 32'	124° 0'	60
F8425	P786	East Vancouver Island	49° 42'	125° 5'	80	P824	Lower Mainland	49° 22'	123° 0'	950
F8426	P854	Submaritime BC	50° 23'	122° 44'	570	P907	South Vancouver Island	48° 28'	124° 6'	580
F8427	P854	Submaritime BC	50° 23'	122° 44'	570	P1153	Washington Coast	47° 51'	122° 50'	200
F8428	P933	Submaritime BC	49° 18'	121° 22'	700	P557	Northern California	41° 50'	124° 22'	20
F8429	P933	Submaritime BC	49° 18'	121° 22'	700	P854	Submaritime BC	50° 23'	122° 44'	570
F8430	P1041	Central Oregon	44° 24'	122° 34'	240	P660	Northern Oregon	45° 54'	123° 23'	300
F8431	P1041	Central Oregon	44° 24'	122° 34'	240	P933	Submaritime BC	49° 18'	121° 22'	700
F8432	P1116	Washington Interior	47° 3'	121° 55'	450	P830	Lower Mainland	49° 21'	123° 1'	480
F8433	P1149	Washington Coast	47° 49'	122° 55'	50	P1041	Central Oregon	44° 24'	122° 34'	240
F8434	P1153	Washington Coast	47° 51'	122° 50'	200	P1149	Washington Coast	47° 49'	122° 55'	50

Table 10: Seedling provenances tested in 2015/16 experiments.

Provenances and Seedlots tested

ID Code	Type	Origin	Region	Latitude (DM)	Longitude (DM)	Elevation (m)
PROV55	Provenance	Upper Incomappleaux River, BC	Interior BC	50° 59’	117° 40’	1410
PROV75	Provenance	Upper Dore River, BC	Interior BC	53° 17’	120° 16’	1120
PROV80	Provenance	Purden Mountain, BC	Interior BC	53° 54’	121° 54’	990
PROV84	Provenance	Elk River, ID	Idaho	46° 50’	116° 10’	900
PROV85	Provenance	Lochsa River, ID	Idaho	46° 23’	115° 14’	710
PROV91	Provenance	Albert River, BC	Interior BC	50° 37’	115° 33’	1300
PROV957	Provenance	Big Falls Creek, BC	North Coast BC	53° 58’	129° 35’	150
PROV960	Provenance	Kumois, BC	Haida Gwaii, BC	53° 45’	132° 06’	20
PROV966	Provenance	Joffre Creek, BC	Submaritime BC	50° 20’	122° 33’	900

Table 11: Subset of families/provenances tested for cold hardiness in 2015/16.

Code	Cold hardiness measurement type	Seedling type
F406	Individual	Controlled cross family
F8422	Individual	Provenance
F8423	Individual	Controlled cross family
F8434	Individual	Provenance
PROV55	Individual	Provenance
PROV91	Individual	Provenance
PROV957	Individual	Provenance
PROV960	Individual	Provenance
F398 & F685	Tested as combined pair	Controlled cross family
F583 & PROV966	Tested as combined pair	Controlled cross family & Provenance
F8420 & F8424	Tested as combined pair	Controlled cross family
F8429 & F8432	Tested as combined pair	Controlled cross family
PROV75 & PROV80	Tested as combined pair	Provenance
PROV84 & PROV85	Tested as combined pair	Provenance

2017/18 and 2018/19

2017/18 and 2018/19 material were also tested for cold hardiness as detailed in Chapter 2. In 2017/18, 64 cloned genotypes from across the range of redcedar were tested for cold hardiness, rhodoxanthin and other carotenoids (Appendix H). 20 of these clones were the parents of the families tested in 2015/16. The source material for these clones came from grafts aged 20- to 30-years-old planted outside in the arboretum at CLRS. One ramet of each clone was sampled. Foliar samples were collected using 10 m pruning poles to obtain foliage from as high in the crown as possible and on the south facing or more exposed side of the tree. Tissue was sampled in fall (Nov. 20, 2017) and winter (Jan. 16, 2018) and spring (March 12, 2018). Rhodoxanthin and other carotenoids were tested in the fall and winter seasons but not in spring due to budget limitations. Electrolyte leakage experiments for cold hardiness were also performed with methods described in Chapter 2. After sampling, foliage tissue was placed into a plastic zipper-sealed bag and placed on

ice in a polystyrene cooler before transport to the respective lab for rhodoxanthin or cold hardiness testing.

In 2018/19, 19 cloned, 20- to 30-year-old clones were tested for cold hardiness, rhodoxanthin and other carotenoids (Appendix H). 15 clones were repeated from the 2017/18 experiments and four additional clones were selected to match the objectives of the cold hardiness study (Chapter 2). Clones were sampled in fall (Dec. 4, 2018), winter (Jan. 21, 2019) and spring (Mar. 24, 2019). Sampling protocols and transport were the same as in 2017/18 experiments. Rhodoxanthin and carotenoids were tested for all seasons.

High Performance Liquid Chromatography (HPLC)

All HPLC work to analyze carotenoids including rhodoxanthin was conducted by the B.C. Ministry of Environment and Climate Change Analytical Lab (MOE Lab) in Saanich, B.C following a modified version of methodology described in Barba et al. (2006). Samples from all experiments were transported from CLRS to the MOE Lab. Upon arrival at the MOE Lab, they were transferred to a -80°C freezer and stored until processing. During processing, samples were ground in liquid nitrogen and then lyophilized. 4 ml of a 90% acetone 10% distilled water solution was added to lyophilized material and then samples were shaken at 4°C for one hour. Samples were allowed to cold steep at 4°C overnight and then returned to a -80°C freezer until analysis.

All samples were analyzed using HPLC on a C18 column using a three-phase solvent system of acetonitrile, methanol and a solution of water and 10% anhydrous acetic acid.

Absorbance spectra were determined using a photodiode array detector that generated chromatograms. Detection peaks were noted and peak areas were integrated to determine

compound concentration per redcedar sample. Six carotenoid compounds were detected in redcedar tissue. Carotenoid identities were confirmed when possible against standards available. The standards available for identification were astaxanthin, β-carotene, lutein, lycopene, rhodoxanthin and zeaxanthin. Four additional compounds were quantified but their identities differed from the standards available. At 470 nm, fucoxanthin eluted first, followed by unknown carotenoid #1, astaxanthin, zeaxanthin, lutein. At 450 nm, lycopene eluted first followed by unknown carotenoids #3, beta-carotene and carotenoid #4. At 497 nm only rhodoxanthin eluted.

For the 2015/16 experiments, only rhodoxanthin concentration was determined.

Freeze-induced electrolyte leakage (EL)

Cold damage was quantified by freeze-induced electrolyte leakage as described in Chapter 2. Freeze temperatures and treatments for the 2015/16, 2017/18 and 2018/19 experiments with clones/families that were used for rhodoxanthin and carotenoid concentration comparisons are described in Table 12.

Table 12: Sampling dates (seasons) and associated freeze treatments for cold hardiness testing.

Date	**Freeze treatment (held at temperature for one hour)**
2015/16	
Fall (Nov. 23, 2015)	-8°C, -13°C, -18°C
Winter (Jan. 25, 2016)	-23°C, -28°C, -33°C
Spring (Mar. 18, 2016	-8°C, -13°C, -18°C
2017/18	
Fall (Nov. 20, 2017)	-8°C, -13°C, -18°C
Winter (Jan. 22, 2018)	-18°C, -23°C, -28°C
Spring (Mar. 12, 2018)	-8°C, -13°C, -18°C
2018/19	
Fall (Dec. 4, 2018)	-13°C, -18°C, -23°C
Winter (Jan. 21, 2019)	-23°C, -28°C, -33°C
Spring (Mar. 24, 2019)	-8°C, -13°C, -18°C

Data and statistical analysis

Statistical analysis of the data was completed using R version 3.6.3 (R Core Team, 2020), ASReml-R version 4.1.0.110 for linear mixed effect modelling (Butler, 2019) and the R package psych version 2.0.9 which includes correction factors when conducting multiple correlation tests (Revelle, 2020). Data visualization was performed using the ggplot2 R package (Wickham, 2016) and skewness and kurtosis were calculated using the moments R package (Komsta & Novometsky, 2015). The significance threshold for all inferential tests was $p < 0.05$.

In 2015/16, the fall rhodoxanthin levels were substantially lower than in winter and spring (mean of 20.6 µg/g versus 324.9 µg/g and 320.9 µg/g, respectively). Fall data also strongly deviated from normal, being heavily right skewed (skewness of 2.8) and kurtotic (kurtosis = 14.9) (Clewer & Scarisbrick, 2001). By comparison, winter and spring were more normally distributed as determined by visual assessment of histograms, quartile-quartile plots and smaller skew and kurtosis values (Appendix I).

The variances between fall and winter and fall and spring were tested using an F test and found to differ significantly ($p < 0.01$ for both seasons), but winter and spring did not differ significantly ($p = 0.05$). Because of the non-normality of the fall data combined with the heteroskedasticity with winter and spring data, fall was modelled using a univariate model. To correct for the right skew and kurtosis, fall data was natural logarithm transformed (Clewer & Scarisbrick, 2001).

Data from this study was correlated through the inclusion of related germplasm (i.e. parents and progeny) and temporal correlation of repeated measures over multiple seasons and years. Observations therefore could not be considered independent. Linear

mixed effects models (LMMs) with random effects were used to accommodate the violation of the assumption of independence of observations, required for traditional analysis of variance (Faraway, 2005; Crawley, 2015). LMMs are also more robust for unbalanced designs as the Restricted Maximum Likelihood (REML) method can be used to estimate parameters with less bias than ordinary least squares (Faraway, 2005). Furthermore, the LMM can also incorporate the pedigree information in order to both account for the correlation between relatives, as well as predict the effects associated with parents not tested based on their progeny's performance (Isik et al. 2017).

Random effects in an LMM represent an unknown variable whereas fixed effects represent an unknown constant (Faraway, 2005). The LMM instead estimates parameters of the unknown variable (i.e., variance) with an assumed multivariate normal distribution (MVN) from which inferences about the population underlying that unknown variable can be made (Faraway, 2005). Partitioning the variance into components produced by the clones/families and those from the residual (environment) allows estimates of heritability (Isik et al., 2017).

In this study, narrow-sense heritability (h^2) (Falconer & Mackay, 1996) was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{a\times e}^2 + \sigma_e^2}$$

where σ^2_a is the additive genetic variance component (clone/family effect with relationship matrix incorporated), $\sigma^2_{a\,x\,e}$ is the interaction between the additive genetic component and environment and σ^2_e is the environmental (residual) variance component. Standard errors were estimated using the Taylor series approximation (Butler, 2018).

As a rule of thumb, variance components are likely significant when the Z ratio is greater than 2.0 (Isik et al. 2017). The significance of random effects and their associated interactions was formerly tested using a nested log-likelihood test (Self & Liang, 1987) where the likelihood ratio statistic was compared to the associated null χ^2 distribution for χ^2_{n+1} and χ^2_n where n is the number of random effects and $n+1$ is for the additional random effect or interaction.

Best linear unbiased predictors (BLUPs) were predicted for each clone/family tested and for associated, untested, parents as described in the pedigree/relationship matrix. BLUPs represent an individual's scaled and weighted deviance from the overall population mean (μ).

Because of the incorporation of the pedigree relationship matrix ($\boldsymbol{A}$), BLUPs can be predicted for parents of the seedling families tested based on their progeny's rhodoxanthin concentrations despite having not been phenotyped themselves. As a result, BLUPs were calculated for 24 parents. For the fall 2015 model, coefficients of random effects for family/provenance were back transformed to produce BLUPs in the same scale as the raw observations and as the winter/spring model. Back-transformed BLUPs for fall were then compared with those from the bivariate model (winter and spring), although it is acknowledged this method over-estimates the correlation between traits (Houslay & Wilson, 2017).

Fall 2015 model

For analysis of the fall 2015 rhodoxanthin data, a REML method was used to estimate variance components from a LMM described as:

$$\boldsymbol{y} = \mu + \boldsymbol{Zu} + \boldsymbol{\varepsilon}$$

where,

$$\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{\varepsilon} \end{bmatrix} \sim MVN\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \boldsymbol{G} & 0 \\ 0 & \boldsymbol{\Sigma} \end{bmatrix}\right)$$

and,

$$\boldsymbol{G} = \boldsymbol{A}\sigma_a^2$$

Where $\boldsymbol{y}$ is vector of the natural log-transformed fall rhodoxanthin observations. The overall mean, μ, is the only fixed effect. $\boldsymbol{Z}$ is the design incidence matrix of random effects (families/provenances), $\boldsymbol{u}$ is the vector of random effect described by the family/provenance code, and $\boldsymbol{\varepsilon}$ is the vector of residual deviations. Vectors $\boldsymbol{u}$ and $\boldsymbol{\varepsilon}$ are assumed to be multivariate normal (MVN) with a mean of 0 and a variance-covariance described by matrices $\boldsymbol{G}$ and $\boldsymbol{\Sigma}$. $\boldsymbol{G}$ is described by matrix $\boldsymbol{A}$ which represents the genetic relationship matrix between families thus accounting for the estimate correlation between relatives (i.e., the offspring of a parent has an estimated coancestry of 0.5).

Winter and spring 2016 model

Given that winter and spring were repeated measures on the same families/provenances and had similar variances, they were modelled using a bivariate model (Holland, 2006) where winter rhodoxanthin level was considered trait one and spring rhodoxanthin level was considered trait two. The model fitted was:

$$\begin{bmatrix} \boldsymbol{y}_{winter} \\ \boldsymbol{y}_{spring} \end{bmatrix} = \begin{bmatrix} \mu_{winter} \\ \mu_{winter} \end{bmatrix} + \begin{bmatrix} \boldsymbol{X}_{winter} & 0 \\ 0 & \boldsymbol{X}_{spring} \end{bmatrix} \begin{bmatrix} \boldsymbol{g}_{winter} \\ \boldsymbol{g}_{spring} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_{winter} & 0 \\ 0 & \boldsymbol{Z}_{spring} \end{bmatrix} \begin{bmatrix} \boldsymbol{ge}_{winter} \\ \boldsymbol{ge}_{spring} \end{bmatrix} + \begin{bmatrix} \boldsymbol{\varepsilon}_{winter} \\ \boldsymbol{\varepsilon}_{spring} \end{bmatrix}$$

where $\boldsymbol{y}_{\mathbf{winter}}$ and $\boldsymbol{y}_{\mathbf{spring}}$ are the vectors of rhodoxanthin observations of the winter and spring season, μ_{winter} and μ_{spring} are the constant, overall means for each season; $\boldsymbol{g}_{winter}$ and $\boldsymbol{g}_{spring}$ are the vectors of family/provenance effects; $\boldsymbol{ge}_{winter}$ and $\boldsymbol{ge}_{spring}$ are the vectors of family/provenance by associated season effect and $\boldsymbol{\varepsilon}_{winter}$ and $\boldsymbol{\varepsilon}_{spring}$ are the vectors of the residual effects by associated season. $\boldsymbol{X}_{winter}$, $\boldsymbol{X}_{spring}$, $\boldsymbol{Z}_{winter}$, and $\boldsymbol{Z}_{spring}$ are the corresponding incidence matrices. Data was balanced therefore $\boldsymbol{X}_{winter} = \boldsymbol{X}_{spring}$, and $\boldsymbol{Z}_{winter} = \boldsymbol{Z}_{spring}$.

Variance covariance matrices on the family/provenance interaction were modelled using a correlated heterogeneous structure where:

$$V\begin{bmatrix}\boldsymbol{g}_{winter}\\ \boldsymbol{g}_{spring}\end{bmatrix} = \begin{bmatrix}\sigma^2_{winter} & \rho\sigma_{winter}\sigma_{spring}\\ \rho\sigma_{winter}\sigma_{spring} & \sigma^2_{spring}\end{bmatrix}$$

$$V\begin{bmatrix}\boldsymbol{ge}_{winter}\\ \boldsymbol{ge}_{spring}\end{bmatrix} = \begin{bmatrix}\sigma^2_{winter} & \rho\sigma_{winter}\sigma_{spring}\\ \rho\sigma_{winter}\sigma_{spring} & \sigma^2_{spring}\end{bmatrix}$$

$$V\begin{bmatrix}\boldsymbol{\varepsilon}_{winter}\\ \boldsymbol{\varepsilon}_{spring}\end{bmatrix} = \begin{bmatrix}\sigma^2_{winter} & \rho\sigma_{winter}\sigma_{spring}\\ \rho\sigma_{winter}\sigma_{spring} & \sigma^2_{spring}\end{bmatrix}$$

Genetic (Type A, ρ_A) correlations (Falconer & Mackay, 1996) between winter and spring rhodoxanthin levels were calculated as:

$$\rho_A = \frac{Cov_{g_{winter,spring}}}{\sqrt{\sigma^2_{winter} \times \sigma^2_{g_{spring}}}}$$

where $Cov_{g_{winter,spring}}$ is the estimated family/provenance covariance between winter and spring rhodoxanthin, and

$$Cov_{g_{winter,spring}} = \frac{\sigma^2_{g_{winter,spring}} - \sigma^2_{g_{winter}} - \sigma^2_{g_{spring}}}{2}$$

Comparison of rhodoxanthin concentration and electrolyte leakage

Comparison of rhodoxanthin and electrolyte leakage was done by calculating Pearson correlations and significance by season and family/provenance for mean rhodoxanthin concentration and mean index of injury. Genetic correlations were not estimated due to the small number of families (eight) that had individual rhodoxanthin concentrations and index of injury data, as eight was far below the recommended threshold of 75 (Holland, 2006).

Geographic clines

Parental rhodoxanthin BLUPs from the families tested in 2015/16 and the BLUPs from the provenances also tested that year were used to assess the extent of geographic or climatic clines associated with rhodoxanthin concentration. Climate data for each parent/ provenance's origin coordinates were acquired from the ClimateNA program (Wang et al. 2016) which provides downscaled Parameter-elevation Regressions on Independent Slopes Model (PRISM) data (Daly et al. 2018) for western North America. ClimateNA uses the normal period 1961 to 1990 as the baseline dataset for ClimateNA because spatial coverage is best for this time period (Wang et al. 2016). Although older climate data would have been preferred, as redcedar can live over one-thousand years (Minore, 1981) and may be adapted to historical climates, I considered the greater spatial accuracy to be more important, particularly given the mountainous location of origin for several provenances tested. Geographic variables (latitude, longitude and elevation) were also included. In total, 26 variables were compared (Table 13).

Table 13: List of geographic and climate variables used in individual Pearson correlations with best linear unbiased predictors (BLUPs) for rhodoxanthin levels.

Variable acronym	**Variable description**	**Origin**
Lat	Latitude of origin (to degree and minutes)	Obtained from CLRS parent tree and provenance collection records
Long	Longitude of origin (to degrees and minutes)	
Elevation	Elevation of origin (m)	
MAT	Mean Annual Temperature (°C)	Climate NA, directly calculated variable
MWMT	Mean warmest month temperature (°C)	
MCMT	Mean coldest month temperature (°C)	
TD	Continentality (difference between MWMT and MCMT (°C)	
MAP	Mean annual precipitation (mm)	
MSP	Mean annual summer (May to September precipitation) (mm)	
AHM	Annual heat-moisture index (MAT+10)/(MAP/1000)	
SHM	Summer heat-moisture index (MWMT)/(MSP/1000)	
DD<0	Degree-days below 0°C – chilling degree days	ClimateNA, derived variable
DD>5	Degree-days above 5°C – growing degree days	
DD<18	Degree-days below 18°C – heating degree days	
DD>18	Degree-days above 18°C – cooling degree-days	
NFFD	Number of frost-free days	
FFP	Frost-free period	
bFFP	Day of year which FFP begins (beginning frost-free period)	
eFFP	Day of year which FFP ends (end frost-free period)	
PAS	Precipitation as snow (mm)	
EMT	Extreme minimum temperature over 30 years	
EXT	Extreme maximum temperature over 30 years	
Eref	Hargreaves reference evaporating (mm)	
CMD	Hargreaves moisture deficit (mm)	
MAR	Mean annual solar radiation (MJ m^{-2} d^{-1})	
RH	Mean annual relative humidity (%)	

Individual Pearson correlations between BLUPs and the geographic and climatic variables were calculated and tested for significance. Because of the large number of

climate variables compared, the Benjamini & Hochberg (1995) false discovery rate test was used to account for the error associated with multiple comparisons as it has greater power than the Bonferroni false discovery method when applied to a large number of tests (Benjamini & Hochberg, 1995).

Results

Rhodoxanthin

2015/16 experiments

Rhodoxanthin was present in every seedling measured in each season (fall, winter, spring). Mean rhodoxanthin concentration increased substantially from fall (20.6 µg/g) to winter (324.9 µg/g) and then was similar from winter to spring (320.9 µg/g) (Figure 12).

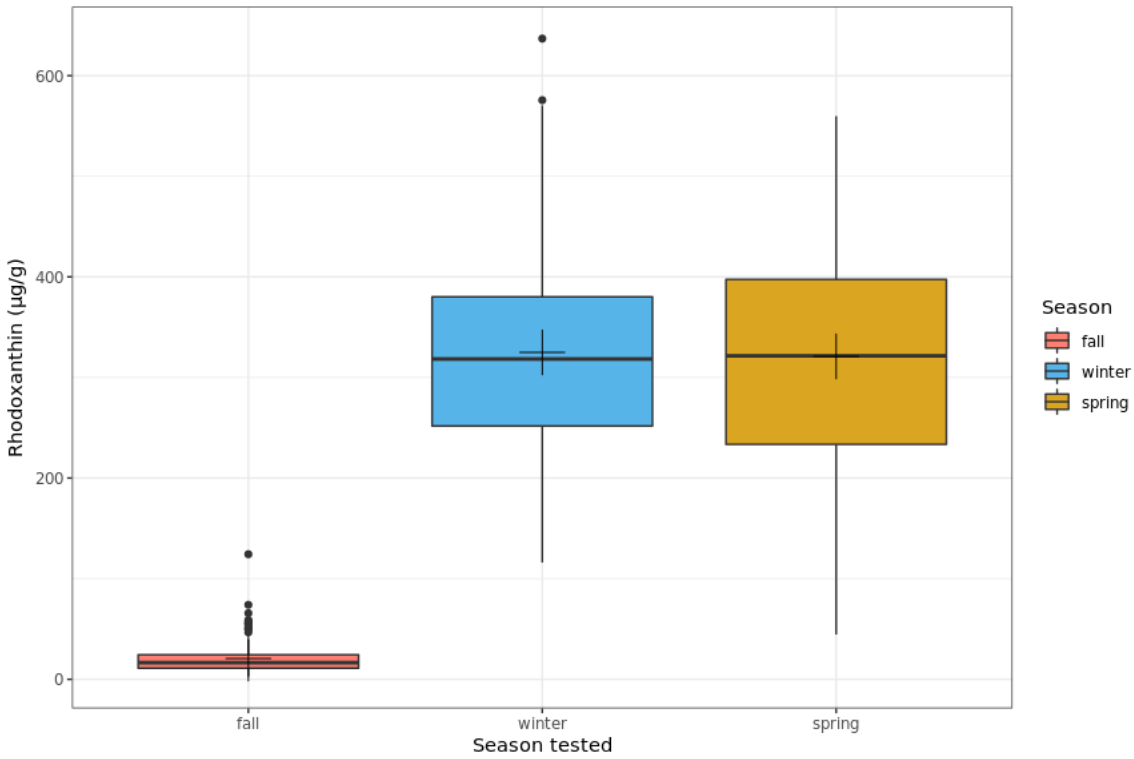

Figure 12: Boxplot of rhodoxanthin concentration (µg/g) from seedlings tested in three seasons in 2015/16. Bolded, black midlines within boxes are median rhodoxanthin levels, black crosses indicate mean rhodoxanthin levels, box dimensions are 25th percentile and 75th percentile, and whiskers indicate minimum and maximum values.

Phenotypic correlations between fall and winter rhodoxanthin concentrations were not significant. Winter and spring rhodoxanthin concentrations in seedlings were significantly positively correlated ($\rho = 0.50$, $p < 0.01$). Fall and spring were also significantly positively correlated ($\rho = 0.19$, $p = 0.02$), although much more weakly than the winter-spring correlation.

The univariate, natural logarithm transformed fall rhodoxanthin concentration data provided an estimated fall heritability of 0.30 ± 0.09 (Table 14).

Table 14: Variance components from the natural log transformed mixed effect model for fall rhodoxanthin concentration.

	Component	**Standard error**	**Z ratio**
Family/provenance	0.13	0.05	2.6
Residual	0.29	0.04	7.8

The bivariate model of winter and spring calculated from the variance components for the respective seasons (Table 15) found heritability for winter to be 0.42 ± 0.09 and for spring to be 0.28 ± 0.09. The Type A, genetic, correlation between winter and spring was 0.76 ± 0.14 which was also greater than the phenotypic correlation.

Table 15: Variance components from the bivariate mixed effect model for winter and spring rhodoxanthin concentration.

	Component	**Standard error**	**Z ratio**
Winter season: Family/provenance	4180.1	1397.0	5.6
Spring season: Family/provenance	3840.9	1546.9	2.5
Winter season residual	5849.5	754.1	7.8
Spring season residual	10053.3	1292.2	7.8

Plotting by BLUPs suggested variation between different family/provenance rhodoxanthin levels and demonstrated family/provenance rank changes between winter and spring (Figure 13). Family/provenances generally maintained similar rhodoxanthin BLUPs between winter and spring but rank changes in fall were more common (see Appendix J for plot of BLUPs by family/provenance and season). BLUPs in fall were minimal, with values ranging from 0.52 to 1.79 (see Appendix J for plot of fall BLUPs). In winter, the highest BLUP family/provenance was F8434 with a BLUP of 151.6 and the lowest was PROV960 with a BLUP of -99.7. In spring, F8434 remained the highest with a BLUP of 101.4 but F8430 was the lowest with a BLUP of -101.7. BLUPs are listed for each family/provenance by season in Appendix K.

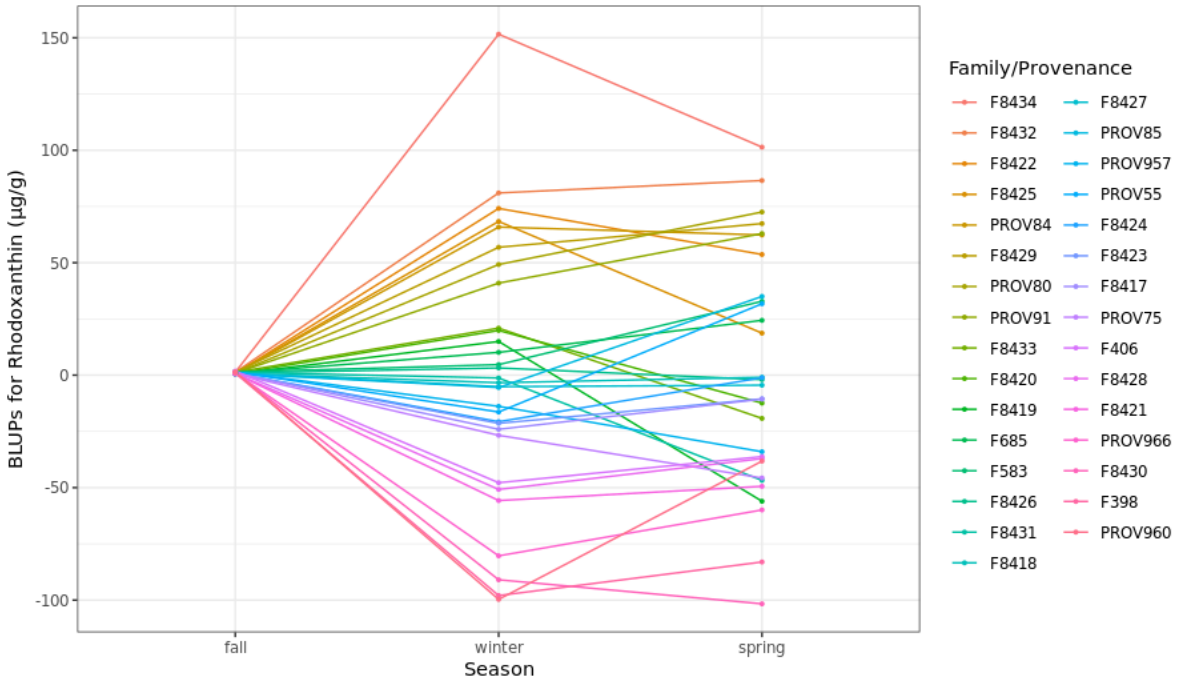

Figure 13: Best linear unbiased predictors (BLUPs) from fall univariate and winter and spring bivariate models for each family/provenance tested in 2015/16. Fall BLUPs, produced from the natural logarithmic model have been back transformed to be on the same scale as the bivariate model BLUPs. Families/provenances are coloured by winter ranking.

2017/18 and 2018/19 experiments

Unlike the one-year-old seedlings tested in 2015/16, rhodoxanthin levels of clones were below the detection limit (less than 2 µg/g) for every clone tested in all seasons. Germplasm tested in these experiments came from approximately 20-year-old clonal grafts. Other carotenoids were detected, however. For all clones tested in both years, zeaxanthin, fucoxanthin and four unknown carotenoids were detected.

Cold hardiness versus rhodoxanthin

2015/16 experiment

Eight families/provenances were individually tested for EL and rhodoxanthin in fall, winter and spring of 2015/16. Seasonal index of injury was not significantly correlated with rhodoxanthin levels (fall correlation: $p = 0.31$, winter correlation: $p = 0.25$, and spring correlation: $p = 0.67$). Appendix L lists all correlations for rhodoxanthin levels with index of injury.

2017/18 and 2018/19 experiment

Because rhodoxanthin concentration was below the detection limit (2 µg/g) for every genotype in every season tested, it was not possible to compare the rhodoxanthin concentration with the EL for clones. Chapter 2 reviews EL results in detail for these clones.

Geographic clines for rhodoxanthin

Since the heritability of rhodoxanthin concentration was highest in the winter, BLUPs from winter rhodoxanthin levels were compared against the geographic and climate variables. Of the three geographic and 23 climate variables, only longitude was significantly correlated with rhodoxanthin levels (correlation coefficient of 0.42, $p=0.02$); Table 16). Figure 15 shows a comparison of winter rhodoxanthin BLUPs with longitude.

Table 16: Correlation coefficients and p values for the 26 geographic and climate variables assessed with winter rhodoxanthin best linear unbiased predictors (BLUPs). Longitude, in red, corresponds to the significant correlation.

Variable	Correlation Coefficient	P Value
Longitude	0.42	0.02
AHM	0.19	0.31
bFFP	0.03	0.89
CMD	0.08	0.66
DD_0	0.07	0.69
DD_18	0.03	0.88
DD18	0.13	0.48
DD5	0.04	0.84
eFFP	-0.08	0.65
Elevation	0.15	0.44
EMT	-0.10	0.59
Eref	0.09	0.63
EXT	0.13	0.47
FFP	-0.05	0.77
Latitude	-0.08	0.69
MAP	-0.05	0.77
MAR	0.12	0.53
MAT	-0.02	0.91
MCMT	-0.10	0.60
MSP	0.06	0.73
MWMT	0.16	0.40
NFFD	-0.07	0.70
PAS	0.03	0.87
RH	-0.15	0.41
SHM	0.06	0.75
TD	0.17	0.36

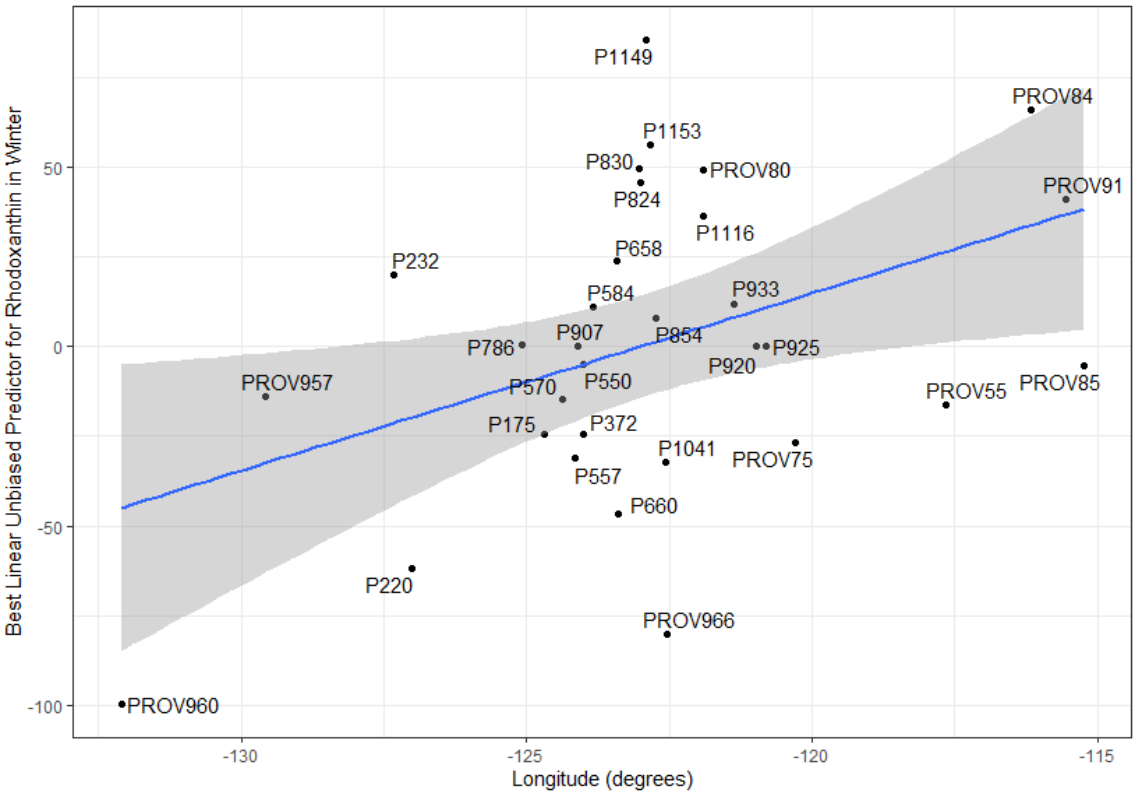

Figure 14: Best Linear Unbiased Predictors (BLUPs) for winter rhodoxanthin concentration compared to longitude of origin for the parents, provenances and seedlots tested in 2015/16 with line of best fit (blue). Grey band corresponds to the 0.95 confidence band around fit.

Discussion

Rhodoxanthin and cold hardiness

Based on the comparison of rhodoxanthin concentration with EL results, it does not appear there is evidence of phenotypic correlations of family or provenance means between rhodoxanthin levels and cold hardiness. This supported by the lack of rhodoxanthin in the clones, which still develop cold hardiness. A major limitation to this study was the small numbers of families/provenances for which there was both EL and rhodoxanthin data; however, given the absence of rhodoxanthin in the older clones as found in the 2017/18 and 2018/19 experiments, it suggests that even if there had been a stronger experimental design with more direct comparisons between rhodoxanthin and EL data, it is unlikely that there would have been a significant relationship.

Electrolyte leakage measures freeze damage occurring when cellular membranes are lysed by the formation of ice crystals, releasing their cytoplasm. Rhodoxanthin is a pigment theorized to provide photoprotection within the chloroplast. Given the different cellular structures and physiological processes associated with these two attributes, it is perhaps unsurprising that there is no evidence of a relationship between the two traits, aside from occurring concurrently in winter. Cold tolerance in redcedar has been found to be a purely temperature-driven process (Silim & Lavender, 1994). When Weger et al. (1993) tested rhodoxanthin levels in redcedar, they did so by comparing two treatments, one with normal temperatures and daylength and the other with colder temperatures and shorter daylengths. Rhodoxanthin levels increased during the colder temperature, shorter daylength treatment. Although most studies in conifers link rhodoxanthin content to low

temperatures, Ida (1981b) found rhodoxanthin was also present in summer, light stressed leaves of *C. japonica*. In plants other than conifers, Merzylak et al. (2005) found increased rhodoxanthin in high light and drought stressed *Aloe arborescens* plants in winter in the desert in Israel.

Given the lesser amount of rhodoxanthin produced in shade leaves (Ida, 1981a) and the observation of rhodoxanthin produced in warm, but sunny conditions (Ida, 1981b), this may suggest that light stress rather than colder temperature is the main driver of rhodoxanthin accumulation. Although light levels in winter are lower due to the shorter day lengths and lower solar angle, the reduction in photosynthetic capabilities (Taiz & Zeiger, 2002) creates the light stress, rather than high light, itself. Future work could expose seedlings to colder temperatures in a variety of light conditions to confirm that rhodoxanthin production is correlated with light intensity in the winter in redcedar.

A surprising result from this study was the absence of rhodoxanthin in the redcedar clones tested in 2017/18 and 2018/19. Although the foliage collected from these trees was visibly green, it was assumed that rhodoxanthin would still be present. Ida (1981a) found rhodoxanthin levels to be lower in *C. japonica* 30-year-old trees compared to 2-year-old seedlings, and that 30-year-old shade leaves had trace amounts. While several clones at CLRS were too tall to sample from the crown, many clones were short enough to acquire foliage from the crown and from the south-facing side, which was assumed to be representative of sun leaves. The absence of rhodoxanthin in these clones suggests other pigments or alternate mechanisms are being used to tolerate winter light stress in these plants.

One possible explanation for the difference in rhodoxanthin levels between seedlings and clones could be an age-effect associated with rhodoxanthin production in redcedar and *C. japonica* (Ida, 1981a). Another type of age effect has been observed in another Cupressaceae, yellow cypress (*Callitropsis nootkatensis*). Steckling rootability decreases as the donor plant ages in this species, although age effects were not observed for photosynthetic parameters or cold hardiness (Krakowski et al. 2005). Anecdotally, I have observed winter reddening is highly visible in one-year-old redcedar seedlings, moderately visible in six-year-old redcedar saplings, and not visible in older trees, although this has not been formally tested or analyzed. It would be beneficial for future work to examine rhodoxanthin concentrations in redcedar seedlings and saplings of varying ages to confirm if rhodoxanthin concentrations diminish as redcedar ages.

Season by family/provenance interaction in rhodoxanthin

Fall rhodoxanthin levels were considerably lower than levels in both winter and spring in terms of mean and variance. This suggests that the fall measurement was taken not long after the redcedar seedlings had begun to accumulate rhodoxanthin. The winter measurement appeared to represent close to the peak rhodoxanthin level for some of the families/provenances tested, although for others rhodoxanthin levels continued to increase with the spring measurement higher than winter.

This suggests, consistent with work done by others both on redcedar (Weger et al. 1993) and other gymnosperms such as *Ephedra monosperma* (Sofranova et al. 2014) that rhodoxanthin accumulation is a cyclical phenomenon, starting in fall, peaking in winter, and declining until full dissipation in spring. This study appeared to have missed the final declining stage for all of the families/provenances tested, as the lowest rhodoxanthin

concentration in spring was 174.7 µg/g (from family F8430) compared to fall measurements closer to 20 µg/g. Weger et al. (1993) noted a sharp decline in rhodoxanthin levels upon resumption of longer days and warmer temperatures. The higher amount of rhodoxanthin in spring observed in my study may be a result of sampling time. In mid March, at CLRS, the average overnight low temperature was 2.6°C and night length was just over 12 hours (Coster, A. personal comm.), which is substantially cooler and darker compared to the 15°C overnight temperatures and six hour night length of Weger et al.'s (1993) warmer treatment regime.

This cyclical cycle of rhodoxanthin can be considered as a 'wave' type pattern. The peak winter rhodoxanthin levels of some families/provenances suggest that both the wavelength and amplitude of rhodoxanthin concentration varies depending on family. One can consider each family to have an individual 'wave function' associated with its changing rhodoxanthin concentration with a unique peak and wavelength associated. It is unclear from this study if there are family/provenance differences in the starting and ending times of rhodoxanthin production and accumulation. Although winter and spring rhodoxanthin concentrations were strongly genetically correlated (Type A correlation of 0.76 ± 0.14), the spring measurement period in this study did not capture the decline or cessation of rhodoxanthin accumulation in the seedlings. If a later measurement (i.e., late-spring), when rhodoxanthin levels had decreased further, had been compared, it may not have found such a strong correlation with winter concentrations. Weger et al. (1993) found a sharp decline in rhodoxanthin concentrations after prompt exposure to warmer temperatures and longer days. In the natural environment where light and temperature increase more gradually into spring and summer, this decrease in rhodoxanthin levels

may not be so abrupt. Future work should measure rhodoxanthin concentration on seedlings more frequently (e.g. weekly) and over a longer period of time to ascertain family/provenance differences in both the amplitude and wavelength of rhodoxanthin production.

A limitation of this study was the attempt to apply linear methods to a non-linear biological process. This required separate models for fall and winter/spring and prevented the accurate estimation of genetic correlations between fall and winter rhodoxanthin production. If future studies incorporate more frequent measurements and determine individual family/provenance rhodoxanthin production time, period (wavelength) and peak concentration (amplitude), these potentially could be analyzed as two traits using a bivariate approach and genetic correlations could be estimated. Alternatively, this may represent an area of further study for quantitative genetic modelling of non-parametric traits.

Geographic and climatic clines

Longitude was the only geographic or climate variable significantly correlated with BLUPs for rhodoxanthin concentration in the winter. Longitude (in decimal degrees) was weakly positively correlated ($\rho = 0.42$; Figure 14), with provenances/parents originating further east having higher rhodoxanthin levels. This concurred with the initial observations (Figure 11) of seedling colour change, where seedlings from interior B.C. appeared darker purple than those from the coast.

Longitude and continentality (TD) are generally correlated in B.C. TD is derived from the difference between MWMT and MCMT (Wang et al. 2016) and is therefore also a measure of temperature. TD was not significantly correlated with rhodoxanthin levels.

This may indicate that temperature is not the main contributor to natural selection for rhodoxanthin production. Winters in the interior of B.C. tend to be colder with greater frequencies of clear skies and sunny days. The relationship between longitude and rhodoxanthin BLUPs may reflect these differences in light levels in the winter, with interior trees requiring greater photoprotection and producing more rhodoxanthin as a result. Further work should be done to verify this relationship.

The large standard errors for the BLUPs are due to a small number of progeny (five per family) and each parent represented in two or fewer families. The original intent of this study was to use the clonal grafts to explore geographic and climatic associations with rhodoxanthin. This was not possible due to the absence of rhodoxanthin in these clonal grafts. Future work to explore and verify these patterns should therefore consider using a more robust design comparing multiple seedlings from multiple provenances across the range rather than using a small number of controlled-cross progeny.

Other carotenoids

Although not specifically studied in this work, other carotenoids were detected in the clonal grafts tested in 2017/18 and 2018/19, particularly zeaxanthin, fucoxanthin and four unknown carotenoids. β-carotene and lutein were not detected. Antheraxanthin and violaxanthin, as part of the xanthophyll cycle, may be two of the four unknown carotenoids identified. Other possible identities for the unknown carotenoids could include the yet uncharacterized three intermediary compounds between zeaxanthin and rhodoxanthin (Royer et al. 2020).

Weger et al. (1993) detected β-carotene and lutein but not zeaxanthin in hardened redcedar. They also detected neoxanthin and α-carotene. My study did not assess

zeaxanthin in younger seedlings (2015/16), only in the clones where rhodoxanthin was not detected (2017/18 and 2018/19). As zeaxanthin is the precursor to rhodoxanthin (Hudon et al. 2007; Royer et al. 2020), perhaps the lack of zeaxanthin in the redcedar seedlings studied by Weger et al. (1993) reflects the seedlings' complete conversion of zeaxanthin to rhodoxanthin. With the clones in my study, it is possible zeaxanthin was detected because it had not been converted to rhodoxanthin. Future work examining other carotenoids in rhodoxanthin-producing redcedar seedlings would be helpful to learn more about carotenoid pools and rhodoxanthin accumulation.

Variation in the xanthophyll pool (sum of zeaxanthin, antheraxanthin and violaxanthin) or in individual proportions of xanthophylls have not been studied for different redcedar populations or clones. *Arabidopsis thaliana* (L.) Heynh. with over-expression of β-carotene hydroxylase leading to increased xanthophyll content, particularly zeaxanthin, was found to be more drought and heat stress tolerant than the wildtype (Davison et al. 2002). This may suggest that variation in xanthophyll pool size and content could contribute to stress resilience (or sensitivity) for redcedar, particularly in relation to drought and heat stress. Future studies should consider examining this observed variation in xanthophylls in redcedar and test if they are associated with enhanced drought or other stress resistance.

The presence of fucoxanthin in redcedar may be a novel finding. Fucoxanthin is an important carotenoid in the light harvesting system of brown algae (Mikami & Hosokawa, 2013) and diatoms (Gelzinis et al. 2015). The literature appears to exclusively refer to fucoxanthin within brown algae and diatoms as I could not find mention of fucoxanthin occurring in trees or higher plants. The explanation for the presence of

fucoxanthin in redcedar is unclear. Fucoxanthin is thought to be synthesized either from violaxanthin either via neoxanthin or diadinoxanthin (Mikami & Hosokawa, 2013; Wang et al. 2014). Neither neoxanthin nor diadinoxanthin were detected in this study, but they may perhaps be two of the four unknown carotenoids identified. Fucoxanthin, like many carotenoids, has antioxidant capabilities (Nomura et al. 1997) and like the xanthophylls may act to promote stress resilience against reactive oxygen species created by light stress. Future work should confirm the presence of fucoxanthin and its precursors in redcedar using both HPLC and mass spectrometry. Exploration for fucoxanthin in other members of Cupressaceae may also help elucidate its presence in other conifers. It may be that fucoxanthin and other novel carotenoids are more common in higher plants but have never been specifically examined.

Conclusions

The main objective of this study was to determine if rhodoxanthin concentration is correlated with fall, winter or spring cold hardiness in order to use it as a proxy trait for cold hardiness. Based on the results of this study, it does not appear that cold hardiness and rhodoxanthin levels are correlated, aside from both occurring concurrently in the winter. Although there were experimental limitations in this study, given the lack of relationship observed between the two traits, further investigation to examine the linkage between cold hardiness and rhodoxanthin are not expected to be fruitful. An unexpected result of this study was the absence of rhodoxanthin in foliage from older, grafted clones of redcedar. Further work testing an age gradient of redcedar seedlings to saplings and assessing rhodoxanthin levels could confirm the existence of an age-effect for rhodoxanthin and help elucidate the ages and changes associated with such an effect.

The secondary objective of this work was to investigate seasonal changes in rhodoxanthin concentration and explore any associated geographic clines. Rhodoxanthin accumulation and loss follows a cyclic pattern. It was evident from the changes in BLUPs that the peak time of rhodoxanthin concentration changes depending on family and likely each family/provenance would have a unique curve associated with its rhodoxanthin accumulation. Geographic trends suggested longitude was positively correlated with rhodoxanthin in the winter although temperature measures of continentality were not, nor were other climatic or geographic variables. Further work should incorporate a more robust set of provenances across a longitudinal transect to confirm this. As light stress has been shown to affect rhodoxanthin levels, acquiring data for these provenances related to light levels at their respective locations of origin and comparing that data to rhodoxanthin levels would be beneficial. Han and Mukai (1999) note that *C. japonica* with high levels of rhodoxanthin are favoured for reforestation in Japan, although they do not specify why. As rhodoxanthin is likely a photoprotectant, it is possible trees that produce more rhodoxanthin experience less damage to their photosystems during the winter and are able to more quickly resume photosynthesis and active growth in the spring. It may be beneficial to compare rhodoxanthin concentration with seedling growth traits to assess whether any relationships exist.

Finally, it appears there is variation in a variety of carotenoids in redcedar including unusual carotenoids (e.g., fucoxanthin). Future work could explore the variation of these carotenoids and investigate if they are associated with stress tolerance.

Chapter 4: Conclusions and Future Perspectives

This research has concluded that strong geographic or climatic clines for cold hardiness do not appear to exist in western redcedar (*Thuja plicata* Donn ex D. Don.; redcedar). As a result, from a cold hardiness perspective, there does not appear to be a need to impose hard geographic or climatic limits onto assisted gene flow for the species. Individual genotypes from south of B.C. could be incorporated into B.C. provincial seed orchards to support assisted gene flow and Climate Based Seed Transfer of the species. The major limitation of this study is that it focused entirely on cold hardiness and did not examine other traits that may impact redcedar survival, for example, damage due to snow press (Degner, 2019; unpublished) or foliar blights (Russell et al. 2007). I therefore propose that while no hard threshold be applied for cold hardiness, only trees tested in field conditions in the area of deployment be incorporated.

Several novel findings regarding cold hardiness in redcedar have occurred as a result of this research. Firstly, the positive genetic correlation between fall and spring index of injury suggest that similar genes are regulating fall and spring cold hardiness in redcedar. This is different from the weak or negative (adverse) correlations found in other species (Hannerz et al. 1999, O'Neill et al. 2001; Bower & Aitken, 2006). This suggests that unique genetic pathways support cold hardiness in redcedar (and likely other indeterminate species in Cupressaceae) compared to what has been found in determinant species in Pinaceae. While the genes responsible for cold hardiness are unknown, work in Italian Cypress (*Cupressus sempervirens* L.) has identified putative genes involved in cold hardiness for this species (Baldi et al. 2011). Future work for redcedar could identify

genes involved in cold hardiness and compare these results with Italian cypress. This will improve understanding of genetic pathways for cold tolerance in Cupressaceae and indeterminant species in general.

No effect of reciprocal cross or specific parent on cold hardiness was found. The initial larger variance associated with the male parent in the 2014/15 data was found to be an artifact of the lack of orthogonality in the crossing scheme of the 2014/15 families tested and not a true paternal effect. This is perhaps unsurprising, as with the notable exception of the *Eucalyptus* genus (Tibbits et al. 1991, He et al. 2012), cold hardiness in trees is typically an additive trait (Howe et al. 2003), thus finding a significant reciprocal or parental effect would have been very unusual.

The study did find additive genetic variation in cold hardiness in redcedar with an overall heritability of 0.17 which was lower than the heritability of spring cold hardiness of 0.38 in yellow cypress (*Callitropsis nootkatensis* (D. Don) Orsted (syn. *Chamaecyparis nootkatensis* (D. Don) Spach.) (Russell, 1993) and of 0.28 for overall cold hardiness in Patagonian cypress (*Austrocedrus chilensis* (D. Don). Pic. Ser. Et. Bizzarri) (Aparicio et al. 2012). Redcedar can be considered a generalist species and utilizes its indeterminate growth strategy to capitalize on favourable growing conditions (El-Kassaby, 1999). This may be reflected in the somewhat lower heritability observed in this study and may suggest that the larger environmental (residual) variance is a reflection of the phenotypic plasticity that El-Kassaby (1999) suggests is characteristic of redcedar.

Rhodoxanthin accumulation was not correlated with cold hardiness, and it appears that rhodoxanthin accumulation in redcedar is only produced and accumulated in seedlings

but not in older plants. Age effects in evergreen trees are not well-studied but work on yellow cypress (*Callitropsis nootkatensis* (D. Don) Orsted (syn. *Chamaecyparis nootkatensis* (D. Don) Spach.) for example found age effects in rootability of stecklings, with steckling rootability decreasing with donor plant age (Krakowski et al. 2005). Further work to determine if and how rhodoxanthin production is lost as a redcedar ages may illustrate the causes of this observed effect.

This study identified heritability and family differences in rhodoxanthin production suggesting that the other carotenoids identified may also have such variation. Genetic variation in carotenoids has not been studied before and may represent a new avenue of research into stress resilience. For example, upregulation of β-carotene in *Arabidopsis thaliana* (L.) Heynh. leading to increased xanthophylls, specifically zeaxanthin, was found to be associated with increased drought and heat stress resilience (Davison et al. 2002). Given that the heritability of rhodoxanthin was moderate (ranging between 0.27 and 0.42), it is possible that the same may be true for zeaxanthin, a precursor of rhodoxanthin. With high heritability, tree breeding can lead to trait improvement (White et al002E 2007) therefore selecting for increased zeaxanthin could possibly produce more drought resistant redcedar. This is a novel avenue of research which should be considered.

In conclusion, this study met the objective of informing assisted gene flow decisions for redcedar into B.C. seed orchards. It also has opened several new avenues of inquiry into both cold hardiness and carotenoid variation in this species. It is hoped that the results of this study will support continued, successful reforestation of redcedar across B.C.

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Appendix A: List of material tested by year for cold hardiness experiments (Chapter 2)

Table 17: List of germplasm for freeze testing by year. Note under type, seedling corresponds to family and clone corresponds to clonal graft. Under ID, codes starting with "F" correspond to seedlings/families and codes starting with "P" correspond to clones.

ID (Pedigree Code)	Type	Subtype	2014/15	2017/18	2018/19	2019/20
F392	Seedling	Reciprocal cross family				All seasons
F398	Seedling	USA, BC families	All seasons			
F399	Seedling	USA, BC families	All seasons			All seasons
		Reciprocal cross				
F401	Seedling	USA, BC families	All seasons			
F583	Seedling	USA, BC families	All seasons			
F689	Seedling	Reciprocal cross family				All seasons
F708	Seedling	Reciprocal cross family				All seasons
F863	Seedling	Reciprocal cross family				All seasons
F8182	Seedling	USA, BC families	All seasons			
F8192	Seedling	Reciprocal cross family				All seasons
F8252	Seedling	Reciprocal cross family				All seasons
F8417	Seedling	USA, BC families	All seasons		All seasons	
F8418	Seedling	USA, BC families	All seasons			
F8419	Seedling	USA, BC families	All seasons			
F8420	Seedling	USA, BC families	All seasons			
F8421	Seedling	USA, BC families	All seasons			
F8422	Seedling	USA, BC families	All seasons			
F8423	Seedling	USA, BC families	All seasons			
F8424	Seedling	USA, BC families	All seasons			
F8425	Seedling	USA, BC families	All seasons			
F8426	Seedling	USA, BC families	All seasons		All seasons	
F8427	Seedling	USA, BC families	All seasons		All seasons	
F8428	Seedling	USA, BC families	All seasons		All seasons	
F8429	Seedling	USA, BC families	All seasons		All seasons	
F8432	Seedling	USA, BC families	All seasons		All seasons	
F8434	Seedling	USA, BC families	All seasons			
F8708	Seedling	Reciprocal cross family				All seasons
F8709	Seedling	Reciprocal cross family				All seasons
F8710	Seedling	Reciprocal cross family				All seasons
F8711	Seedling	Reciprocal cross family				All seasons

F8713	Seedling	Reciprocal cross family			All seasons
F8714	Seedling	Reciprocal cross family			All seasons
F8716	Seedling	Reciprocal cross family			All seasons
F8717	Seedling	Reciprocal cross family			All seasons
F8718	Seedling	Reciprocal cross family			All seasons
P175	Clone	Clone	Fall		
P220	Clone	USA, recip parent	Fall	All seasons	All seasons
P232	Clone	Clone	Fall		
P261	Clone	USA/ Reciprocal parent	Fall	All seasons	All seasons
P415	Clone	USA parent	Fall	All seasons	
P527	Clone	Clone	Fall	All seasons	
P550	Clone	USA parent	All seasons	All seasons	
P557	Clone	USA parent	Fall	All seasons	
P566	Clone	Clone	All seasons		
P570	Clone	USA parent	All seasons	All seasons	All seasons
P584	Clone	USA parent	All seasons	All seasons	
P604	Clone	Clone	Fall	All seasons	
P658	Clone	USA parent	Fall	All seasons	
P660	Clone	Clone	Fall		
P664	Clone	Clone	Fall		
P671	Clone	Clone	All seasons		
P676	Clone	Clone	All seasons		
P716	Clone	Clone	Fall		
P721	Clone	Clone	Fall		
P728	Clone	Clone	All seasons		
P737	Clone	Clone	All seasons		
P759	Clone	Clone	Fall		
P764	Clone	Clone	Fall		
P772	Clone	Clone	Fall		
P779	Clone	Clone	Fall		
P786	Clone	USA parent	Fall	All seasons	
P824	Clone	USA parent	Fall	All seasons	
P830	Clone	USA parent	All seasons	All seasons	
P832	Clone	Clone	All seasons		
P847	Clone	Clone	Fall		
P854	Clone	USA parent	Fall	All seasons	
P907	Clone	USA parent	All seasons	All seasons	All seasons
P915	Clone	Clone	All seasons		
P920	Clone	USA parent	All seasons		
P925	Clone	USA parent	Fall		
P933	Clone	USA parent	Fall	All seasons	
P1041	Clone	Clone	All seasons		

P1101	Clone	Clone	Fall		
P1113	Clone	Clone	All seasons		
P1115	Clone	Clone	Fall		
P1116	Clone	USA parent	All seasons	All seasons	
P1124	Clone	Clone	Fall		
P1146	Clone	Clone	All seasons		
P1147	Clone	Clone	Fall		
P1149	Clone	USA parent	Fall	All seasons	
P1153	Clone	USA parent	Fall	All seasons	
P1185	Clone	Clone	Fall		
P1206	Clone	Clone	Fall		
P1368	Clone	Reciprocal parent			All seasons
P1372	Clone	Reciprocal parent	Fall		
P1409	Clone	Clone		All seasons	
P1413	Clone	Clone	Fall		
P1424	Clone	Clone	Fall		
P1434	Clone	Clone	Fall		
P1438	Clone	Clone	Fall		
P1439	Clone	Clone	Fall		
P1457	Clone	Clone	Fall		
P1458	Clone	Clone	Fall		
P1481	Clone	Clone	Fall	All seasons	
P1483	Clone	Clone		All seasons	
P1497	Clone	Clone	Fall		
P1670	Clone	Clone	Fall		
P1870	Clone	Clone		All seasons	
P1921	Clone	Clone	Fall		
P1961	Clone	Clone	Fall		
P1970	Clone	Clone	Fall		

Appendix B: U.S.A., B.C. families' pedigrees (Chapter 2)

Table 18: List of seedling families (Families) and their associated parents with parental geographic region of origin. Note under type, seedling corresponds to family and graft corresponds to clone. Under ID, codes starting with "F" correspond to seedlings/families and codes starting with "P" correspond to clonal grafts/parents.

ID (Pedigree Code)	Subtype	Female parent	Female Origin	Male parent	Male Origin
F398	USA, BC families	P220	North Vancouver Island	P220	North Vancouver Island
F399	USA, BC families	P220	North Vancouver Island	P261	Central Coast BC
F401	USA, BC families	P261	Central Coast BC	P261	Central Coast BC
F583	USA, BC families	P925	Submaritime BC	P920	Submaritime BC
F8182	USA, BC families	P261	Central Coast BC	P415	Central Coast BC
F8417	USA, BC families	P550	Northern California	P570	Southern Oregon
F8418	USA, BC families	P550	Northern California	P854	Submaritime BC
F8419	USA, BC families	P557	Northern California	P550	Northern California
F8420	USA, BC families	P557	Northern California	P830	Lower Mainland
F8421	USA, BC families	P584	Central Oregon	P557	Northern California
F8422	USA, BC families	P584	Central Oregon	P658	Northern Oregon
F8423	USA, BC families	P658	Northern Oregon	P786	East Vancouver Island
F8424	USA, BC families	P786	East Vancouver Island	P550	Northern California
F8425	USA, BC families	P786	East Vancouver Island	P824	Lower Mainland
F8426	USA, BC families	P854	Submaritime BC	P907	South Vancouver Island
F8427	USA, BC families	P854	Submaritime BC	P1153	Washington Coast
F8428	USA, BC families	P933	Submaritime BC	P557	Northern California
F8429	USA, BC families	P933	Submaritime BC	P854	Submaritime BC
F8432	USA, BC families	P1116	Washington Interior	P830	Lower Mainland
F8434	USA, BC families	P1153	Washington Coast	P1149	Washington Coast

Appendix C: Geographic origins of clones tested (Chapter 2)

Table 19: List of clones tested and their geographic region of origin, associated latitude and longitude coordinates and elevation. Under ID, codes starting with "P" correspond to clonal grafts. Latitude and longitude reported to the second when possible.

ID (Pedigree Code)	Type	Region	Latitude (DMS- N)	Longitude (DMS – W)	Elevation (m)
P175	Clone	South Vancouver Island	48° 56'	124° 42'	503
P220	Clone	North Vancouver Island	50° 31'	127° 0'	120
P232	Clone	Central Coast BC	51° 18' "	127° 20'	110
P261	Clone	Central Coast BC	52° 26'"	127° 42'	165
P415	Clone	Central Coast BC	50° 42'	126° 2'	183
P527	Clone	Interior BC	51° 45'	119° 9'	960
P550	Clone	Northern California	41° 32'	124° 0'	60
P557	Clone	Northern California	41° 50' 30"	124° 22'	20
P566	Clone	Northern California	40° 31' 48"	124° 10' 48"	50
P570	Clone	Southern Oregon	42° 16' 48"	124° 22' 12"	50
P584	Clone	Central Oregon	44° 1' 12"	123° 49' 48"	100
P604	Clone	Northern Oregon	45° 48'	123° 22'	430
P658	Clone	Northern Oregon	46° 3'	123° 25'	476
P660	Clone	Northern Oregon	45° 54'	123° 23'	300
P664	Clone	Central Oregon	45° 19'	123° 38'	369
P671	Clone	Northern Oregon	45° 46'	123° 47'	230
P676	Clone	Northern Oregon	45° 47'	123° 52'	123
P716	Clone	Northern Oregon	45° 55'	123° 11'	215
P721	Clone	Northern Oregon	45° 55'	123° 12'	338
P728	Clone	Southern Washington	46° 17'	123° 16'	123
P737	Clone	Central Oregon	45° 5'	122° 29'	246
P759	Clone	Central Washington	46° 45'	123° 45'	30
P764	Clone	Central Washington	47° 8'	123° 53'	15
P772	Clone	Northern Washington	47° 52'	124° 21'	15
P779	Clone	East Vancouver Island	49° 44'	125° 14'	950
P786	Clone	East Vancouver Island	49° 42'	125° 5'	80
P824	Clone	Lower Mainland	49° 22'	123° 0'	950
P830	Clone	Lower Mainland	49° 21'	123° 1' 12"	480
P832	Clone	Lower Mainland	49° 21'	123° 1' 12"	475
P847	Clone	Submaritime BC	50° 23'	122° 45'	920
P854	Clone	Submaritime BC	50° 23'	122° 44'	570
P907	Clone	South Vancouver Island	48° 28'	124° 6'	580

P915	Clone	Submaritime BC	49° 34' 48"	120° 58' 12"	1150
P920	Clone	Submaritime BC	49° 34' 48"	120° 58' 12"	1150
P925	Clone	Submaritime BC	49° 33' 24"	120° 48' 36"	1100
P933	Clone	Submaritime BC	49° 18'	121° 22'	700
P1041	Clone	Central Oregon	44° 24'	122° 34'	240
P1101	Clone	Central Oregon	44° 8'	123° 54'	308
P1113	Clone	Washington Interior	47° 28'	121° 55'	420
P1115	Clone	Washington Interior	47° 29'	121° 54'	420
P1116	Clone	Washington Interior	47° 3'	121° 55'	450
P1124	Clone	Washington (Cascades)	48° 2'	122° 6'	220
P1146	Clone	Washington (Cascades)	48° 17'	122° 3'	480
P1147	Clone	Washington Coast	47° 48'	122° 54'	280
P1149	Clone	Washington Coast	47° 49'	122° 55'	50
P1153	Clone	Washington Coast	47° 51'	122° 50'	200
P1185	Clone	Southern Washington	46° 21' 50"	123° 37' 26"	600
P1206	Clone	Central Washington	46° 56' 15"	123° 26' 31"	80
P1368	Clone	North Coast BC	54° 14'	129° 37'	140
P1372	Clone	North Coast BC	54° 16' 50"	130° 16' 33"	65
P1409	Clone	North Coast BC	54° 16' 50"	130° 16' 33"	65
P1413	Clone	North Coast BC	54° 14'	129° 37'	140
P1424	Clone	West Vancouver Island	49° 1'	125° 35'	50
P1434	Clone	Submaritime BC	49° 47'	123° 8'	100
P1438	Clone	North Coast BC	54° 16' 50"	130° 16' 33"	65
P1439	Clone	Interior BC	53° 17'	128° 3'	1300
P1457	Clone	Submaritime BC	49° 47'	123° 8'	100
P1458	Clone	North Coast BC	54° 0'	132° 0'	20
P1481	Clone	Interior BC	53° 17'	120° 20'	1300
P1483	Clone	Interior BC	55° 15'	128° 3'	370
P1497	Clone	Interior BC	53° 17'	120° 20'	1300
P1670	Clone	Interior BC	50° 59' 37"	117° 40' 1"	1410
P1870	Clone	Interior BC	53° 17' 43"	120° 16' 48"	1210
P1921	Clone	Interior BC	53° 54' 01"	121° 53' 34"	1005
P1961	Clone	Idaho	46° 50'	116° 10'	900
P1970	Clone	Idaho	46° 23'	115° 24' 5"	715
P2024	Clone	Interior BC	50° 37' 28"	115° 33' 4"	1300

Appendix D: Reciprocal cross families and groups (Chapter 2)

Table 20: List of seedling families and associated pedigree for reciprocal crosses. Families are listed and ordered by their assigned reciprocal group. Model coding also included.

ID (Pedigree Code)	Subtype	Female parent	Male parent	Reciprocal Group	Model Coding
F8252	Reciprocal	P10	P784	A	1
F708	Reciprocal	P784	P10	A	-1
F8192	Reciprocal	P144	P340	B	1
F392	Reciprocal	P340	P144	B	-1
F863	Reciprocal	P158	P779	C	1
F8708	Reciprocal	P779	P158	C	-1
F689	Reciprocal	P220	P261	D	1
F399	Reciprocal BC Cross	P261	P220	D	-1
F8716	Reciprocal	P331	P505	E	1
F8709	Reciprocal	P505	P331	E	-1
F8713	Reciprocal	P449	P483	F	1
F8710	Reciprocal	P483	P449	F	-1
F8714	Reciprocal	P468	P504	G	1
F8711	Reciprocal	P504	P468	G	-1
F8718	Reciprocal	P1368	P1372	H	1
F8717	Reciprocal	P1372	P1368	H	-1

Appendix E: BLUPs from overall models (Chapter 2)

Table 21: Best linear unbiased predictors (BLUPs) predicted from the overall index of injury linear mixed model. Standard errors and Z ratio scores are also reported. Families refer to seedlings and clones to grafted clones. Parent refers to the prediction of BLUPs for reciprocal parents (material not tested) based on the performance of their progeny and relationship coefficients. BLUPs are ranked from smallest (lower index of injury; more cold hardy) to largest (higher index of injury; less cold hardy).

ID (Pedigree Code)	Type	Subtype	BLUP	Standard Error	Z Ratio	BLUP Rank
F392	Family	Reciprocal cross family	15.3	3	5.1	112
F398	Family	USA, BC families	-0.2	2.6	-0.1	50
F399	Family	USA, BC families/ Reciprocal cross	-2	2.2	-0.9	38
F401	Family	USA, BC families	-7.5	2.6	-2.9	7
F583	Family	USA, BC families	-5.5	2.6	-2.1	17
F689	Family	Reciprocal cross family	-3.3	2.9	-1.1	28
F708	Family	Reciprocal cross family	1.1	3	0.4	57
F863	Family	Reciprocal cross family	6.5	3	2.2	95
F8182	Family	USA, BC families	-4.2	2.6	-1.6	22
F8192	Family	Reciprocal cross family	11.9	3	4	110
F8252	Family	Reciprocal cross family	4.2	3	1.4	82
F8417	Family	USA, BC families	9.5	2.2	4.4	106
F8418	Family	USA, BC families	-0.9	2.6	-0.3	46
F8419	Family	USA, BC families	11.4	2.6	4.3	109
F8420	Family	USA, BC families	2.6	2.6	1	73
F8421	Family	USA, BC families	7.7	2.7	2.9	99
F8422	Family	USA, BC families	1.8	2.7	0.7	65
F8423	Family	USA, BC families	-3.5	2.6	-1.3	25
F8424	Family	USA, BC families	6.7	2.6	2.5	96
F8425	Family	USA, BC families	-0.8	2.6	-0.3	48
F8426	Family	USA, BC families	-6.6	2.2	-3	10
F8427	Family	USA, BC families	1.9	2.3	0.8	68
F8428	Family	USA, BC families	5.8	2.2	2.6	93
F8429	Family	USA, BC families	-3.6	2.2	-1.6	24
F8432	Family	USA, BC families	-0.5	2.3	-0.2	49
F8434	Family	USA, BC families	1.2	2.6	0.4	58
F8708	Family	Reciprocal cross family	4.4	3	1.5	85
F8709	Family	Reciprocal cross family	4.6	3	1.5	88
F8710	Family	Reciprocal cross family	5.4	3	1.8	92

F8711	Family	Reciprocal cross family	3.8	3	1.3	77
F8713	Family	Reciprocal cross family	8.8	3	3	103
F8714	Family	Reciprocal cross family	0.9	3	0.3	56
F8716	Family	Reciprocal cross family	-4.9	3	-1.7	19
F8717	Family	Reciprocal cross family	-7.3	2.9	-2.5	8
F8718	Family	Reciprocal cross family	-7.7	2.9	-2.6	6
P175	Clone	Clone	1.3	5.1	0.2	59
P220	Clone	USA, recip parent group D	1.7	2.1	0.8	63
P232	Clone	Clone	1.6	5.8	0.3	60
P261	Clone	USA, recip parent group D	-7.9	2.1	-3.8	5
P415	Clone	USA parent	0	2.7	0	53
P527	Clone	Clone	-1.4	2.8	-0.5	41
P550	Clone	USA parent	4	2.2	1.8	79
P557	Clone	USA parent	8.1	2.6	3.1	100
P566	Clone	Clone	4	3	1.3	80
P570	Clone	USA parent	16.2	1.9	8.5	113
P584	Clone	USA parent	2.1	2.2	0.9	69
P604	Clone	Clone	5.3	2.9	1.8	91
P658	Clone	USA parent	-2.6	2.7	-1	33
P660	Clone	Clone	4.5	5.1	0.9	86
P664	Clone	Clone	9.9	5.1	1.9	107
P671	Clone	Clone	-6.1	3	-2	12
P676	Clone	Clone	-2.2	3	-0.7	35
P716	Clone	Clone	-2	5.1	-0.4	39
P721	Clone	Clone	2.4	5.8	0.4	71
P728	Clone	Clone	-5.6	3	-1.9	15
P737	Clone	Clone	-5.6	3	-1.9	16
P759	Clone	Clone	-1.9	5.1	-0.4	40
P764	Clone	Clone	2.1	5.1	0.4	70
P772	Clone	Clone	10.3	5.1	2	108
P779	Clone	Clone	6.1	4.7	1.3	94
P786	Clone	USA parent	-3.4	2.6	-1.3	27
P824	Clone	USA parent	-3	2.7	-1.1	30
P830	Clone	USA parent	-1.4	2.2	-0.6	42
P832	Clone	Clone	-5.8	3	-2	14
P847	Clone	Clone	12.6	5.1	2.5	111
P854	Clone	USA parent	-6	2.6	-2.3	13
P907	Clone	USA parent	-5.4	2	-2.8	18
P915	Clone	Clone	-3.1	3	-1	29
P920	Clone	USA parent	-3.7	2.9	-1.3	23
P925	Clone	USA parent	-6.3	3	-2.1	11
P933	Clone	USA parent	-8.8	2.7	-3.3	4

P1041	Clone	Clone	-2.2	3	-0.8	36
P1101	Clone	Clone	8.2	5.1	1.6	101
P1113	Clone	Clone	-4.4	3	-1.5	20
P1115	Clone	Clone	1.7	5.8	0.3	64
P1116	Clone	USA parent	-1.4	2.2	-0.6	43
P1124	Clone	Clone	2.9	5.8	0.5	74
P1146	Clone	Clone	-6.8	3	-2.3	9
P1147	Clone	Clone	7.2	5.1	1.4	98
P1149	Clone	USA parent	-3	2.7	-1.1	31
P1153	Clone	USA parent	8.3	2.7	3.1	102
P1185	Clone	Clone	-2.7	5.8	-0.5	32
P1206	Clone	Clone	-4.4	5.8	-0.8	21
P1368	Clone	Reciprocal parent group H	-17	2.6	-6.6	1
P1372	Clone	Reciprocal parent group H	-1.2	4.4	-0.3	45
P1409	Clone	Clone	4.3	3	1.5	84
P1413	Clone	Clone	-0.9	5.1	-0.2	47
P1424	Clone	Clone	0.8	5.1	0.2	55
P1434	Clone	Clone	-2.2	5.1	-0.4	37
P1438	Clone	Clone	3.5	5	0.7	76
P1439	Clone	Clone	4.2	5.1	0.8	83
P1457	Clone	Clone	0.6	5.1	0.1	54
P1458	Clone	Clone	3.9	5.1	0.8	78
P1481	Clone	Clone	-3.5	2.9	-1.2	26
P1483	Clone	Clone	4.5	3	1.5	87
P1497	Clone	Clone	7	5.1	1.4	97
P1670	Clone	Clone	-1.4	5.1	-0.3	44
P1870	Clone	Clone	-10.9	3	-3.6	3
P1921	Clone	Clone	2.9	5.1	0.6	75
P1961	Clone	Clone	-2.5	5.1	-0.5	34
P1970	Clone	Clone	4	5.8	0.7	81
P2024	Clone	Clone	-11	2.4	-4.6	2
P10	Parent	Parent of Recip group A	1.8	5.9	0.3	66
P144	Parent	Parent of Recip group B	9.1	5.9	1.5	104
P158	Parent	Parent of Recip group C	2.4	5.7	0.4	72
P331	Parent	Parent of Recip group E	-0.1	5.9	0	51
P340	Parent	Parent of Recip group B	9.1	5.9	1.5	105
P449	Parent	Parent of Recip group F	4.7	5.9	0.8	89
P468	Parent	Parent of Recip group G	1.6	5.9	0.3	61
P483	Parent	Parent of Recip group F	4.7	5.9	0.8	90
P504	Parent	Parent of Recip group G	1.6	5.9	0.3	62
P505	Parent	Parent of Recip group E	-0.1	5.9	0	52
P784	Parent	Parent of Recip group A	1.8	5.9	0.3	67

Appendix F: Geographic and climate variable correlations (Chapter 2)

Table 22: Annual climate variables with Pearson correlations for overall model best linear unbiased predictors (BLUPs) and associated p value. Bolded correlations are significant ($p < 0.05$).

Variable	Variable description	Variable type	Correlation	P value
AHM	Annual heat-moisture index	Temperature:Precipitation	-0.090	0.464
bFFP	Beginning of frost-free Period	Temperature	**-0.343**	0.010
CMD	Hargreaves moisture deficit	Precipitation	0.091	0.461
DD_0	Degree days below 0°C	Temperature	**-0.279**	0.018
DD_18	Degree days below 18°C	Temperature	**-0.302**	0.012
DD18	Degree days above 18°C	Temperature	0.189	0.117
DD5	Degree days above 5°C	Temperature	**0.280**	0.018
eFFP	End frost-free Period	Temperature	**0.329**	0.010
Elevation	Elevation (m)	Geographic	**-0.250**	0.033
EMT	Extreme minimum temperature, past 30 years	Temperature	**0.329**	0.010
Eref	Hargreaves reference evaporation (mm)	Temperature	0.200	0.095
EXT	Extreme maximum temperature, past 30 years	Temperature	0.073	0.556
FFP	Frost-free period	Temperature	**0.339**	0.010
Latitude	Latitude (°)	Geographic	-0.213	0.073
Longitude	Longitude (°)	Geographic	-0.227	0.054
MAP	Mean annual precipitation (mm)	Precipitation	0.073	0.556
MAR	Mean annual solar radiation	Radiation	0.125	0.314
MAT	Mean annual temperature (°C)	Temperature	**0.301**	0.012
MCMT	Mean coldest month temperature (°C)	Temperature	**0.330**	0.010
MSP	Mean summer precipitation (mm)	Precipitation	-0.038	0.763
MWMT	Mean warmest month temperature (°C)	Temperature	0.128	0.302
NFFD	Number of frost-free days	Temperature	**0.322**	0.010
PAS	Precipitation as snow (mm)	Precipitation	**-0.263**	0.024
RH	Mean annual relative humidity (%)	Temperature:Precipitation	0.128	0.302
SHM	Summer - heat moisture index	Temperature:Precipitation	0.113	0.363
TD	Continentality	Temperature	**-0.348**	0.010

Table 23: Monthly climate variables with Pearson correlations for overall model best linear unbiased predictors (BLUPs) and associated p values. Bolded correlations are significant ($p < 0.05$). Correlations in red are significant and the largest magnitude correlation for the variable group (e.g., DD_0_01 is the largest, significant correlation for degree days below 0°C).

Variable	Variable description	Month	Correlation	P value
CMD01	Hargreaves Moisture Deficit	January	NA	NA
CMD02		February	NA	NA
CMD03		March	0.087	0.478
CMD04		April	-0.121	0.331
CMD05		May	-0.019	0.880
CMD06		June	0.116	0.353
CMD07		July	0.096	0.438
CMD08		August	0.097	0.436
CMD09		September	0.160	0.187
CMD10		October	**-0.236**	0.045
CMD11		November	NA	NA
CMD12		December	NA	NA
DD_0_01	Degree days below 0°C	January	**-0.289**	0.015
DD_0_02		February	**-0.278**	0.018
DD_0_03		March	**-0.268**	0.022
DD_0_04		April	**-0.258**	0.027
DD_0_05		May	-0.185	0.127
DD_0_06		June	NA	NA
DD_0_07		July	NA	NA
DD_0_08		August	NA	NA
DD_0_09		September	-0.204	0.087
DD_0_10		October	**-0.269**	0.022
DD_0_11		November	**-0.267**	0.023
DD_0_12		December	**-0.279**	0.018
DD_18_01	Degree days below 18°C	January	**-0.331**	0.010
DD_18_02		February	**-0.324**	0.010
DD_18_03		March	**-0.306**	0.011
DD_18_04		April	**-0.279**	0.018
DD_18_05		May	-0.225	0.056
DD_18_06		June	-0.172	0.156
DD_18_07		July	-0.123	0.325
DD_18_08		August	-0.144	0.238
DD_18_09		September	**-0.271**	0.021
DD_18_10		October	**-0.315**	0.010
DD_18_11		November	**-0.324**	0.010
DD_18_12		December	**-0.326**	0.010
DD18_01	Degree days above 18°C	January	0.206	0.084
DD18_02		February	**0.311**	0.010
DD18_03		March	**0.306**	0.011
DD18_04		April	**0.290**	0.015
DD18_05		May	0.189	0.117
DD18_06		June	0.154	0.209
DD18_07		July	0.084	0.493
DD18_08		August	0.092	0.461
DD18_09		September	**0.289**	0.015
DD18_10		October	**0.351**	0.010
DD18_11		November	**0.311**	0.010
DD18_12		December	**0.268**	0.022
DD5_01	Degree days above 5°C	January	**0.362**	0.010

DD5_02	Degree days above 5°C	February	**0.345**	0.010
DD5_03		March	**0.329**	0.010
DD5_04		April	**0.279**	0.018
DD5_05		May	0.224	0.056
DD5_06		June	0.177	0.144
DD5_07		July	0.118	0.342
DD5_08		August	0.134	0.278
DD5_09		September	**0.277**	0.018
DD5_10		October	**0.328**	0.010
DD5_11		November	**0.350**	0.010
DD5_12		December	**0.363**	0.010
Eref01	Hargreaves Reference Evaporation (mm)	January	**0.329**	0.010
Eref02		February	**0.300**	0.012
Eref03		March	**0.308**	0.011
Eref04		April	0.192	0.112
Eref05		May	0.101	0.417
Eref06		June	0.051	0.687
Eref07		July	0.038	0.763
Eref08		August	0.047	0.716
Eref09		September	0.198	0.099
Eref10		October	**0.251**	0.032
Eref11		November	**0.280**	0.018
Eref12		December	**0.324**	0.010
NFFD01	Number of frost-free days	January	**0.378**	0.010
NFFD02		February	**0.340**	0.010
NFFD03		March	**0.320**	0.010
NFFD04		April	**0.269**	0.022
NFFD05		May	**0.258**	0.027
NFFD06		June	**0.259**	0.027
NFFD07		July	0.167	0.168
NFFD08		August	**0.311**	0.010
NFFD09		September	**0.258**	0.027
NFFD10		October	**0.281**	0.018
NFFD11		November	**0.315**	0.010
NFFD12		December	**0.354**	0.010
PAS01	Precipitation as Snow (mm)	January	**-0.314**	0.010
PAS02		February	**-0.258**	0.027
PAS03		March	-0.153	0.209
PAS04		April	-0.158	0.193
PAS05		May	-0.078	0.527
PAS06		June	-0.095	0.447
PAS07		July	-0.146	0.235
PAS08		August	-0.100	0.422
PAS09		September	-0.189	0.118
PAS10		October	-0.227	0.054
PAS11		November	**-0.240**	0.041
PAS12		December	**-0.290**	0.015
PPT01	Precipitation (mm)	January	0.090	0.461
PPT02		February	0.111	0.373
PPT03		March	0.170	0.161
PPT04		April	0.068	0.581
PPT05		May	0.083	0.493
PPT06		June	-0.034	0.781
PPT07		July	-0.102	0.417
PPT08		August	-0.055	0.670
PPT09		September	-0.072	0.557
PPT10		October	0.003	0.990
PPT11		November	0.102	0.417
PPT12		December	0.127	0.309
Rad01	Radiation (MJ m^{-2} d^{-1})	January	**0.303**	0.012
Rad02		February	0.172	0.155

Rad03		March	0.001	0.997
Rad04		April	-0.037	0.763
Rad05		May	0.051	0.687
Rad06		June	0.045	0.727
Rad07		July	-0.030	0.804
Rad08		August	0.005	0.984
Rad09		September	0.176	0.144
Rad10		October	0.223	0.058
Rad11		November	**0.302**	0.012
Rad12		December	**0.308**	0.011
RH01	Relative humidity (%)	January	0.181	0.135
RH02		February	0.175	0.148
RH03		March	0.179	0.139
RH04		April	0.187	0.121
RH05		May	0.166	0.170
RH06		June	0.116	0.353
RH07		July	0.065	0.600
RH08		August	0.091	0.461
RH09		September	0.000	0.999
RH10		October	-0.019	0.880
RH11		November	0.085	0.489
RH12		December	0.164	0.174
Tave01	Mean average temperature (°C)	January	**0.330**	0.010
Tave02		February	**0.323**	0.010
Tave03		March	**0.307**	0.011
Tave04		April	**0.277**	0.018
Tave05		May	0.228	0.053
Tave06		June	0.179	0.139
Tave07		July	0.115	0.357
Tave08		August	0.135	0.277
Tave09		September	**0.276**	0.019
Tave10		October	**0.320**	0.010
Tave11		November	**0.324**	0.010
Tave12		December	**0.325**	0.010
Tmax01	Mean maximum temperature (°C)	January	**0.335**	0.010
Tmax02		February	**0.321**	0.010
Tmax03		March	**0.291**	0.015
Tmax04		April	0.231	0.050
Tmax05		May	0.147	0.229
Tmax06		June	0.086	0.481
Tmax07		July	0.037	0.765
Tmax08		August	0.040	0.756
Tmax09		September	0.232	0.050
Tmax10		October	**0.297**	0.013
Tmax11		November	**0.319**	0.010
Tmax12		December	**0.333**	0.010
Tmin01	Mean minimum temperature (°C)	January	**0.322**	0.010
Tmin02		February	**0.316**	0.010
Tmin03		March	**0.309**	0.011
Tmin04		April	**0.297**	0.013
Tmin05		May	**0.277**	0.018
Tmin06		June	**0.235**	0.045
Tmin07		July	0.180	0.138
Tmin08		August	0.205	0.086
Tmin09		September	**0.266**	0.023
Tmin10		October	**0.312**	0.010
Tmin11		November	**0.319**	0.010
Tmin12		December	**0.316**	0.010

Table 24: Seasonal climate variables with Pearson correlations with overall model best linear unbiased predictors (BLUPs) and associated p values. Bolded correlations are significant ($p < 0.05$). Correlations in red are significant and the largest magnitude correlation for the variable group (e.g., DD_0_wt is the largest, significant correlation for degree days below 0°C).

Variable	Variable description	Month	Correlation	P value
CMD_at	Hargreaves Moisture Deficit	Autumn	0.129	0.302
CMD_sm		Summer	0.105	0.406
CMD_sp		Spring	-0.043	0.731
CMD_wt		Winter	NA	NA
DD_0_at	Degree days below 0°C	Autumn	**-0.268**	0.022
DD_0_sm		Summer	NA	NA
DD_0_sp		Spring	**-0.265**	0.024
DD_0_wt		Winter	**-0.283**	0.018
DD_18_at	Degree days below 18°C	Autumn	**-0.312**	0.010
DD_18_sm		Summer	-0.150	0.221
DD_18_sp		Spring	**-0.282**	0.018
DD_18_wt		Winter	**-0.327**	0.010
DD18_at	Degree days above 18°C	Autumn	**0.325**	0.010
DD18_sm		Summer	0.103	0.415
DD18_sp		Spring	**0.241**	0.041
DD18_wt		Winter	**0.375**	0.010
DD5_at	Degree days above 5°C	Autumn	**0.322**	0.010
DD5_sm		Summer	0.144	0.238
DD5_sp		Spring	**0.276**	0.019
DD5_wt		Winter	**0.358**	0.010
Eref_at	Hargreaves Reference Evaporation	Autumn	**0.242**	0.040
Eref_sm	(mm)	Summer	0.044	0.727
Eref_sp		Spring	0.228	0.053
Eref_wt		Winter	**0.315**	0.010
NFFD_at	Number of frost-free days	Autumn	**0.294**	0.014
NFFD_sm		Summer	**0.271**	0.021
NFFD_sp		Spring	**0.293**	0.014
NFFD_wt		Winter	**0.355**	0.010
PAS_at	Precipitation as Snow (mm)	Autumn	**-0.244**	0.039
PAS_sm		Summer	-0.100	0.422
PAS_sp		Spring	-0.151	0.215
PAS_wt		Winter	**-0.293**	0.014
PPT_at	Precipitation (mm)	Autumn	0.031	0.799
PPT_sm		Summer	-0.064	0.606
PPT_sp		Spring	0.121	0.330
PPT_wt		Winter	0.110	0.375
Rad_at	Radiation (MJ m-2 d-1)	Autumn	**0.239**	0.042
Rad_sm		Summer	0.000	0.999
Rad_sp		Spring	0.003	0.990
Rad_wt		Winter	**0.256**	0.028
RH_at	Relative humidity (%)	Autumn	0.027	0.824
RH_sm		Summer	0.094	0.450
RH_sp		Spring	0.181	0.135
RH_wt		Winter	0.168	0.166
Tave_at	Mean average temperature (°C)	Autumn	**0.313**	0.010
Tave_sm		Summer	0.145	0.235
Tave_sp		Spring	**0.282**	0.018
Tave_wt		Winter	**0.327**	0.010
Tmax_at	Mean maximum temperature (°C)	Autumn	**0.295**	0.014

Tmax_sm	Mean maximum temperature (°C)	Summer	0.052	0.682
Tmax_sp		Spring	**0.240**	0.041
Tmax_wt		Winter	**0.330**	0.010
Tmin_at	Mean minimum temperature (°C)	Autumn	**0.306**	0.011
Tmin_sm		Summer	0.210	0.078
Tmin_sp		Spring	**0.299**	0.013
Tmin_wt		Winter	**0.318**	0.010

Appendix G: Variance components from 2014/15 parental effects models (Chapter 2)

Table 25: Base model for 2014/15 cold hardiness data (all seasons). Random effects were season:treatment interaction and family (described by A matrix/pedigree).

Random effect	**Component**	**Standard error**	**Z ratio**
Season: treatment	584.4	292.5	2.0
Family (pedigree)	20.0	7.4	2.7
Residual	122.4	5.9	20.8

Table 26: Paternal effect model for 2014/15 cold hardiness data (all seasons). Random effects were season:treatment interaction, family (described by A matrix/pedigree) and effect of male parent (with its familial relationships included per A matrix). A likelihood ratio test was not significant compared to base model ($p = 0.16$) (Table 25).

Random effect	**Component**	**Standard error**	**Z ratio**
Season: treatment	584.4	292.5	2.0
Family (pedigree)	2.6	4.0	0.7
Male (pedigree)	19.6	9.9	2.0
Residual	122.4	5.9	20.8

Table 27: Maternal effect model for 2014/15 cold hardiness data (all seasons). Random effects were season:treatment interaction (described by A matrix/pedigree) and effect of female parent (with its familial relationships included per A matrix). The female component was too small to estimate standard errors or calculate the associated Z ratio. A likelihood ratio test was not significant when compared to base model ($p = 0.50$) (Table 25).

Random effect	**Component**	**Standard error**	**Z ratio**
Season: treatment	584.4	292.5	2.0
Family (pedigree)	20.0	7.4	2.7
Female (pedigree)	0.00001	NA	NA
Residual	122.4	5.9	20.8

Appendix H: List of clones tested 2017/18 and 2018/19 (Chapter 3)

Table 28: List of clones tested for rhodoxanthin and other carotenoids in 2017/18 and 2018/19 with their origin information. Latitude and longitude are provided to degree and minute level. Elevation is in meters.

	Testing year		Origin information			
Code	**2017/18**	**2018/19**	**Region**	**Latitude (DM – N)**	**Longitude (DM – W)**	**Elevation (m)**
P175	Fall & winter		South Vancouver Island	48° 56'	124° 42'	503
P220	Fall & winter		North Vancouver Island	50° 31'	127° 0'	120
P232	Fall & winter	All seasons	Central Coast BC	51° 18'	127° 20'	110
P261	Fall & winter		Central Coast BC	52° 26'	127° 42'	165
P415	Fall & winter	All seasons	Central Coast BC	50° 42'	126° 2'	183
P527	Fall & winter	All seasons	Interior BC	51° 45'	119° 9'	960
P550	Fall & winter	All seasons	Northern California	41° 32'	124° 0'	60
P557	Fall & winter		Northern California	41° 50'	124° 22'	20
P566	Fall & winter	All seasons	Northern California	40° 31'	124° 10'	50
P570	Fall & winter		Southern Oregon	42° 16'	124° 22'	50
P584	Fall & winter		Central Oregon	44° 1'	123° 49'	100
P604	Fall & winter	All seasons	Northern Oregon	45° 48'	123° 22'	430
P658	Fall & winter	All seasons	Northern Oregon	46° 3'	123° 25'	476
P660	Fall & winter	All seasons	Northern Oregon	45° 54'	123° 23'	300
P664	Fall & winter		Central Oregon	45° 19'	123° 38'	369
P671	Fall & winter		Northern Oregon	45° 46'	123° 47'	230
P676	Fall & winter		Northern Oregon	45° 47'	123° 52'	123
P716	Fall & winter		Northern Oregon	45° 55'	123° 11'	215
P721	Fall & winter		Northern Oregon	45° 55'	123° 12'	338
P728	Fall & winter		Southern Washington	46° 17'	123° 16'	123
P737	Fall & winter		Central Oregon	45° 5'	122° 29'	246
P759	Fall & winter		Central Washington	46° 45'	123° 45'	30
P764	Fall & winter		Central Washington	47° 8'	123° 53'	15
P772	Fall & winter		Northern Washington	47° 52'	124° 21'	15
P779	Fall & winter		East Vancouver Island	49° 44'	125° 14'	950
P786	Fall & winter		East Vancouver Island	49° 42'	125° 5'	80
P824	Fall & winter	All seasons	Lower Mainland	49° 22'	123° 0'	950
P830	Fall & winter	All seasons	Lower Mainland	49° 21'	123° 1'	480
P832	Fall & winter		Lower Mainland	49° 21'	123° 1'	475
P847	Fall & winter		Submaritime	50° 23'	122° 45'	920
P854	Fall & winter		Submaritime	50° 23'	122° 44'	570
P907	Fall & winter	All seasons	South Vancouver Island	48° 28'	124° 6'	580
P915	Fall & winter		Submaritime	49° 34'	120° 58'	1150
P920	Fall & winter		Submaritime	49° 34'	120° 58'	1150
P925	Fall & winter		Submaritime	49° 33'	120° 48'	1100
P933	Fall & winter		Submaritime	49° 18'	121° 22'	700
P1041	Fall & winter	All seasons	Central Oregon	44° 24'	122° 34'	240
P1101	Fall & winter		Central Oregon	44° 8'	123° 54'	308
P1113	Fall & winter		Washington Interior	47° 28'	121° 55'	420

P1115	Fall & winter		Washington Interior	47° 29'	121° 54'	420
P1116	Fall & winter		Washington Interior	47° 3'	121° 55'	450
P1124	Fall & winter		Washington Coast	48° 2'	122° 6'	220
P1146	Fall & winter		Washington Coast	48° 17'	122° 3'	480
P1147	Fall & winter		Washington Coast	47° 48'	122° 54'	280
P1149	Fall & winter		Washington Coast	47° 49'	122° 55'	50
P1153	Fall & winter	All seasons	Washington Coast	47° 51'	122° 50'	200
P1185	Fall & winter	All seasons	Southern Washington	46° 21'	123° 37'	600
P1206	Fall & winter		Central Washington	46° 56'	123° 26'	80
P1372	Fall & winter		North Coast BC	54° 16'	130° 16'	65
P1409	Fall & winter		North Coast BC	54° 16'	130° 16'	65
P1413		All seasons	North Coast BC	54° 14'	129° 37'	140
P1424	Fall & winter		West Vancouver Island	49° 1'	125° 35'	50
P1434	Fall & winter		Submaritime	49° 47'	123° 8'	100
P1438	Fall & winter		North Coast BC	54° 16'	130° 16'	65
P1439	Fall & winter		Interior BC	53° 17'	128° 3'	1300
P1457	Fall & winter		Submaritime	49° 47'	123° 8'	100
P1458	Fall & winter		North Coast BC	54° 0'	132° 0'	20
P1481	Fall & winter		Interior BC	53° 17'	120° 20'	1300
P1483	Fall & winter	All seasons	Interior BC	55° 15'	128° 3'	370
P1497		All seasons	Interior BC	53° 17'	120° 20'	1300
P1670	Fall & winter		Interior BC	50° 59'	117° 40'	1410
P1870	Fall & winter		Interior BC	53° 17'	120° 16'	1210
P1876		All seasons	Interior BC	53° 17'	120° 16'	1210
P1921	Fall & winter		Interior BC	53° 54'	121° 53'	1005
P1961	Fall & winter		Idaho	46° 50'	116° 10'	900
P1970	Fall & winter		Idaho	46° 23'	115° 24'	715
P2024	Fall & winter		Interior BC	50° 37'	115° 33'	1300

Appendix I: 2015/16 Rhodoxanthin raw data summary (Chapter 3)

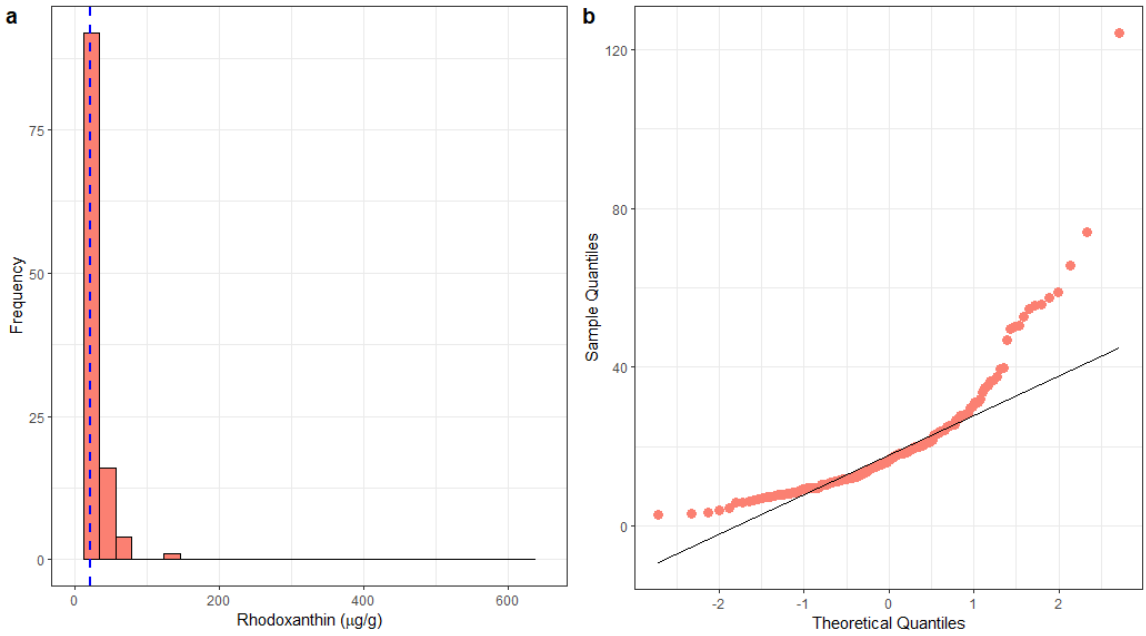

Figure 15: Histogram (plot a) and quantile-quantile plot (b) for fall 2015 rhodoxanthin concentration (µg/g). Blue dashed line on histogram equals the mean.

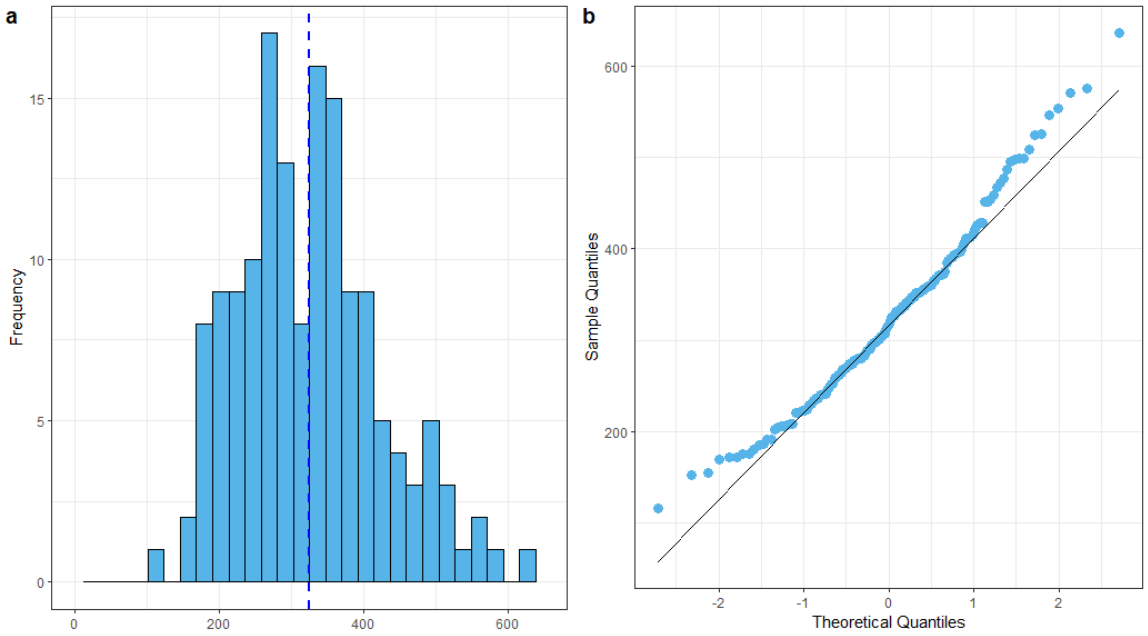

Figure 16: Histogram (plot a) and quantile-quantile plot for winter 2016 rhodoxanthin concentration (µg/g). Blue dashed line on histogram equals the mean.

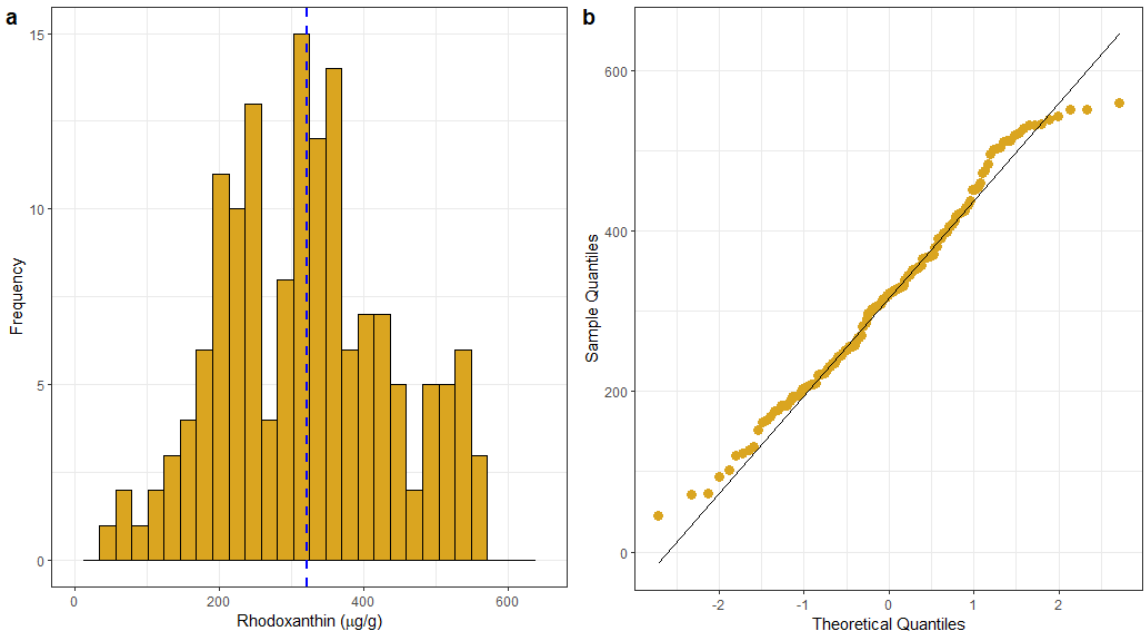

Figure 17: Histogram (plot a) and quantile-quantile plot (plot b) for spring 2016 rhodoxanthin concentration. Blue dashed line on histogram equals the mean.

Table 29: Summary statistics for rhodoxanthin (2015/16) observations by season of measurement.

Season	**Fall**	**Winter**	**Spring**
Mean (µg/g)	20.64	324.92	320.92
Median (µg/g)	16.55	318.38	321.48
Variance $(\mu g/g)^2$	252.07	9986.75	13807.96
Standard deviation (µg/g)	15.88	99.93	117.51
Skewness	2.75	0.54	0.09
Kurtosis	14.86	3.05	2.44

Appendix J: Bar plots of rhodoxanthin BLUPs (Chapter 3)

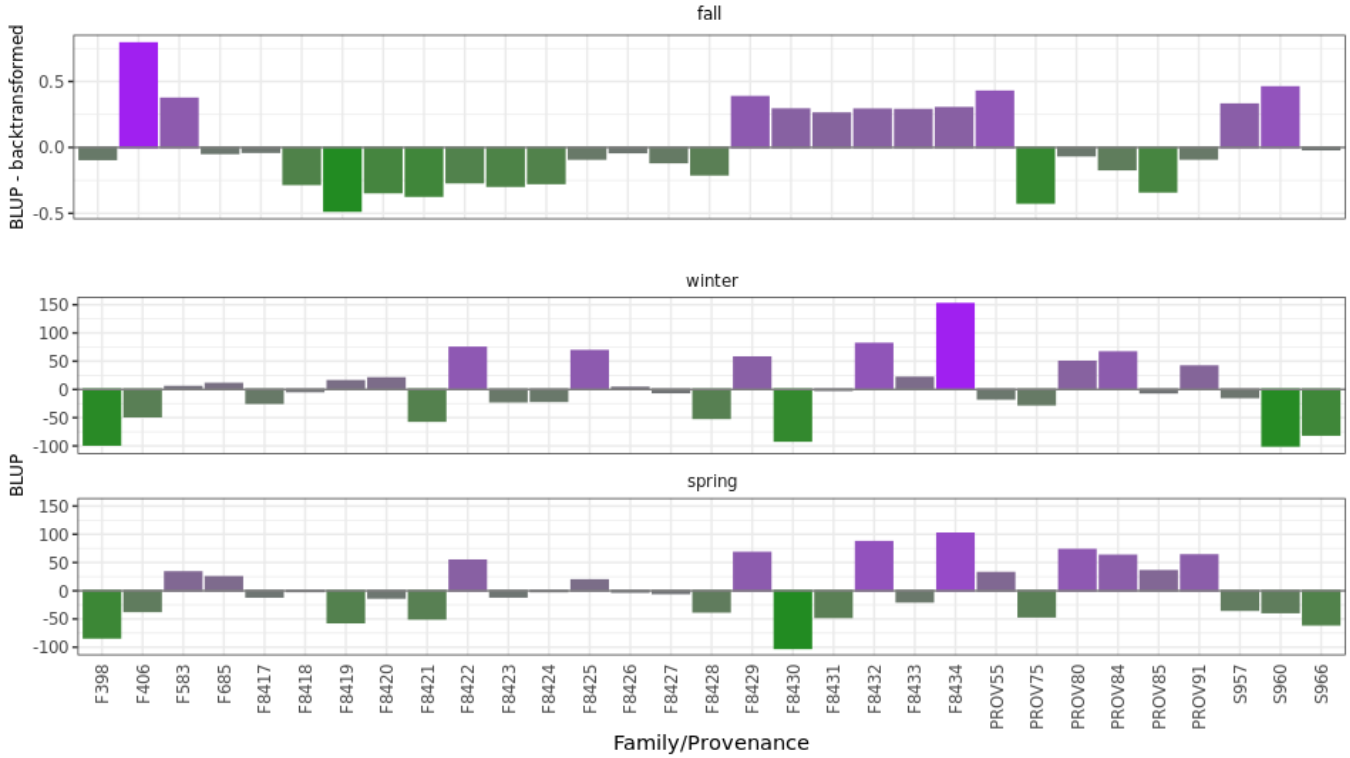

Figure 18: Bar plot of best linear unbiased predictors (BLUPs) for rhodoxanthin (µg/g) by family/provenance and season (fall, winter and spring measurements). Fall BLUPs are back-transformed from the natural logarithmic transformation applied and for purposes of visual comparison also re-centred with a mean of zero, the same as winter and spring seasons. Bar sizes between fall and winter/spring do not correspond to the same magnitude of change. Bars coloured in purple indicate increased BLUP from zero relative to other families/provenances by season, and bars in green indicate decreased BLUP from mean. More saturated shade indicates greater differences.

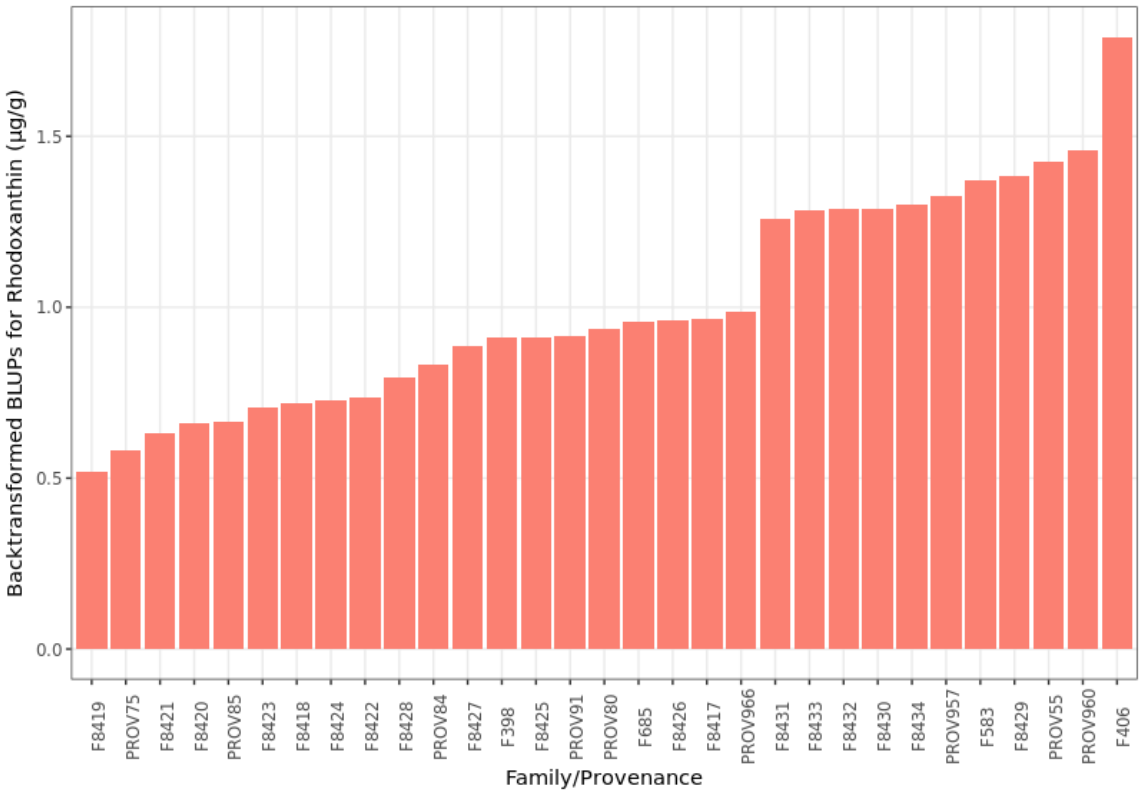

Figure 19: Best linear unbiased predictors (BLUPs) from fall univariate model for rhodoxanthin (µg/g) for each family/provenance tested in 2015/16. BLUPs have been back-transformed and are in the original scale of the data.

Appendix K: Lists of rhodoxanthin BLUPs (Chapter 3)

Table 30: Fall season rhodoxanthin back-transformed from natural logarithmic transformation best linear unbiased predictors (BLUPs) predicted from univariate fall 2015 model. Standard errors and Z ratios for each BLUP are reported. Rank (1 to 59) refers to rank within the season with lower ranks corresponding to lower rhodoxanthin BLUPs (i.e. lowest BLUP = 0.52).

Code	Type	BLUP	Standard error	Z Ratio	Rank
F398	F1	0.91	0.22	-0.09	18
F406	F1	1.79	0.21	0.58	53
F583	F1	1.37	0.21	0.32	49
F685	F1	0.96	0.21	-0.04	23
F8417	F1	0.97	0.21	-0.04	25
F8418	F1	0.72	0.21	-0.33	9
F8419	F1	0.52	0.21	-0.66	1
F8420	F1	0.66	0.21	-0.42	5
F8421	F1	0.63	0.21	-0.46	4
F8422	F1	0.73	0.21	-0.31	11
F8423	F1	0.71	0.21	-0.35	7
F8424	F1	0.73	0.21	-0.32	10
F8425	F1	0.91	0.21	-0.09	19
F8426	F1	0.96	0.21	-0.04	24
F8427	F1	0.89	0.21	-0.12	17
F8428	F1	0.79	0.21	-0.23	13
F8429	F1	1.38	0.21	0.32	50
F8430	F1	1.29	0.21	0.25	44
F8431	F1	1.26	0.21	0.23	41
F8432	F1	1.29	0.21	0.25	43
F8433	F1	1.29	0.21	0.25	42
F8434	F1	1.30	0.21	0.26	45
P175	Parent	1.34	0.33	0.29	47
P220	Parent	0.94	0.25	-0.07	21
P232	Parent	0.99	0.32	-0.01	29
P372	Parent	1.34	0.33	0.29	48
P550	Parent	0.72	0.27	-0.33	8
P557	Parent	0.59	0.27	-0.54	3
P570	Parent	1.09	0.32	0.09	33
P584	Parent	0.83	0.30	-0.19	14
P658	Parent	0.79	0.30	-0.23	12
P660	Parent	1.11	0.32	0.11	34
P786	Parent	0.83	0.28	-0.19	15
P824	Parent	1.00	0.32	0.00	30
P830	Parent	1.01	0.30	0.01	31
P854	Parent	0.97	0.27	-0.03	26
P907	Parent	0.99	0.32	-0.01	28
P920	Parent	1.17	0.33	0.16	35
P925	Parent	1.17	0.33	0.16	36
P933	Parent	1.23	0.28	0.21	40
P1041	Parent	1.21	0.29	0.19	38
P1116	Parent	1.18	0.33	0.17	37
P1149	Parent	1.22	0.30	0.20	39
P1153	Parent	1.03	0.30	0.03	32

PROV55	Provenance	1.42	0.21	0.35	51
PROV75	Provenance	0.58	0.21	-0.54	2
PROV80	Provenance	0.94	0.21	-0.06	22
PROV84	Provenance	0.83	0.21	-0.18	16
PROV85	Provenance	0.66	0.21	-0.41	6
PROV91	Provenance	0.91	0.21	-0.09	20
PROV957	Provenance	1.33	0.26	0.28	46
PROV960	Provenance	1.46	0.21	0.38	52
PROV966	Provenance	0.98	0.21	-0.02	27

Table 31: Winter rhodoxanthin best linear unbiased predictors (BLUPs) predicted from bivariate winter/spring 2016 model. Standard errors and Z ratios for each BLUP are reported. Rank (1 to 59) refers to rank within the season with lower ranks corresponding to lower rhodoxanthin BLUPs (i.e. lowest BLUP = -99.70).

Code	Type	BLUP	Standard error	Z Ratio	Rank
F398	F1	-97.98	31.72	-3.09	2
F406	F1	-47.82	31.72	-1.51	7
F583	F1	4.68	31.72	0.15	26
F685	F1	10.14	31.72	0.32	27
F8417	F1	-24.06	31.72	-0.76	12
F8418	F1	-3.36	31.72	-0.11	23
F8419	F1	14.97	31.72	0.47	28
F8420	F1	19.88	31.72	0.63	30
F8421	F1	-55.75	31.72	-1.76	5
F8422	F1	74.13	31.72	2.34	48
F8423	F1	-21.41	31.72	-0.68	13
F8424	F1	-20.68	31.72	-0.65	14
F8425	F1	68.36	31.72	2.16	46
F8426	F1	3.21	31.72	0.10	25
F8427	F1	-5.12	31.72	-0.16	22
F8428	F1	-50.81	31.72	-1.60	6
F8429	F1	56.86	31.72	1.79	43
F8430	F1	-90.98	31.72	-2.87	3
F8431	F1	-1.25	31.72	-0.04	24
F8432	F1	81.02	31.72	2.55	50
F8433	F1	20.91	31.72	0.66	31
F8434	F1	151.59	31.72	4.78	53
P175	Parent	-18.05	76.39	-0.24	15
P220	Parent	-31.36	52.33	-0.60	9
P232	Parent	53.43	74.81	0.71	41
P372	Parent	-18.05	76.39	-0.24	16
P550	Parent	31.48	58.71	0.54	35
P557	Parent	-5.78	58.80	-0.10	20
P570	Parent	-10.60	75.14	-0.14	19
P584	Parent	22.83	68.21	0.33	32
P658	Parent	50.76	68.42	0.74	40
P660	Parent	-41.36	75.43	-0.55	8
P786	Parent	36.04	63.39	0.57	37
P824	Parent	55.48	75.43	0.74	42
P830	Parent	81.03	68.66	1.18	51
P854	Parent	58.44	59.11	0.99	44
P907	Parent	19.41	75.15	0.26	29
P920	Parent	28.21	76.39	0.37	33

P925	Parent	28.21	76.39	0.37	34
P933	Parent	35.22	62.40	0.56	36
P1041	Parent	-29.64	63.58	-0.47	10
P1116	Parent	71.19	75.81	0.94	47
P1149	Parent	95.13	68.42	1.39	52
P1153	Parent	77.88	68.21	1.14	49
PROV55	Provenance	-16.38	31.72	-0.52	17
PROV75	Provenance	-26.75	31.72	-0.84	11
PROV80	Provenance	49.20	33.54	1.47	39
PROV84	Provenance	65.84	31.72	2.08	45
PROV85	Provenance	-5.48	31.72	-0.17	21
PROV91	Provenance	40.93	31.72	1.29	38
PROV957	Provenance	-13.86	41.87	-0.33	18
PROV960	Provenance	-99.70	31.72	-3.14	1
PROV966	Provenance	-80.33	31.72	-2.53	4

Table 32: Spring rhodoxanthin best linear unbiased predictors (BLUPs) predicted from bivariate winter/spring 2016 model. Standard errors and Z ratios for each BLUP are reported. Rank (1 to 59) refers to rank within the season with lower ranks corresponding to lower rhodoxanthin BLUPs (i.e. lowest BLUP = -101.67).

Code	Type	BLUP	Standard error	Z Ratio	Rank
F398	F1	-83.06	35.48	-2.34	2
F406	F1	-36.20	35.48	-1.02	11
F583	F1	32.86	35.48	0.93	36
F685	F1	24.37	35.48	0.69	34
F8417	F1	-10.62	35.48	-0.30	17
F8418	F1	-0.94	35.48	-0.03	26
F8419	F1	-56.07	35.48	-1.58	4
F8420	F1	-12.41	35.48	-0.35	16
F8421	F1	-49.42	35.48	-1.39	5
F8422	F1	53.65	35.48	1.51	43
F8423	F1	-10.54	35.48	-0.30	18
F8424	F1	-1.13	35.48	-0.03	25
F8425	F1	18.68	35.48	0.53	29
F8426	F1	-1.98	35.48	-0.06	24
F8427	F1	-4.46	35.48	-0.13	23
F8428	F1	-37.09	35.48	-1.05	10
F8429	F1	67.39	35.48	1.90	48
F8430	F1	-101.67	35.48	-2.87	1
F8431	F1	-46.76	35.48	-1.32	6
F8432	F1	86.53	35.48	2.44	52
F8433	F1	-19.23	35.48	-0.54	15
F8434	F1	101.38	35.48	2.86	53
P175	Parent	-9.34	67.01	-0.14	19
P220	Parent	-34.66	46.06	-0.75	12
P232	Parent	37.01	65.63	0.56	38
P372	Parent	-9.34	67.01	-0.14	20
P550	Parent	43.34	51.75	0.84	40
P557	Parent	-8.21	51.85	-0.16	21
P570	Parent	-4.49	65.91	-0.07	22
P584	Parent	18.27	59.91	0.30	28
P658	Parent	44.17	60.11	0.73	41
P660	Parent	-39.44	66.16	-0.60	8

P786	Parent	22.04	55.80	0.40	31
P824	Parent	40.72	66.16	0.62	39
P830	Parent	77.09	60.32	1.28	50
P854	Parent	44.18	52.15	0.85	42
P907	Parent	9.13	65.91	0.14	27
P920	Parent	23.07	67.01	0.34	32
P925	Parent	23.07	67.01	0.34	33
P933	Parent	21.28	54.88	0.39	30
P1041	Parent	-32.80	55.98	-0.59	14
P1116	Parent	62.52	66.50	0.94	46
P1149	Parent	82.37	60.11	1.37	51
P1153	Parent	58.75	59.91	0.98	44
PROV55	Provenance	28.08	30.64	0.92	35
PROV75	Provenance	-45.72	35.48	-1.29	7
PROV80	Provenance	72.53	35.63	2.04	49
PROV84	Provenance	62.33	35.48	1.76	45
PROV85	Provenance	35.01	35.48	0.99	37
PROV91	Provenance	62.96	35.48	1.77	47
PROV957	Provenance	-34.05	44.33	-0.77	13
PROV960	Provenance	-38.21	35.48	-1.08	9
PROV966	Provenance	-59.91	35.48	-1.69	3

Appendix L: Correlations between rhodoxanthin and cold hardiness (Chapter 3)

Table 33: Correlations between rhodoxanthin levels (µg/g) and cold hardiness (index of injury) using individual comparisons (n=8). Data from 2015/16 experiments.

	Fall	
Freeze treatment	**Correlation**	**P value**
-8°C	-0.59	0.16
-13°C	-0.36	0.43
-18°C	-0.38	0.39
Average all treatments	-0.45	0.31
	Winter	
-23°C	0.62	0.14
-28°C	0.51	0.24
-33°C	0.42	0.35
Average all treatments	0.51	0.25
	Spring	
-8°C	0.03	0.95
-13°C	0.10	0.83
-18°C	-0.09	0.85
Average all treatments	0.03	0.94