Cold Hardiness Screening of Grape Seedlings for the Prairies

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1.0 Introduction

The North American grape berry (*Vitis spp.*) industry is estimated to be worth greater than $160 billion annually (ARS, 2007). This high value and culturally important fruit crop has a long history of use dating back some 10,000 years (Hyams, 1965; McGovern, 2003). The species of most economic importance is currently (and historically) *Vitis vinifera* L., the European wine grape. *Vitis vinifera* was the first grape species to be domesticated, and as such, contains the quality characteristics favoured by consumers and processors. The North American continent is home to as many as 30 native grape species that also carry many novel and desirable traits (Mullins et al, 1992).

The riverbank grape, *Vitis riparia* Michx., has the largest habitat range of all the American species, growing on riverbanks south through Texas all the way north to the Riding Mountain region of Manitoba Canada (NRCS, 2008). This species and its hybrids are of most use to a northern breeding program because of its extreme tolerance to winter cold (Hemstad and Luby, 2000). The University of Minnesota and South Dakota State University grape breeding programs have exploited this cold adaptability and have incorporated the riverbank grape into higher quality cultivars with extreme cold hardiness. The two most important cultivars for the prairies resulting from this breeding work are ‘Beta’ and ‘Valiant’. Valiant is hardier than Beta and represents the highest quality grape of sufficient hardiness for the Canadian Prairie Provinces. However, in comparison to established varieties available in warmer zones, both Beta and Valiant are of sub-standard quality. More breeding is necessary on the Canadian Prairies to incorporate quality traits into new cultivars while maintaining extreme cold hardiness.

Cold hardiness is a complex interaction of many physiological pathways (Hamman et al, 1996; Wake and Fennell, 2000; Pierquet and Stushnoff, 1980) involving multiple genes (Xiao et al, 2006) so it is a trait difficult to predict for using basic genetic tools. In a breeding program generating thousands of seedlings per year, predictive tools may assist in early screening resulting in saved time and space and ultimately more efficiency. Screening for cold hardiness would be of particular interest for a northern-prairie plant breeder as winter survival is the trait of greatest initial importance in hardy perennial crops (Bors, 2008). In a grape breeding program, knowledge of a seedling's hardiness could allow for appropriate planting and training of vines in test plots. As well, tender vines could be identified and given winter protection so they can be utilized in further breeding; knowing hardiness would be most useful the first autumn after planting.

Many researches have confirmed the correlation between a plant's dormancy responses in autumn to its winter survival or hardiness (Wake and Fennell, 2000; Hamman et al., 1996; Andrews et al., 1984). The hypothesis of this study is that like other extremely hardy woody plants (including *Vitis riparia* M) interspecific grape seedlings derived from hardy stock will also initiate dormancy response early. That is, seedlings that initiate dormancy earlier will be hardier than seedlings that are actively growing in autumn. In addition, it is also assumed that the hardy parent 'Valiant' will initiate a dormancy response very similar to *Vitis riparia* as the two are nearly equal in hardiness. If a strong relationship exists between observed dormancy response and hardiness, then it may be possible to effectively predict a seedling's hardiness thus adding greater efficiency to a northern-prairie grape breeding program.

2.0 Literature Review

2.1 *Vitis riparia* M: Species Description

Vitis riparia Michx. belongs to the botanical order Vitales and the family Vitaceae. The growth habit is that of a vine with alternate leaves that vary in shape from distinctly maple leaf-like to deeply lobed (Mullins et al, 1992). Like many other vining plants and other members of the Vitaceae, *V. riparia* exhibits tendrils opposite to its leaves; the post juvenile growth phase in *Vitis spp.* allows for the development of some inflorescences in place of tendrils (Srinivasan and Mullins, 1981). Dioecious flowers are bourne on a paniculate inflorescence up to 6" long producing true-berry fruits of about 10-12mm (Mullins et al, 1992). The berries are usually blue to black (although white-fruited clones have been observed) with a heavy bloom on the skin. *Vitis riparia* occupies the largest natural range of any North American grape species, covering Texas in the extreme south, the Canadian Maritime Provinces to the east, Montana to the west and the Riding Mountain region of Manitoba in its northern extreme (NRCS, 2008; Catling and Mitrow, 2005). The wide distribution of the species shows a range of adaptability to moisture requirements (Catling and Mitrow, 2005), soil pH (Rombough, 2002), disease pressure, altitude and extreme climactic conditions (Hemstad and Luby, 2000). A testament to the riverbank grape's adaptability, are reports (after its introduction as a rootstock) of its escape and naturalization along the banks of the Rhone and Garonne rivers of France (Mullins et al, 1992).

As its Latin name contends, the Riverbank grape is a *Vitis* species that has adapted itself to the many riparian ecozones of North America. Morphologically, this species exhibits similar traits found in grape species that occupy similar riparian habitats such as *Vitis rupestris* and *Vitis acerifolia* (Catling and Mitrow, 2005). The 'riparian' species can have a trailing/ vining growth habit or an upright bush-like habit as found in *Vitis rupestris* and certain ecotypes of *V. riparia* (Rombough, 2002). The fruits of this group tend to be small on small to medium bunches with a range of flavours usually combined with high acidity (≤4% TA) (Pierquet, 1978). The small-fruited riparian grapes tend to be foraged by birds whereas forest species such as *V. aestivalis* and *V. labrusca* are preferred by mammals (Rombough, 2002). Indeed, *V. labrusca* has evolved to produce in its low acid berries certain flavour and aroma compounds that are highly attractive to mammals; these flavours/aromas are traditionally referred to as ‘foxy’ and can be explained by the grape’s unusually high production of a compound called methyl anthranilate (Moio and Etievant, 1995). Fruit of the common labrusca cultivar ‘Concord’ is widely utilized in the production of grape juice and is what the average North American consumer associates as *the* ‘grape’ flavour. The pronounced influence of too much methyl anthranilate is a negative characteristic for wine grapes as the resulting wines will be overcome with this flavour; generally vines descendent from *V. labrusca* are only minimally incorporated within a wine-grape breeding program (Reynolds et al., 1982). Conversely, the characteristic flavour found in *V. riparia* is often described as vegetal or ‘herbaceous’; the off-flavours vary greatly within this species and can easily be eliminated through breeding (Rombough, 2002). A fruit characteristic that the riparian species exhibit which is of great benefit in grape breeding is their ability to produce high sugar (≥20º Brix) berries (Hemstad and Luby, 1997). This trait is particularly important in breeding juice and wine grapes.

2.1.1 *Vitis riparia* on the Canadian Prairies

The riverbank grape is only native grape species found in western Canada east of the Rockies and is represented by indigenous populations in two of the three Canadian Prairie Provinces. In Saskatchewan, *Vitis riparia* occurs in the extreme southeast corner of the Province growing along the banks of the Souris River (Catling and Mitrow, 2005). Manitoba’s native population, which is the northern most distribution of the species, occurs in the Riding Mountain National Park region (Pierquet, 1978). These two populations represent distinct ecotypes that have adapted to Canadian plant hardiness zones 1b-3a; the Manitoba population regularly survives winter lows of >-45°C. The ability of the Canadian *V. riparia* to ripen their fruit in a short growing season and survive extreme winter lows prompted the University of Minnesota to incorporate this ecotype into their grape breeding program (Hemstad and Luby, 2000). The U of MN's newly released wine grape 'Marquette' is a descendent of Manitoba riparia #37 and ultimately derives its hardiness from this wild clone (Hemstad and Luby, 1997). The Saskatchewan ecotypes have yet to be utilized by plant breeders; they too possess extreme hardiness and possibly other novel traits worth exploiting. From an ecological perspective, Saskatchewan *V. riparia* may represent a vulnerable population due to susceptibility to phenoxy-type herbicides and loss of riparian habitat (Rombough, 2002; PFRA, 2003).

2.1.2 Hybrids with potential for Prairie Breeding

In an attempt to produce a grapevine that was well adapted to the climates of the Great Plains, fruit breeders at South Dakota State University utilized a unique *V. riparia* ecotype from the foothills of Montana (Rombough, 2002; Nixon, 2001). This population was said to display extreme drought and cold tolerance (-57ºC) and it was for this reason that Dr. Peterson chose to cross the Montana *V. riparia* (accession SD62-9-39) to 'Fredonia' instead of the more northerly Manitoba clones (which are adapted to insulating winter snow cover) (Nixon, 2001; Marshall, 1993). This resulted in the development of the popular and extremely hardy grapevine 'Valiant' (Marshall, 1993) which is the only grape sufficiently/reliably hardy and suited for cultivation on the Canadian Prairies. Valiant seems to have inherited most of the favourable traits of its *V. labrusca* parent Fredonia (except for mildew susceptibility) with none of the herbaceous flavours of it's *V. riparia* parent. Indeed, Valiant possess many favourable attributes including extreme winter hardiness which is almost equivalent to *V. riparia* itself, but unlike *V. riparia*, Valiant's fruit can attain moderate acidities (<0.8% TA) (Rombough, 2003). With a brix of up to 20° and a mild *V. labrusca* flavour, Valiant has been touted as one of the best juice grapes (Marshall, 1993; Nixon, 2001). From the perspective of a northern Prairie grape breeding program, the use of Valiant as a parent represents an opportunity to combine extreme drought and cold tolerance while maintaining acceptable acidities in progeny. Montana *V. riparia* could also be utilized directly in new crosses with higher quality parents than Fredonia in an attempt to produce Valiant-type F1's with similar acidities but none of the *V. labrusca* flavours, possibly creating a new line of hardy wine grapes suited to the Canadian Prairies. Some of the key physiological traits that all northern *V. riparia* ecotypes share are their ability to initiate early dormancy responses in preparation for winter extremes (Pierquet and Stushnoff, 1980; Wake and Fennell, 2000).

2.2 Dormancy responses in *Vitis spp.*

The ability for a woody plant to acclimate in autumn has been linked positively to its winter survival or hardiness (Wolpert and Howell, 1985). Different plant families or plant species initiate dormancy in different ways in response to environmental cues of shortened daylength and lower temperatures (Welling et al., 2004; Arora et al., 2003; Rinne et al., 1994). The dormancy responses of *Vitis riparia* have been observed in detail and involve a combination of many physiological and morphological changes within vine (Wake and Fennell, 2000).

2.2.1 Physiological changes

Hamman et al (1996) noted that the level of soluble sugars raises steadily in *Vitis spp.* tissues in response to dormancy/hardening and may serve a cryprotectant function within the plant cells. The accumulation of carbohydrates in the stem tissues of the *Vitis vinifera* L.

cultivars 'Chardonnay' and 'Riesling' were strongly associated with their cold hardiness (Hamman et al., 1996). Specific sugars belonging to the raffinose family oligosaccharides (RFO) were shown to have the most influence on tolerance to cold temperatures; the very hardy *Vitis riparia* hybrid 'Valiant' accumulates much higher amounts of RFO in its tissues than non-hardy *V. vinifera* cultivars (Hamman et al., 1996).

An important survival mechanism found in *V. riparia* is its ability to dehydrate its bud cells of water (Pierquet and Stushnoff, 1980). This adaptation in response to dormancy cues like shortening day-lengths and lower temperatures allows the buds to be exposed to extremely low winter temperatures without any loss to bud viability (Wolpert and Howell, 1985). The translocation of freezable water outside of the bud cells during dormancy makes the bud tissues much hardier than stem tissues; based on low temperature exotherm (LTE) release canes are hardy to a maximum of ~-47°C (Pierquet and Stushnoff, 1980). Shortening day length and/or lower temperatures initiates other physiological changes within *V. riparia* such as the accumulation of bark storage proteins (polypeptides), which may be related to cold acclimation (Coleman et al., 1993; Wake and Fennell, 2000).

2.2.2 Morphological changes

Aside from the translocation of carbohydrates from leaves to stems and roots (which causes the abscission of the leaves during dormancy), the grape vine also changes in appearance through cane browning/periderm development in autumn (Mullins et al., 1992). Wake and Fennell (2000) described other morphological changes to *Vitis riparia* in response to short day dormancy cues such as reduced cane elongation, lower bud numbers and abscission of terminal meristems (cane tips).

3.0 Experiment 1

3.1 Hypothesis and Objectives of Experiment 1

Within the framework of a northern plant hybridizing system, early hardiness evaluations may offer the opportunity to improve a breeding program's overall efficiency. Predicting a plant's capacity for winter survival or fitness in a northern prairie climate could impact on its placement within the growing plots (test or advanced trial) and removal of non-hardy genotypes will make room for new material the following spring. Perhaps of most importance, a predictor of hardiness in autumn could indicate which seedlings need winter protection (if they are to be utilized as parents); moderately hardy F1's may carry many desirable traits such as low acidity, firm flesh, recessive skin colours and seedlessness that may be expressed in the F2's in combination with increased hardiness.

The predictive tool used in assessing the hardiness of grapevine seedlings in this experiment was a dormancy response described by Wake and Fennell (2000). Terminal meristem abscission (tip abscission) was the chosen response because it was an easily identifiable morphological change and it is a characteristic dormancy response of *V. riparia* (Wake and Fennell, 2000); *V. riparia* is the species from which the seedlings will derive their winter hardiness from. The hypothesis is that seedlings that initiate tip abscission earliest will also be the hardiest genotypes. To be an effective tool, the dormancy response of tip abscission in the *V. riparia* x seedlings must be positively correlated to their winter hardiness; hardiness will be assessed via an in vitro tissue controlled-freeze test.

3.2 Materials and Methods:

3.2.1 Plant Material & Crosses

In this study, four groups of ten vines per group were selected to monitor their dormancy response. The four groups consisted of hybrids with the fully hardy *V. riparia* x F1 cultivar 'Valiant' serving as the emasculated female (seed) parent and other higher quality (but less hardy) cultivars serving as pollen parents. Emasculations and pollinations were carried out as described by Janick and Moore (1975). The pollen parents listed in order of hardiness (greatest to least) were an unnamed male Manitoba *V. riparia* clone, 'Kay Gray', 'Himrod', and 'Suffolk Red'. The *V. riparia* was a male Manitoba ecotype clone, Kay Gray is a hardy white bred by Elmer Swenson of Osceola Wisconsin (Swenson, 1985) and Himrod and Suffolk Red are seedless grapes bred at the Agriculture Experiment Station, Geneva New York (Reisch , 2001). Seed obtained from these various interspecific crosses made in 2006 were cold stratified for three months in damp peat at aprox 4ºC. The resulting seeds were pre-germinated in Ziplock© bags (the same bags they were stratified in) with exposure to far red light (400W high pressure sodium) which resulted in temperature fluctuation (~30°C daytime/ 20°C nighttime) that aids germination (Ellis et at.,1983).

On Dec.1/ 2006 the germinated seeds were sewn in planting cells and placed in the University of Saskatchewan poly greenhouse which operates on a 'flood floor' irrigation system. On Jan. 15/07 the seedlings were transplanted from the planting cells to 2L pots containing Sunshine #4 potting media and ~5g of slow release Osmocoat© fertilizer. The 2L potted vines were then transferred to a greenhouse bench supplemented with HPS lighting; a manual irrigation regime was employed. One to two foot bamboo stakes were used to train the young vines vertically; after aprox two months the bamboo stakes were replaced with four-foot wooden stakes. On May 15/2007 the seedlings had attained vertical growth averaging 182 cm (6') so were cut back to 91 cm at this time; periderm development was evident on the trunks and older canes in all the vines which combined with the development of tendrils is suggestive of physiological aging (Xiao, 2006). The result of pruning (and leaf removal of some diseased vines) was that ~10% of the seedling population developed fertile inflorescences. Vines that flowered were retained in the greenhouse to allow for pollen/seed collection and were not included in the field study.

On June 1/2007 the 6-month old vines were transferred to the University Horticulture shade house where they remained for two weeks to acclimate. On June 15/2007 the vines were planted at 61cm spacing in east-west oriented rows within the Horticulture field plots and hand watered for the remaining summer months. Each vine was trained to one central leader guided by a 213 cm bamboo stake on a wire trellising system.

3.2.2 Tip Abscission Data

Monitoring the progression of growth cessation and meristem abscission as described by Wake and Fennell (2000) was carried out every seven days for one month beginning Sept 1/07. Data was collected until Sept 27/07 which was the week previous to the first major (killing) frost event; a total of 5 readings were taken for each of the 40 vines studied plus seven vines of Valiant. A visual rating scale similar to that used in tracking the terminal bud set of *Malus* (Lu, 2004) was employed. Tip abscission was rated on a scale of 0-5; 0 representing total abscission while a rating of 5 indicates active growth.

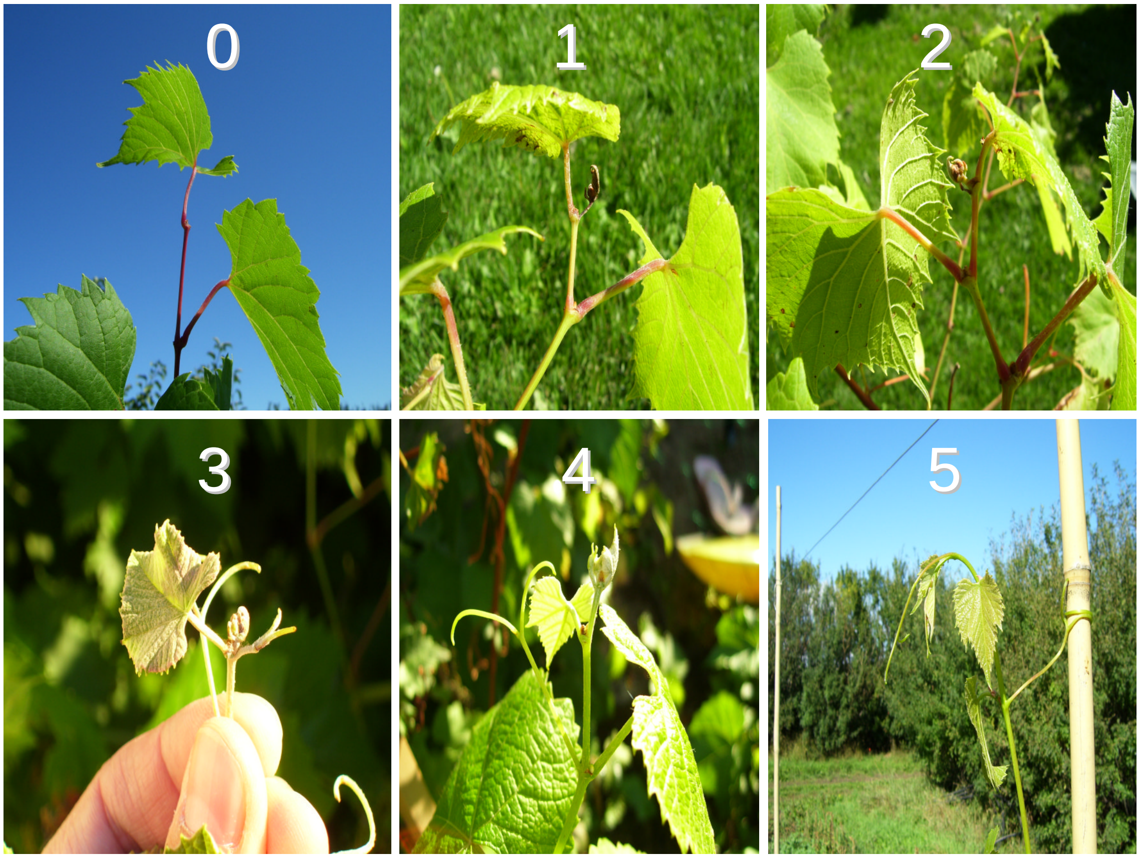

Figure 3.1 Tip abscission/growth cessation visual rating scale (0-5). 0= total tip abscission, 1= brown necrotic tip, 2= tip browning & growth cessation, 3= no tendrils extending, 4= slowed growth but tendril growth still apparent, 5= out-stretched tendrils & active growth

3.2.3 Controlled Freeze Test

December lows had already gone down to ~-30°C by the sampling date so some genotypes were already injured. The focus of this test was to observe tissue survival after prolonged exposure to low temperatures as the minimum temperature of -29.3°C observed for the Saskatoon area was only maintained for one hour on December 8, 2007 (Weatheroffice, 2008). Dormant, hardwood central leader canes of the 40 genotypes were collected on Jan 5/08. The length of the canes averaged about 46 cm and were removed two buds from the base of the cane.

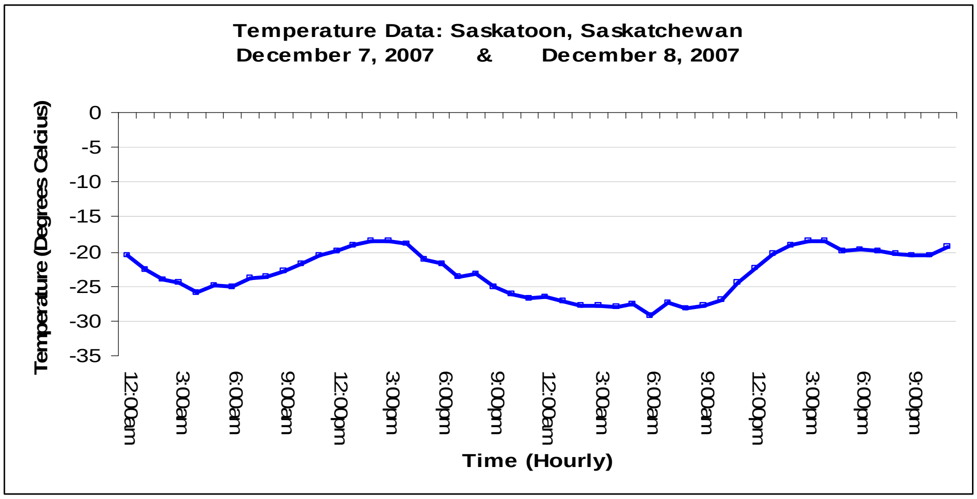

Figure 3.2 Saskatoon weather data for Dec. 2007. www.weatheroffice.gc.ca

Die-back was evident in all the canes to aprox 5 buds from the tips. Samples for the freeze test consisted of the aprox lower 10 cm of the cane samples (which did not appear to display die-back). Preliminary visual evaluation of the 10 cm samples confirmed that eleven of the forty samples had suffered winter injury and were considered 'dead' and were rated '0' in the data analysis.

The samples were cut into two equal portions and placed into a screw-cap glass test tube containing 1ml of ddH_2O. The samples were then placed into a Neslab Endocal LT-50DD refrigerated circulating bath set to -3.5°C, once this temperature was reached the samples were held there overnight (~18 hrs). Starting at 11:00am on Jan 6/08, the cooling bath was set to -8.5°C and reduced by -5°C every hour until a temperature of -30°C was reached. The samples were held at this minimum temp for 48hrs, removed and allowed to thaw at 4°C overnight. On Jan 9/08 the samples were removed from refrigeration and placed on the laboratory workbench at room temperature (~21°C) for two days. On Jan 11/08 the samples were removed from the test tubes and freeze tissue damage was evaluated. A visual rating scale was again employed.

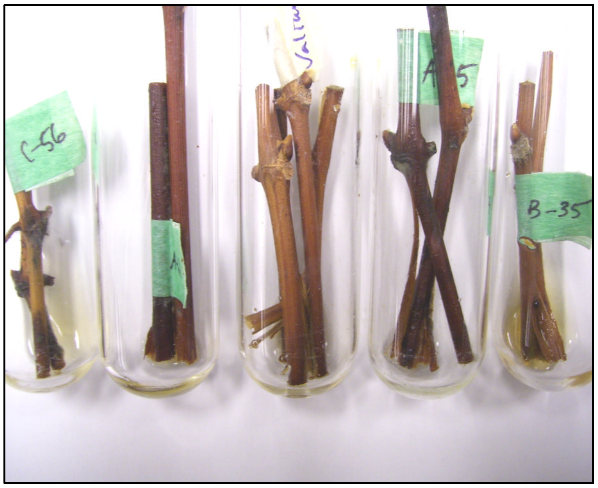

Figure 3.3 In-vitro freeze tissue samples

Figure 3.4 Live (left) and dead (right) canes

The scale is reflective of tissue colour which indicates live (green) tissues or dead (brown tissues). 1=live or green, 0=dead or brown & 0.5 represented intermediates (100%, 0% and 50% live tissue respectively).

3.2.4 Statistical analysis

Statistical analysis software (SAS Institute) was used to analyze the dormancy and hardiness data. An analysis of variance (ANOVA) was generated for each data set using the general linear model (GLM) procedure. The least square means (lsmeans) comparison procedure was used to separate the means of treatments.

4.0 Results

Within the pollen parent group, the Valiant/Kay Gray F1's displayed significantly different dormancy responses (Table 4.1) than the rest of the male parents. The interaction data between time (weeks) and dormancy suggests that the first three weeks of data collection revealed significant variation in response whereas weeks 4 & 5 appeared less significant (Table 4.2).

Table 4.1 Dormancy response (index) and winter hardiness (% live tissue) of 40 seedlings from four different crosses with 'Valiant' as the seed parent; 10 seedlings represent each population.

Variable	Pollen Parent			
	Suffolk Red	Himrod	Kay Gray	*V. riparia*
Dormancy Response	3.98 a*	4.16 a	3.56 b	3.96 a
Hardiness	2.5 c	12.5 c	12.5 c	70.0 d

* lsmean multiple comparison at P= 0.05
Different letters represent significantly different lsmean

Table 4.1 shows significant variation among pollen parents in relation to tissue survival; only the Valiant/*V. riparia* group exhibited a significant difference in tissue survival compared to the three other crosses. Regression data in relation to dormancy response & tissue injury showed no correlation.

Table 4.2 Dormancy response (index) of 40 seedlings from four different crosses with 'Valiant' as the seed parent; recorded over a 5 week period in Sept 2007 in Saskatoon, SK.

Variable	Week				
	1	2	3	4	5
Dormancy Response	4.88 a*	4.30 b	3.73 c	3.45 d	3.23 d

* lsmean multiple comparison at P= 0.05
Different letters represent significantly different lsmean

5.0 Discussion

The results of this study did not find a statistically significant correlation between the dormancy response and freeze test data. That is, the dormancy response triggering tip abscission/growth cessation in *Vitis* x seedlings does not appear to be an effective early predictor of cold hardiness in young grape seedlings. The freeze test data does however appear to correlate to the known hardiness of the pollen parents in each group of crosses. As expected, the Valiant x *V. riparia* F1 seedlings had the highest tissue survival when subjected to the prolonged -30ºC cooling bath test. F1's of the Valiant x Suffolk Red and Valiant x Himrod crosses showed the greatest tissue damage; these two crosses involved the most tender/ least adapted pollen parents. Conversely, one accession (A-71) from the Valiant x Himrod group appeared to suffer little freeze damage at the end of the trial.

Interestingly, the Valiant x Kay Gray F1's showed greater growth cessation in comparison to the Valiant x *V. riparia* F1's and were the progeny of two very hardy parents but displayed much greater tissue damage in the freeze tests (almost parallel to injury in the two previously mentioned non-hardy crosses). Both Valiant and Kay Gray are reported to be hardy to >-40 ºC (Rombough, 2002), but injury in their F1 progeny is suggestive of a much more complex interaction of genes involved in cold hardiness. Grape species are considered to be highly heterozygous (Velasco et al., 2007) and the aforementioned cross would result in interspecific seedlings drawing traits from three different *Vitis spp*.; Valiant x Kay Gray F1 seedlings would have genomes consisting of aprox. 16% *V. vinifera*, 44% *V. labrusca*, and 40% *V. riparia* (Pollefeys and Bousquet, 2003). The large percentage of genes from non-adapted *Vitis spp.* (*Vitis vinifera* and *Vitis labrusca*) may lessen the probability of a Prairie-hardy seedling emerging after recombination. The genomes of the two non-hardy crosses are composed of only 25% *V. riparia* genes whereas the Valiant/*V. riparia* F1's would theoretically carry *V. riparia* genes in 75% of their genome. Lack of hardiness in some of the Valiant/*V. riparia* F1's may be due to the inheritance of unfit traits from Valiant's non-adapted (zone 5 hardy) *V. labrusca* parent 'Fredonia'.

5.1 Observations

5.1.1 Recessive Fruit Colours

Fredonia is a black-fruited *V. labrusca* cultivar developed in Geneva, NY. Barrit and Einset (1969) hypothesized that the genotype of Fredonia (based on fruit colour) was BbRR and their work involving the two-gene hypothesis suggested dominant epistasis; black fruit colour is dominant to both red and white in grapes. Valiant is a cross between Fredonia and *V. riparia* (BbRR x BB_ _). The *V. riparia's* genotype is hard to predict because the species is predominately black-fruited but white clones have been observed, to the best of this author's knowledge no red-fruited *V. riparia* have been reported; BBRR, BBRr, BBrr are three possible genotypes. One of the results of the genetic pairing between the black-fruited Valiant and the white-fruited Kay Gray was the emergence of the red-fruited phenotype; clone accession A-11 exhibited red fruit. If this skin colour is stable and not due to environmental influences (late frost), then the genotype of Valiant could be assumed (like Fredonia) to be BbRR (or more accurately BbR_) (Barrit and Einset, 1969). A simple Punnett square dihybrid testcross confirms Valiant's possible genotype and further, the cross to Kay Gray (BbR_ x bbrr) will indeed produce red-fruited offspring; if Valiant is BbRr then white fruited genotypes should also emerge (if they do not, then Valiant would be confirmed to be BbRR). As such, the prescence of the red-fruited F1 accession A-11 would appear to confirm Barrit and Einset's genotypic assumptions about Fredonia and provide new insight into Valiant's genotype.

Valiant (Fredonia x *V. riparia*)				
BbRR x BB_ _	B_	B_	B_	B_
BR	BBR_	BBR_	BBR_	BBR_
BR	BBR_	BBR_	BBR_	BBR_
bR	BbR_	BbR_	BbR_	BbR_
bR	BbR_	BbR_	BbR_	BbR_
Valiant x Kay Gray				
BbR_x bbrr	br	br	br	br
BR	BbRr	BbRr	BbRr	BbRr
B_	Bb_r	Bb_r	Bb_r	Bb_r
bR	bbRr	bbRr	bbRr	bbRr
b_	bb_r	bb_r	bb_r	bb_r

Figure 5.1 Punnett squares predicting 'Fredonia' and 'Valiant' fruit colour genotype

Although beyond the scope of this experiment, knowing the genotype of Valiant in regards to colour may be useful in a breeding program in predicting phenotypes.

5.1.2 Water Stress in Valiant

Anecdotal reports describe an unusually high drought tolerance in the Valiant cultivar and this adaptation to water stress seems to make Valiant particularly suited to the arid climates of the Canadian Prairies (Marshall, 1993). Valiant's dehydration tolerance was put to the test in the freeze trials of this experiment. According to Tanino (2008) the conditions of the cooling bath/prolonged periods of -30°C places considerable water stress on plant cells. Valiant and some of its progeny such as accessions A-71, B-27, 32, 34, 36, and 39 displayed little tissue browning (injury) after the hardiness trial. Perhaps Valiant's extreme winter hardiness is in partly due to its ability to adapt to water stress; a plant's dehydration tolerance and winter hardiness have also been positively correlated (Pierquet and Stushnoff, 1980; Wolpert and Howell, 1985).

5.1.3 Shortened Juvenility

Unexpectedly, the materials preparation phase of this study showed that the juvenility in *Vitis* can be drastically reduced. Under normal cultural conditions *Vitis spp.* seedlings will take 2-5yrs to flower and fruit (Calonje et al., 2004). The cause of this shortened juvenility can be explained through the work of various researchers and the resulting greenhouse environment combined with timely training/pruning may have created favourable conditions for flowering. The flowering response in *Vitis* has been correlated to higher cytokinin concentrations in buds and tendrils and grape tendrils have been induced to convert into inflorescences by the application of exogenous cytokinin (Srinivasan and Mullins, 1981). Cytokinin is largely produced in root cells and correspondingly, grapevines grown under cultural conditions that supplied warmer temperatures to their root zone have shown an increase the frequency and quantity of inflorescence growth (Kliewer, 1975). Dormant hardwood cuttings of *Vitis* canes have been induced to flower and fruit (despite limited root growth) by removal of leaves basal to the developing inflorescence (which re-directs cytokinin from the leaf-sink to the flowering apparatus) (Mullins and Rajasekaran, 1981). One may propose that the shortened juvenility of the of the *Vitis* x seedlings was due to the greenhouse environment; vine roots were kept warm thus ensuring adequate supply of cytokinin (Kliewer, 1975) and, in pruning/removing leaves redirected cytokinin from leaves (sinks) to buds which are now a strong enough cytokinin sink to convert developing tendrils into inflorescences (Mullins and Rajasekaran, 1981). Adequate or optimum fertilizer/nutrition, moisture and far-red spectrum and long daylength lighting supplementation (HPS) may have also contributed to the shortened juvenility response.

Perhaps these cultural conditions could be exploited in a breeding program as a means of 'fast-tracking' or speeding up the breeding cycles between generations of grape crosses.

5.2 Lab vs. Outdoor Conditions

Wake and Fennell (2000) conducted their dormancy response observations within the confines of a greenhouse environment. Many uncontrolled factors affect the plants in a field situation like untimely precipitation, warm spells and irregular soil texture and fertility (just to name a few). Similarly, both the greenhouse experiments (Wake and Fennell, 2000) and the outdoor trials were subjected to the stimulus of shortened day lengths. Both trials confirmed that Manitoba *V. riparia* does indeed initiate dormancy responses (tip abscission/growth cessation) in response to shortening daylight hours during the fall months. Tip abscission was not observed in Valiant or its progeny in the field. One could speculate that tip abscission may eventually have been observed if not for the killing frost after Sept. 27/07; perhaps this response would have been noted under greenhouse short day conditions. Another similarity between the two studies was the observation that *V. riparia* F1's have a delayed dormancy response in comparison to the wild species. Valiant itself being a *V. riparia* cross F1 showed this delay in growth cessation compared to native *V. riparia* grown under the same cultural conditions. It could also be expected that Valiant x F1's would have an even greater delay in responsiveness.

The field dormancy/hardiness trials were under the context of a breeding program so different cultural methods were employed. The reasoning behind training the field vines to one central leader lies in the need to assess absolute hardiness. That is, under normal growing conditions (wire trellising) the vines would attain a height of at least 6'; the vines must be able to survive the drying effects of the Prairie winter air (at that height) to be considered a viable fruit crop. The drawback of this training system was that unlike the Wake and Fennell (2000) greenhouse trials, percentage of tip abscission/growth cessation based on multiple canes within the vines could not be produced.

5.3 Irregular Dormancy Responses

The seed parent 'Valiant' was not included in the statistical analysis but its dormancy response based on the visual rating scale was irregular/unexpected for a grape of its hardiness. Based on the work of previous researchers (Wake and Fennell, 2000), one would expect that Valiant's growth cessation would parallel that of *V. riparia* which is of comparable hardiness; this assumption/hypothesis appears to be unfounded as the statistical analysis of the seedlings contradicts the early-response/hardy theory. The response ratings of seven different vines of Valiant were averaged to give its dormancy data. Lack of replications in the seedlings, and having only one vertical cane to assess, may have provided limited data. However, within the scope of a breeding program producing replicates of each genotype would prove highly impractical. Within the Valiant group, varying ratings were taken from full growth cessation to very active growth by Sept. 27/07; active growth did not appear to come at the expense of hardiness in Valiant (as suggested by the freeze tests). Like Valiant, accessions A-17, B-27 and B-35 also showed later active growth but no lack of hardiness. It should be noted that when the dormancy data was viewed at the exclusion of tip abscission (rating of 0-2), the responses (growth cessation) between Valiant and *V. riparia* may parallel more closely based on date. The visual observations could then be taken in the context of growth cessation alone based on the remaining 3-5 scale. Active growth was rated visually based on the presence of tendrils; ratings of 4-5 indicated tendril growth. A visual rating of 3 indicated a lack of tendrils and it was noted that tip browning and abscission followed (ratings of 2-0) not active growth. Based on these observations, a visual rating of 3 becomes an indicator of growth cessation as tip abscission was not observed.

5.4 Conclusion

In first year field trials many genetic and environmental factors can affect the dormancy responses in grape seedlings. Anecdotal reports from other northern plant breeders describe difficulties in assessing field hardiness the first year because these responses seem to differ following the initial planting (Sawatzky, 2007). The allotted time frame for this experiment was a major shortcoming of the research; ultimately replications over multiple locations and years are necessary to evaluate a complex quantitative trait such as hardiness. This study failed to find an effective means of correlating meristem tip abscission to cold temperature tissue survival in various *Vitis x* seedlings. The lack of significant statistical correlation between growth cessation and hardiness and the absence of tip abscission all together calls into question the validity of using this morphological marker as an effective hardiness-screening tool. However, in the broader scope of a breeding program, the materials preparation stage of this experiment revealed some very useful and practical tools for a grape hybridizer, perhaps of greater practical use than the dormancy correlations could have provided alone.

The ability to dramatically shorten the juvenility of a seedling means that a breeder can also dramatically shorten the time between seedling generations. Shortening the juvenility of grapes would offer significant advantages in the areas of breeding and research. Srinivasan and Mullins (1979) suggested that the conversion of tendrils to inflorescence with exogenous cytokinin may provide a means of speeding the breeding process. However, the low practicality of this method has prevented its widespread use; four month old vines could be induced to flower but repeated cytokinin applications were required over a period of weeks (Srinivasan and Mullins, 1979). This undergraduate project has revealed that seedlings of comparable age can be induced to flower on their own roots with no tedious hormone applications. In applying the research findings of Srinivasan and Mullins (1979, 1981) and Kliewer (1975), a practical means of exploiting reduced juvenility may be possible in a grape breeding program. The efficiency of this method is being tested as some F1 grape seedlings from the 2006 crosses that germinated in Jan. 2007 flowered under greenhouse conditions produced F2 seed which are germinated and currently growing out (as of Jan. 27/08). Extraordinarily, traits currently unknown in Prairie-hardy grapevines may be incorporated through recombination, in as little as an 18-month period. By exploiting the natural physiology in *Vitis* it may be theoretically possible to produce subsequent breeding generations/crosses every 9-12 months and fast-track a certain percentage until the desired phenotypic traits are combined.

Perhaps seedlings could be started in the greenhouse with the intent of shortening juvenility. After transferred to the field, the young vines will be physiologically aged while producing vigorous vegetative growth. Temporary planting systems such as the pot-in-pot system employed by large nurseries could be used to save on field space and allow for easier winter protection of possible non-hardy genotypes. Although the dormancy response data proved inconclusive, the freeze test analysis may be used directly in hardiness trials. Cane samples for the freeze testing would be collected in the early winter months before minimum temperatures are reached. To make the experiment practical on a large scale (1,000+ canes tested), larger programmable freezers could be used in place of the refrigerator bath method. Once hardy genotypes have been identified by this method, they can removed from the field and placed in a cooler to satisfy chilling hour requirements; non-hardy genotypes types can lay down easily and be forced the following spring to screen for novel traits. The hardy seedlings could then be forced in the greenhouse mid-winter, crossed with other genotypes and later (spring) planted in the field plots in advanced trials. Second-generation pollen/seed from lower quality but hardy genotypes could be collected, then the parents along with male vines could be discarded to save space. A highly efficient selection process could be achieved by alternating the vines between greenhouse and field culture thereby fast-tracking the breeding process.

The preparation phase of this study yielded more useful tools for increasing the efficiency of a grape breeding program. The use of simple resealable Ziplock© bags for the collection of *Vitis* pollen proved to be a much more efficient method of storage and application in hybridizing than those methods described in the literature (Janick and Moore, 1975). Less expensive silica crystals were used as a drying medium which the plastic pollen-storage bags could be buried into; grape pollen proved to stay viable for one year in refrigeration under these conditions. The flexibility of the plastic bags allowed for efficient whole cluster (female or emasculated) pollination rather than tedious individual pollinations with a paint brush. It was also noted that grape seed could be germinated in the same plastic bags which they were stratifying in. Germinating the seeds under an HPS light resulted in rapid germination after only four days out of stratification. This method of stratification/germination allows for planting pre-germinated seed in flats thereby achieving maximum planting efficiency since all cells are filled with viable seedlings.

One of the major objectives for a grape breeding program on the Canadian Prairies is to incorporate many more phenotypic traits into seedlings. All current cultivars (with the exception of ‘Boughen’s White Riparia’) that are hardy to this region have blue/black fruit. The occurrence of recessive colour traits such as red or white skin would be highly marketable to northern growers. The emergence of red-fruited F1 progeny in this experiment sheds new light on the genetic background of Valiant. Breeding for recessive colour traits may now be possible in Valiant x F1’s with this new insight.

6.0 Literature Cited

Andrews, P.K., Sandidge III, C.R., Toyama, T.K. 1984. Deep supercooling of dormant and deacclimating *Vitis* buds. *Am. J. Vitic*. 35 (3): 175-177.

Arora, R., Rowland, L.J., Tanino, K. 2003. Induction and release of bud dormancy in woody perennials: a science comes of age. *Hort. Sci.* 38 (5): 911-921.

ARS, 2007. http://www.ars.usda.gov/is/video/vnr/grapes.htm . Last Modified: 11/02/2007

Barrit, B.H., Einset, J. 1969. The inheritance of three major fruit colors in grapes. *J. Amer. Soc. Hort. Sci.* 94: 87-89

Bors, 2008. Personal Communication.

Calonje, M., Cubas, P., Martinez-Zapater, J.M., Carmona, M.J. 2004. Floral meristem identity genes are expressed during tendril development in grapevine. *Plant Physiol.* 135: 1491-1501.

Catling, P.M., Mitrow, G. 2005. The dune race if *Vitis riparia* in Ontario: taxonomy, conservation and biogeography. *Can. J. Plant Sci.* 85: 407-415.

Coleman, G.D., Englert, J.M., Chen, T.H.H., Fuchigami, L.H. 1993. Physiological and environmental requirements for poplar (*Populus deltoides*) bark storage protein degradation. *Plant Physiol.* 102: 53-59.

Ellis, R.H., Hong, T.D., Roberts, E.H. 1983. A note on the development of a practical procedure for promoting the germination of dormant seed of grape (*Vitis spp.)* Vitis. 22, 211-219.

Hemstad, P.R., Luby, J.J. 2000. Utilization of *Vitis riparia* for the development of new wine varieties with resistance to disease and extreme cold. *Acta Hort*. 528: 487-90.

Hemstad, P.R., Luby, J.J. 1997. University of Minnesota grape breeding annual report 1997. University of Minn. Hort. Research C. Excelsior, MN 55331, USA.

Hamman, R.A. Jr., Dami, I.E., Walsh, T.M., Stushnoff, C. 1996. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. *Am. J. Enol. Vitic*. 47 (1): 31-36.

Hyams, E. 1965. Dionysus. (p.15) New York. The Macmillan Company

Janick, J., Moore, J.N., 1975. Advances in fruit breeding. Purdue University Press. (p. 136-137). ISBN 0-911198-36-9. West Lafayette, Indiana.

Kliewer, W.M. 1975. Effect of root temperature on budbreak, shoot growth and fruit-set of Cabernet Sauvignon grapevines. *Am. J. Enol. Vitic.* 26 (2): 82-89.

Lu, Q. 2004. Feasibility of a tip grafting system for fruit breeding and its effects on cold hardiness and juvenility. Masters Thesis. Dept. Plant Sci. UofS, Saskatoon.

Marshall, J. 1993. The Valiant grape: miracle or mistake. *Notes from the North,* MGGA. 19 (4): 1-3.

McGovern, P. E. 2003. Ancient wine. (p. 2) Princeton, NJ .Princeton University Press.

Moio, L., Etievant, P. X. (1995). Ethyl anthranilate, ethyl cinnamate, 2,3-dihydrocinnamate, and methyl anthranilate: four important odorants identified in Pinot noir wines of Burgundy. *Am. J. Enol. Vitic.* 46 (3), 392-398.

Mullins, M.G., Rajasekaran, K. 1979. Flowering in *Vitis*: conversion of tendrils into inflorescences and bunches of grapes. *Planta.* 145, 187-192.

Mullins, M.G., Rajasekaran, K. 1981. Fruiting cuttings: revised method for producing test plants of grapevine cultivars. *Am. J. Enol. Vitic.* 32 (1): 35-40.

Mullins, M. G., Bouquet, A., Williams, L. E. 1992. Biology of the grapevine. (p. 19-21, 32, 54) New York. Cambridge University Press

Nixon, L. 2001. Grape growers have their own language: foxy, earthy, Valiant. *Farm & Home Research.* 52 (3): 1-7.

NRCS, 2008. Vitis riparia species profile. Available online at: http://plants.usda.gov/java/profile?symbol=VIRI

Pierquet, P.L., Stushnoff, C. 1980. Relationship of low temperature exotherms to cold injury in *Vitis riparia* Michx. *Am. J. Enol. Vitic.* 31 (1): 1-6.

Pierquet, P.L. 1978. Fruit quality and freezing resistance mechanisms in northern clones of *Vitis riparia* Michx. Graduate Thesis. U of Minnesota.

Pollefeys, P., Bousquet, J. 2003. Molecular genetic diversity of the French-American grapevine hybrids cultivated in North America. *Genome.* 46: 1037-1048

Prairie Farm Rehabilitation Administration. 2003. Riparian areas: *an undervalued Saskatchewan resource.* Available online at: www.agr.gc.ca/pfra/land/riparea.htm Verified: April 1, 2008.

Reynolds, A.G., Fuleki, T., Evans, W.D. 1982. Inheritance of methyl anthranilate and total volatile esters in *Vitis* spp. *Am. J. Enol. Vitic.* 33 (1): 14-19

Reisch, B.I., Stewart, P. 2001.Grape Varieties Named. Available online at: http://www.nysaes.cornell.edu/hort/faculty/reisch/nyreleases.html

Rinne, P., Saarelainen, A., Olavi, J. 1994. Growth cessation and bud dormancy in relation to ABA level in seedlings and coppice shoots of *Betula pubescens* as affected by short photoperiod, water stress and chilling. *Physiologia Plantarum.* 90 (3): 451-458.

Rombough, L. 2002. The grape grower: a guide to organic viticulture. (p. 187, 192-193, 218-223) Chelsea Green. White River Junction, Vt.

Rombough. 2003. Acidity levels in Valiant. Personal communication: e-mail.

Sawatzky, 2007. Personal communication.

Srinivasan, C., Mullins, G.M. 1981. Physiology of flowering in the grapevine- a review. *Am. J. Enol. Vitic.* 32 (1): 47-63.

Swenson, E.P. 1985. Wild *Vitis riparia* from northern U.S. and Canada: breeding source for winter hardiness in cultivated grapes. *A background of the Swenson hybrids. MGGA. 1985 Annual Report. p. 5-11.*

Tanino, 2008. Personal communication.

Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D.A., Cestaro, A., et al. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE*. 2 (12): e1326: 1-18

Wake, C.M.F., Fennell, C. 2000. Morphological, physiological and dormancy responses of three *Vitis* genotypes to short photoperiod. *Physiol. Plant.* 109: 203-210.

Weatheroffice. 2008. Environment Canada Website. Available online at: www.weatheroffice.gc.ca. Last Modified: 2008-01-22.

Welling, A., Rinne, P., Vihera-Aarnoi, A., Kontunen-Soppela, S., Heino, P., Palva, T. 2004. Photoperiod and temperature differentially regulate the expression of two dehydrin genes during overwintering of birch (*Betula pubescens* Ehrh). *J. Exper. Bot.* 55 (396): 507-516.

Wolpert, J.A., Howell, G.S. 1985. Cold acclimation of Concord grapevines. I. Variation in cold hardiness within the canopy. *Am. J. Enol. Vitic.* 36 (3): 185-188.

Xiao, H., Siddiqua, M., Braybrook, S., Nassuth, A. 2006. Three grape CBF/DREB1 genes respond to low temperature, drought and abscisic acid. *Plant, Cel. Envron*. 29: 1410-21