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20100079

BREEDING OF GRAPES AND APPLES WITH EMPHASIS ON JUICE PRODUCTS AND ROOTSTOCKS

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1. Project title and ADF file number.

2010079-Breeding of grapes and apples with emphasis on juice products and rootstocks (Apple Part)

2. Name of the Principal Investigator and contact information.

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4. Abstract/ Summary*: This must include project objectives, results, and conclusions for use in publications and in the Ministry database. Maximum of 300 words in lay language.*

Apple cultivars, recent releases and selections from our breeding program were evaluated for juice quality for key traits such as juice yield, sugar content, total acidity, pH, and tannins. These were compared with a sampling of BC apples that are used for hard cider production. Within each group of apples promising selections were identified for potential as 100% apple Juice, high sugar/low acid juice for blending with more acidic fruits, and high tannin juice that might be best for quality hard cider. Of U of SK varieties, 'Prairie Rose' and Prairie Sensation' were among the best for 100% apple juice. 'Granny Annie' showed great potential as a hard cider. But several high sugar/low acid selection were discovered that would be good blended with other fruits. About 3000 seedlings were generated that could help the cider industry in the future.

Our hardy rootstock selections were subjected to simultaneous screening with 7 strains of fireblight under greenhouse conditions. Two selections were found to be highly resistant and could become very important rootstocks in the future. Several rootstocks were found to have moderate levels of resistance better than the popular O3 rootstock. An extensive rootstock trial orchard was established to ascertain dwarfing abilities of our better rootstocks which will also serve as field for PBR trials.

5. Introduction: *Brief project background and rationale.*

The goals of this project focus on the creation, and advancement, of industries associated with grapes and apples in Saskatchewan. Although there are two different crops being considered in this project, there exists some overlap which will streamline both grape and apple evaluations. Many of the variables which indicate the suitability of a grape cultivar for wine, for example, are the same as those which indicate the suitability of an apple cultivar for cider. These variables are discussed in detail in the body of this report.

The number of rootstocks and fruit cultivars capable of surviving zone 2 winters is a limiting factor for apple producers. Currently in Saskatchewan, the only rootstock known to be both sufficiently dwarfing and cold hardy is Ottawa 3. While Ottawa 3 is able to survive cold Saskatchewan winters, it known to be susceptible to fire blight (Russo 2007, Ferree 2002, Norelli 2003). In warmer climates growers are able to choose from a wide variety of dwarfing rootstocks, some of which are resistant to infection by fire blight bacteria (Russo, 2007). By screening the U of SK advanced rootstock selections for fire blight resistance, it is hoped that more options will be made available to Saskatchewan growers.

Cider production has emerged as a viable business option for growers across Canada. This is due in large part to the low prices assigned to below grade apples. In established apple growing regions, such as the Okanagan, increased penalties have been imposed on growers for shipping non-fancy grade apples to packing houses. These apples are either too large, too small, are inadequately colored, or have some other superficial blemish which makes them less desirable for fresh eating. Value added processing of these below grade apples is a way for growers to make use of, and extract value from, non-fancy grade apples. In Saskatchewan, growers face the same challenges. Blemished fruit is less desirable, and therefore difficult to sell. Cider production is seen as a viable processing option for this blemished fruit. Evaluating existing cold hardy cultivars, as well as some of the advanced selections on the U of SK Fruit plots for quality traits specific to the production of cider will provide information and options for those pursuing Cider production as a processing option.

Breeding for more cold hardy and disease resistant rootstocks, as well as for fruit characteristics suited for cider production will further the pursuit of the stated goals. These crosses will build on, and improve, the extensive collection of cold hardy germplasm currently available to the U of SK. Being the coldest active fruit breeding facility in North America, the U of SK fruit program is in a unique position to evaluate the cold hardiness of these cultivars.

6. Methodology: *Include approaches, experimental design, methodology, materials, sites, etc.*

1 Dwarfing Rootstock Breeding

The use of rootstocks was originally developed to make the propagation of desirable cultivars easier, but there have emerged many other significant advantages. Rootstocks can influence growth habits, disease resistance and final height of the apple tree. Rootstocks that limit the height of a tree are referred to as dwarfing rootstocks. Dwarfing rootstocks have become a key factor in improving the efficiency of apple production by commercial producers. Dwarf trees are easier to prune, pick and spray. They can also be planted at much higher densities than full size trees, increasing the yield per acre of land.

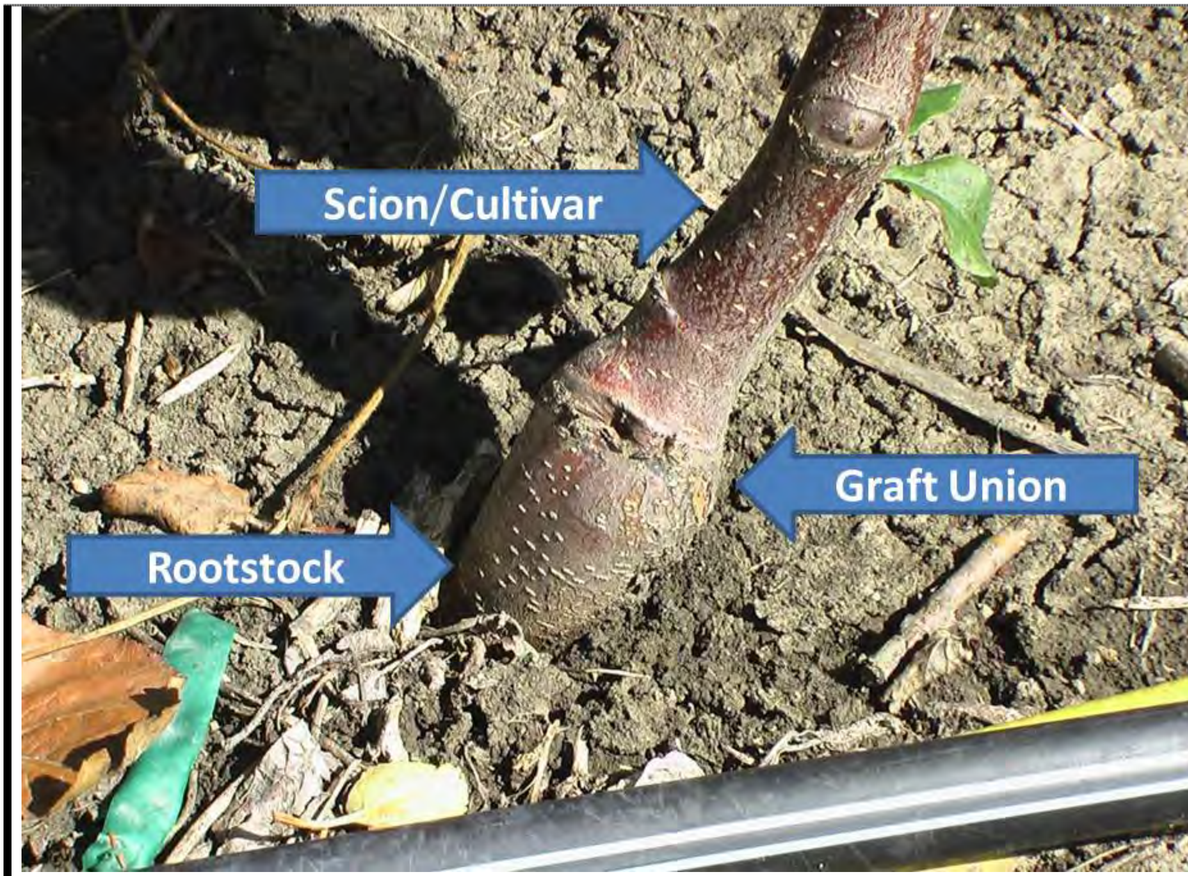


Figure 1. A grafted apple tree. The scion/cultivar, the fruiting portion of the tree, is grafted to the rootstock. The rootstock can affect tree characteristics like vigor, disease resistance, hardiness, and yield.

Currently in Saskatchewan Siberian crabapple seedlings are still used as rootstock. While these seedling rootstocks have proven to be hardy and relatively resistant to disease, they produce less desirable/less efficient, full sized trees. While growers in warmer growing regions can choose between dozens of different dwarfing rootstocks, the only dwarfing rootstock recommended for use in zone 2 is Ottawa 3. However, Ottawa 3 has been known to have problems with hardiness, especially when young and has also been shown to be susceptible to fire blight.

The University of Saskatchewan fruit breeding program has been breeding apple rootstocks in an effort to create more options for zone 2 apple growers. Over the last decade, over 300 seedlings from this rootstock breeding program have been grown in the field. Several have been selected for further propagation and evaluation.

With the funding provided by the ADF the U of SK has been able to bear more resources to the evaluation of these dwarfing rootstocks. To speed up propagation and allow for more screening and experimentation, stool beds of these rootstocks have been established. Rootstocks have then been vegetatively propagated, creating dozens of genetically identical examples of each rootstock cultivar. These rootstocks have then been subjected to tests to measure characteristics like yield, height and fire blight resistance.

1.1 Yield Efficiency

Yield efficiency is determined using a mathematical formula which expresses the yield of fruit as a function of the cross sectional area of the trunk.

$$Yield\ Efficiency = \frac{4M}{\pi d^2}$$

Where: M is the combined mass of fruit harvested from the tree
d is the diameter of the tree at 30cm above the graft union

1.2 Fire blight Screening

Fire blight is caused by infection with the *Erwinia amylovora* bacteria. It is named for the appearance of branches killed by the infection which look blackened as if scorched by fire. It is a potentially serious disease which can kill entire orchards.

Varying levels of natural resistance can be observed in grafted apple cultivars and rootstocks. A susceptible scion grafted to a resistant rootstock demonstrates more resistance than when grafted to a susceptible rootstock. Establishing resistant rootstock cultivars is therefore very important.

The resistance of a rootstock to the *Erwinia amylovora* bacteria is demonstrated through screening methods involving the inoculation of the tree with bacteria. There are a number of variables which affect the susceptibility of a cultivar to the bacteria. These include; environmental conditions(field or greenhouse), age of the tree when inoculated(seedling, 2nd true leaf, muli-year wood, full sized), part of the tree exposed to inoculant(leaf, branch, trunk, flower), method of inoculation(scissors, hypodermic needle, clamps, sprayer or mister), concentration of inoculant(cfu/ml, or colony forming units/ml), and strain of bacteria. In order to get an accurate representation of the resistance of our rootstocks, each of these variables must be carefully considered.

1.2.1 Inoculant

Bacteria are isolated from infected plant tissue using a Crosse and Goodman (Crosse et al, 1973) or CCT (Ishimaru and Klos 1984) selective media and streaked on nutrient agar sucrose(NAS) plates. After approximately 2 days of growth on NAS plates, isolates are added to sterile distilled water in equal portions and the optical density at 600nm is measured. The optical density is correlated to colony forming units per mL(CFU/mL) and the mixture is adjusted until a solution containing $1x10^9$ CFU/ml is achieved. The same procedure has been employed for Erwinia amylovora isolates obtained from Antonet Svircev of Agriculture and Agrifood Canada. Isolates obtained from Antonet Svircev's lab include EA 110, EA G-5, EA 17-1-1, EA 29 -7, EA 6-4 and EA D-7

Figure 2. Strains of Erwinia amylovora used in the inoculant. Long term cryogenic storage is used to maintain the virulence of these cultures.

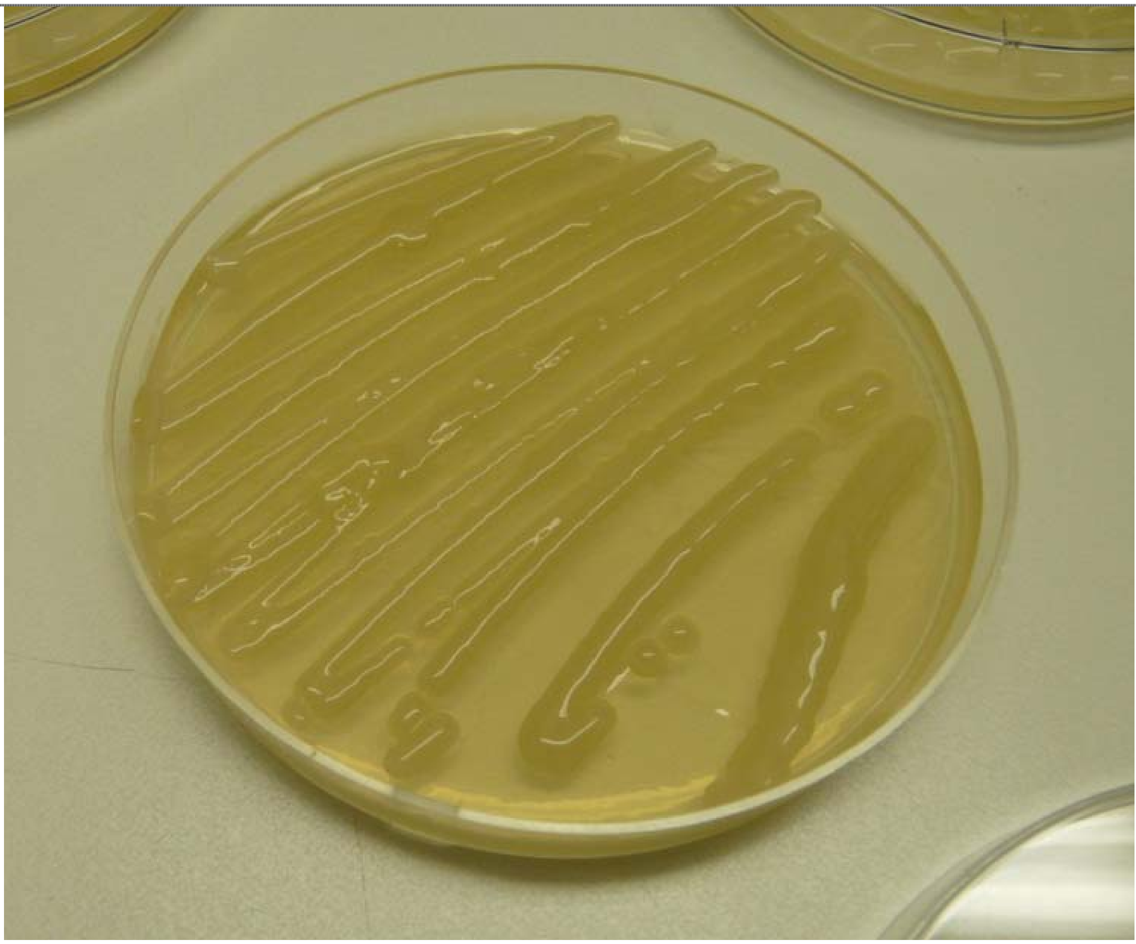


Figure 3. Petri dishes containing cultures of Erwinia amylovora, the bacteria responsible for fire blight infections. Each Petri dish contains a different strain of the bacteria collected from locations across North America. These strains are mixed to form an inoculant which is used to infect the U of SK bred rootstocks under bio-secure greenhouse conditions.

1.2.2 BIO-SECURITY

Level 1 biosecurity must be observed for the entire fire blight screening process. Level 1 is the safety level suitable for working with 'agents not known to cause disease in healthy adult humans.' At this level, standard microbiological practices and decontamination procedures are observed including decontamination of all materials via autoclave, hand washing, and use of appropriate PPE. All laboratory personal handling Fire blight bacteria have had biosecurity specific training from the U of SK Department of Health, Safety and Environment (DHSE) for procedures related to fire blight screening.

1.2.3 TREE SIZE/AGE

Potted 1 – 3 ft tall nursery stock with multi-year wood is used for fire blight screening (Figure 4). It is important not to use young plants as it has been shown (Hampson et al, 2008) that the fire blight susceptibility of young seedling greenhouse plants is poorly correlated to the susceptibility of mature field grown trees.

Figure 4. Potted rootstocks before inoculation with fire blight bacteria. **These trees are 1 – 3ft tall with multi-year wood. Actively growing tissue is inoculated with a mixture of fire blight strains from across North America.**

1.2.4 Environment

Inoculations will be performed in a greenhouse setting. This will allow for inoculation in winter under bio-secure conditions. While greenhouse inoculations have not been shown to be as representative of field resistance as field inoculations (Norelli et al, 2003), the findings will still be a useful indicator and selection tool.

1.2.5 Inoculation

Inoculation will be performed as described by Norelli (2003) by transversally bisecting the young leaves of actively growing shoots with scissors dipped in the inoculant.

1.2.6 Resistance

Resistance of apple rootstock to fireblight infection is determined by measuring the length of necrosis (dead tissue) caused by infection. This measurement, when expressed as a ratio of the total shoot length, can be used as a quantitative measurement of disease resistance. Virulence of *Erwinia* can vary from strain to strain, and even between batches of inoculant. In order to allow for the comparison between the resistance of U of SK rootstocks to those tested at other institutions, check cultivars are used. Check cultivars are rootstocks which are common across North America. Bud 491, V3, and Ott3 will all act as check cultivars for our screening.

2 FRUIT QUALITY

At the U of SK there are about 3000 apple seedlings and 400 selections / cultivars on dwarfing rootstocks that are of fruit bearing age. These were derived from controlled crosses made 5 to 15 years ago. All of these are being observed in the field for plant health, productivity and juice quality. The activity of scouring the fields to find apples worthy of evaluating with lab tests, is a regular activity in September and October. Notes are taken of interesting selections and if that seedling continues to show positive attributes it will be given the elevated status of an 'advanced selection'

There have been a few hundred apple cultivars named and grown on the prairies, but most of these have not stood the test of time. Many of these apples were derived from seeds brought by immigrants. Most of them have been found to not be hardy, and none are notable in the industry as large high quality apples for cider. Of the hardy apples there are about a dozen popular varieties developed by 'Prairie Fruit Breeding Cooperative' that was cancelled in the 1980's and recent releases from the U of SK. The U of SK is the only institution on the prairies involved in breeding apples at this time. In the past the U of SK has focused on the breeding of eating, or "dessert" apples. With this project we hope to investigate apple quality traits which may be useful for juice and cider production. While there is anecdotal and descriptive information regarding the juice quality traits the fruit grown on some cultivars, there is little to no quantitative data, except for sugar content of some selections. Measurements of pH, titratable acidity, sugar content, juice yield and phenolic content is valuable to processors such as juice and wine makers and is being done in this project.

2.1 CULTIVARS VS. SEEDLINGS

Both cultivars and seedlings are being evaluated for fruit quality. Cultivars are types of apple which are generally well known and are common across the prairies. Measuring and reporting the acidity, sugar content, and tannin content of these apples will be useful to individuals wishing to process them. Seedlings are the result of controlled crosses performed by the U of SK fruit program in the past. These seedlings are unique to the U of SK field plots. Evaluating the quality of these apples will assist in the selection of cultivars for advanced testing and release. The results of analysis of both cultivars and seedlings have begun to be posted to the apple page of the U of SK Fruit Program website.

2.2 JUICE EXTRACTION

The yield of juice that an apple produces could be an important factor in cultivar selection when planning for cider production. A greater yield of juice from an apple could increase profitability for a producer.
The juice yields of many cultivars were measured. Juice yield is highly dependent on the type of equipment, and the way the equipment is used. The type of press cloth used, the thickness of the press cake, the type of grinder, and the pressure used to press the cake can all affect the yield obtained from the apples. The equipment variables are further complicated by the variation between cultivars. Pectin content, fruit size, and flesh texture, can all affect juice yield. For this reason, juice yields obtained in this experiment could only be used for qualitative evaluation, or for interpretation on a case by case basis depending on the equipment used.

Apples were fed through a custom grinder consisting of a stainless steel chambered garbage disposal unit which yielded particle sizes ~5mm (Figure 6). This grinder was constructed because the particle size produced is comparable to many larger commercial style grinding units. The ground apples were then transferred to the apple press (Figure 6) for pressing. The weights of the juice and the press cake were recorded and the mass of juice per mass of fruit (w/w) was calculated. These values are displayed in table 3.

Figure 5. Apples after grinding.

The particle size of the ground apples plays a significant role in the amount of juice yielded during pressing. Particle size varies depending on grinder design. We used a garbage disposal mounted on its own stand that allowed us to grind smaller sample sizes yet get a high level of juice extraction.

Figure 6. Small scale apple press. This press uses the same basic principles used in larger commercial presses. Ground apples are loaded into a pressing bag which is in turn loaded into the wooden slatted tub. A screw assembly and plate presses the bag of ground apples. The juice is forced through the bag, between the slats of the tub, and into a collection base which drains into a bucket. Attempting to press too much ground apple at once reduces pressing efficiency.

2.3 Acidity

Titratable Acidity (TA) and pH are both indicators of fruit juice acidity. While pH is a good indicator of effective acidity, TA is more indicative of flavor, or sourness of a juice, and is therefore more relevant to fruit processors.

Acidity is also a very important quality trait for wine and fermented ciders. Cider apples, for example, are categorized by their titratable acidities. 'Sharp' apples are those cultivars with Titratable acidity (TA) above 4.5g/l(0.45%) and 'Sweet' apples are those with TA below 4.5g/l. Generally a blend of sharp and sweet apples is used to create a finished product with a TA around 5g/l.

Table 3 shows the titratable acidities of many prairie hardy cultivars. Samples were measured using a Hanna instruments HI84432 Mini Titrator and pH Meter for fruit juice, shown in Figure 7.

Figure 7. Hanna Instruments Juice Titrator. This equipment makes measure total acidity and pH.

2.4 SUGAR CONTENT

Sugar content was measured using an ATAGO Digital Pocket Refractometer (Figure 8). Results are displayed in table 3.

Figure 8. A digital Pocket Refractometer was used to measure the soluble solids of juice. Soluble solids are most made up of sugars. Refractometers have been widely used for decades to measure sugar levels in the food industry.

2.5 PHENOLS AND POLYPHENOLS

Phenols and Polyphenols are important components of wines, ciders and juices. The quantities and composition of these organic compounds affect quality traits like aroma, flavor and mouthfeel. The class of polyphenols known as tannins, are especially important when considering fermented beverages. Condensed tannins are responsible for mouthfeel and increased condensed tannin concentrations often correlate with increased overall perceived beverage quality.

The two measurements taken to quantify phenol contents in the apple juice and cider samples are the Methyl Cellulose Precipitable Tannin assay and the Lowenthal-Permanganate titration. The tannin contents observed in apples using both measurement techniques are displayed in table 3. Note that due to the difference in the manner in which tannins are measured in these two techniques, these measurements cannot be expressed using the same units.

2.5.1 METHYL CELLULOSE PRECIPITABLE TANNIN ASSAY

The Methyl Cellulose Precipitable (MCP) Tannin assay is a simple and robust method for measuring condensed tannins in a wine or juice sample. This assay was introduced by Sarneckis et al in 2006 and was designed specifically for the wine industry. The assay exploits the ability of tannins to be precipitated by proteins and some polymers. In this procedure, Methyl cellulose molecules bind to tannin molecules and pull them out of solution. The optical density of the solution is measured before and after the precipitation of the tannin molecules by the methyl cellulose, and a mathematical formula calculates concentration based on the difference between the two values.

2.5.2 LOWENTHAL-PERMANGANATE TITRATION

The Lowenthal-Permanganate (LP) titration has historically been the industry standard tannin measurement technique in the apple and cider industry. The LP method uses the antioxidative effects of phenols and polyphenols to determine their quantities in solution. In this procedure, juice samples treated with sulphuric acid and indigo carmine are titrated with an oxidative potassium permanganate solution. The endpoint of the titration occurs when the indigo carmine indicator in the sample turns yellow. The LP method of tannin measurement is more common than the MCP tannin assay, having historically been used by cideries and researchers in England.

Figure 9. Methyl Cellulose Precipitable (MCP) tannin assay lab equipment The MCP Tannin assay allows us to measure the tannins important for juice and cider quality.

3 Controlled Crosses

Crosses were done with the goal of generating a hardy population from which seedlings with fruit containing low TA and high phenolic content can be selected. Germplasm from across Canada has been accumulated to provide genetic material for these crosses. Germplasm collected includes: Yarlington Mill, Michelin, Muscadet de Dieppe, and Tremletts Bitter. These scions have been grafted to dwarfing rootstocks and have been grown in the greenhouse to induce blooming. Pollen from these cultivars was collected and used in controlled field crosses. The following cultivars have been used in breeding.

Table 1. Cultivars used in controlled crosses. Cultivars are selected for breeding based on field performance, appearance, and fruit quality.

Cultivar	Traits	Lineage
Red Sparkle	Hardy. Medium sized attractive fruit. Low acid. Excellent flavour and texture. Disease resistant. Bears annually and heavily	Trail x McIntosh
Exeter	Ripens late summer. Very heavy yield. Good quality for cooking and dessert.	Columbia x Melba
Kerr	Ripens dark red/purple. Good grapefruit/grape flavor	Dolgo x Haralson
Minn 447	Sweet crisp and juicy. Good yield and good keeper.	OP Malinda

Prairie Sensation	Fine, tender, crisp and juicy.	M359 x Brookland
Autumn Delight	Hardy. Good texture. Medium fruit size.	Haralson x Parkland
Norkent	Hardy. Medim to large fruit. Good flavour and texture. Bears heavily	Haralson x Rescue
SX81-69	Hardy. Heavy bearing. Small fruit. Interesting flavour. Low acid.	Unknown. Originated in Minnesota
SX81-48	Hardy. Medium to large fruit. Low acid. Stores well.	Fireside x Chestnut
Stoke Red	Bittersharp cider apple. Good aroma	Unknown. Originated in Somerset, England
Muscadet de Dieppe	Bittersweet cider apple. Sweet and aromatic.	Unknown. Originated in Normandy, France
Yarlington Mill	Bittersweet cider apple. Sweet and mildy bitter. Good aroma and flavor	Unknown. Originated in West Cadbury, England
Michelin	Bittersweet cider apple. Soft tannins, precocious, productive.	Unknown. Originated in France
Brown's Apple	Sharp cider apple	Unknown. Originated in South Devon, England
Kingston Black	UK Cultivar used for cider. Classified as "Bittersweet" meaning it has a low acid and high tannin content. Dark purple skin. Pleasant aroma.	Unknown. Originated in Somerset, England
Tremlett's Bitter	UK Cultivar used for cider. Classified as "Bittersweet" meaning it has a low acid and high tannin content. Dark purple skin. Pleasant aroma.	Unknown. Originated in Devon, England
4-38-34.5	U of Sk seedling with crisp texture, lower acid content, and good flavor	Prairie Sensation x Honeycrisp
4-38-52.0	Very fine, crisp and juicy flesh. Sweet sub-acid.	Prairie Sensation x Honeycrisp

It should be noted that controlled crosses for apples can be very time consuming in comparison to crosses done with small fruit and berries. Large controlled crosses require that entire trees be tented with pollinator resistant netting, and pollen from the pollen parent be introduced inside the tent.

7. Research accomplishments: *(Describe progress towards meeting objectives. Please use revised objectives if Ministry-approved revisions have been made to original objectives.)*	
Objectives	**Progress**
1) Evaluate the new generation for fresh eating, cider and fireblight	Extensive testing was done for juice quality on 54 seedlings of the newest generation. These had been field evaluated for hardiness, lack of disease, and fruit quality.
2) Make controlled crosses specifically for cider	Crosses are being done with the goal of generating a hardy population from which seedlings with fruit containing low TA and high phenolic content can be selected. Germplasm from across Canada has been accumulated to provide genetic material for these crosses. Germplasm collected includes: Yarlington Mill, Michelin, Muscadet de Dieppe, and Tremletts Bitter. These scions have been grafted to dwarfing rootstocks and have been grown in the greenhouse to induce blooming. Pollen from these cultivars has been collected and used in controlled field crosses. In 2013, three trees were tented and over 2000 seeds were obtained.
3) Test Rootstocks for fireblight resistance and compatibility	Fire blight samples from across the Saskatchewan and North America have been accumulated. Strains used for resistance screening by Agriculture Canada have been obtained. Seven strains of *Erwinia amylovora* were mixed to form the inoculant. Potted examples of each rootstock cultivar were inoculated. Infection was observed within days, and the length of the dead tissue was measured 3 weeks after inoculation. Rootstock cultivar V3 showed little to no signs of infection, while Bud 491 showed severe symptoms. Many of the U of SK experimental rootstocks exhibited signs of resistance to Fire blight infection. Ottawa #3 showed moderate to poor resistance to infection.
4) Test apple rootstocks for their ability to convey fireblight resistance to varieties grafted onto it. Also, develop methodology for rapid productions of superior rootstocks and grafted trees such as “stenting” and tissue culture	A large trial of rootstocks was set up that will allow us to see resistance levels in the future and if fireblight resistance from rootstocks will convey to the scion portion. ‘Stenting’, the act of grafting a bud onto a rootstock while simultaneously trying to root the rootstock, was deemed impractical upon further consideration. Rootstock cuttings are done in early summer while grafting is done in early fall. Buds suitable for grafting are not ready so early in the year plus rootstock cuttings are not thick enough so early. In the 1st year of this project we easily put many types apple rootstocks into tissue culture with relative ease. There is much literature on this. The best rootstocks for fireblight resistance were not identified until the last year of the project so it make sense to tissue culture them in 2014.
5) Analytical assessments of sugars and acids useful for wine and cider makers who need the info to choose yeasts and to design fermentation protocols and involve wine and sider juice producers in the selection process.	At the U of SK there are about 3000 apple seedlings and 400 selections / cultivars on dwarfing rootstocks that are of fruit bearing age. These were derived from controlled crosses made 5 to 15 years ago. All of these are being observed in the field for plant health, productivity and juice quality. While there is anecdotal and descriptive information regarding the juice quality traits the fruit grown on some cultivars, there is little to no quantitative data, except for sugar content of some selections. Measurements of pH, titratable acidity, sugar content, juice yield and phenolic content were taken on many cultivars. In addition to cultivars and advanced selections, seedlings were also screened. Of the seedlings screened, several showed traits which indicate suitability for use in fermented beverages.
add additional lines as required	

8. Discussion: *Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

1 YIELD EFFICIENCY

Due to the varying nature of fruit yield as it responds to environmental variables, it is often helpful to use a check cultivar against which these the new rootstocks can be compared. The yield efficiency of the check cultivar is used as a baseline, limiting the effect of environmental variables on rootstock evaluations. In this case Ottawa 3 is used as the check. The yield efficiencies of the new rootstocks can then be expressed as 'percent of Ottawa 3'

$$\% \text{ of Ottawa } 3 = \frac{YE_C}{YE_{Ott3}} ..$$

Where: YE_C is the yield efficiency of the cultivar
YE_{Ott3} is the average yield efficiency of all Ottawa 3 trees

This data is summarized in Table 2 below. A detailed table, including the yield efficiency described as % of Ottawa 3, and height of all the rootstocks in this trial is included in appendix 1. This data, in combination with field observations noted during the trial, was used to choose the rootstocks which would be propagated and screened for fire blight resistance. The yield efficiency of some of the rootstocks chosen for propagation and further testing are described in table 1.

Table 2. Yield efficiency and height of Rootstocks bred at the U of SK. Yield efficiency is the ratio of: the mass of fruit harvested; to the diameter 30cm above the graft union. In this case yield efficiency is expressed as a percent of the production on Ottawa 3 Rootstock in the same orchard. The yield efficiency describes the effects of the rootstock on the fruit production of the scion grafted to it (in this case Prairie Sensation).

Rootstock Cultivar	Average Yield Efficiency (% of Ott3)	Average Height (m)
AR7-1-7.9	60	2.5
AR7-1-6.5	72	2.0
AR7-1-16	97	1.5
AR7-2-36.5	321	1.95
AR7-3-24.3	149	1.7
AR7-3-29.0	47	2.6

2 FIREBLIGHT SCREENING

Fire blight samples from across the Saskatchewan and North America have been accumulated. Strains used for resistance screening by Agriculture Canada have been obtained. Seven strains of *Erwinia amylovora* were mixed to form the inoculant. Potted examples of each rootstock cultivar were inoculated.

Infection was observed within days, and the length of the dead tissue was measured 3 weeks after inoculation. Rootstock cultivar V3 showed little to no signs of infection, while Bud 491 showed severe symptoms. Many of the U of SK experimental rootstocks exhibited signs of resistance to Fire blight infection. Ottawa #3 showed moderate to poor resistance to infection.

Table 3. Fire blight resistance in dwarfing rootstocks.

Test Number	Performance	Fire Blight Susceptibility (1=Low Susceptibility 5=Highly Susceptible)
V3	Resistant check cultivar. Demonstrated significant resistance to infection.	1
O3	Check cultivar. Common hardy rootstock in Saskatchewan	4
Bud 491	Check cultivar. Common commercial rootstock	4
7-1-5.7	Less susceptible than check cultivars Bud491 and O3	3
7-2-30.7	Low susceptibility to infection	1
7-1-16.0	Less susceptible than check cultivars Bud491 and O3.	3
7-2-36.5	High susceptibility to infection	5
7-1-6.5	High susceptibility to infection	5
7-1-7.9	Moderate susceptibility to infection	4
7-3-12.2	Low to moderate susceptibility to infection	2
7-3-29.0	Moderate susceptibility to infection	3
7-3-24.3	Moderate susceptibility to infection	3
7-2-7.8	Highly susceptible to infection	5

Figure 10. Rootstocks showing symptoms of Fireblight infection. The "shepherds crook" branch tips and whitish yellow ooze are typical indicators.

Figure 11. V3 dwarfing rootstock 3 weeks after inoculation with fire blight. V3 showed only slight symptoms of fire blight infection.

3 JUICE QUALITY

Table 4 below shows the results of the tannin, acid, sugar and juice yield measurements. These results would be useful for processors making and blending hard cider or juice. Juice yield was highly variable ranging from 54 to 80% extraction rates. As a general observation, crisp firm apples tend to yield more juice than softer ones. Among traditional apples, 'Prolific' may be have the highest potential for hard cider due to its high juice yield and high tannins yet reasonable sugar acid ratio. 'Prolific' was a U of Sk introduction from the early 1960s. 'Trail' and 'Kerr' are crab apples often used for cider possibly because not much else can be done with them, although 'Kerr' is often blended to add character to a cider. But crab apples would require more labour to harvest. Of the higher juice yielding varieties Minn447 may be the best for juice, while 'Brightness' may be useful for either Juice or hard cider. Of the traditional cultivars grown, very likely 'Norkent', 'Red Sparkle', 'Carlos Queen' and Goodland' are the most widely grown. Unfortunately these popular varieties were on the low side for juice yield.

Regarding recently released U of SK varieties, 'Grannie Annie' and 'Patience' have higher levels of tannins combined with a good sugar acid ration and could be worthwhile for hard cider. 'Autumn Delight' is relatively low in tannins and acidity and would be a good candidate for blending. 'Prairie Rose' has characteristic desirable for a 100% apple juice. Although tannins can be artificially added to ciders, higher quality ciders and wine are usually made if tannins come from the fruit being fermented.

All of the unreleased apple selections have sugar acid ratios exceeding the optimum range of 15 to 20. 'R52T22' was the only selection that had a high juice extraction yield of 77% and may be ideally suited for blending with other fruits. 'SX08-15' should be re-tested since juice yield data was not recorded. It had the highest sugar acid ratio and could potentially be very useful for blending or as a breeding parent for blending.

Table 4. Properties of SK grown apples that seemed to have potential as hard cider apples based on field evaluations. Based on fruit grown at the U of SK orchards in Saskatoon Saskatchewan during 2011, 2012 and 2013. 'SX' designations are breeding selections from other programs that are not available to the public but we are using them as breeding stock. 'R' designations are breeding selections being tested by growers but have not been named and released.

Cultivar	Juice Yield % w/w	%Tannins L-P test	MCP Tannins	pH	Titrateable Acidity	Soluble Solids	Sugar Acid Ratio
			Traditional Prairie Apples				
Minn447	78.4	0.65	12.1	3.27	0.63	13.5	21.4
Brightness	77.3	1.28	35.8	3.30	0.89	16.9	19.0
Kerr	75.4	1.39	9.5	3.21	0.95	16.5	17.4
Prolific	75.2	1.80	73.5	3.60	0.68	15.5	22.8
Norkent	66.0	1.13	37.2	3.77	0.45	14.1	31.3
Trail	65.6	1.34	44.8	3.72	0.59	16.5	28.0
Red Sparkle	65.0	1.31	45.6	3.50	0.37	12.8	34.6
Carlos Queen	58.5	0.83	19.6	3.34	0.68	13.6	20.0
Goodland	53.8	0.95	28.7	3.40	0.62	14.1	22.7
average	68.4	1.2	34.1	3.5	0.7	14.8	24.1
			Recent U of SK Introductions				
Granny Annie	80.1	1.36	75.7	3.29	0.72	12.8	17.8
Autumn Delight	78.9	1.15	28.0	3.31	0.47	12.8	27.2
Patience	77.5	1.41	35.2	3.24	0.66	13	19.7
Prairie Rose	71.1	0.83	11.5	3.13	0.79	13.6	17.2
Prairie Sensation	65.5	0.81	26.1	3.46	0.66	13.2	20.0
Misty Rose	62.0	0.84	37.1	3.38	0.63	14.6	23.2
Anna Gold	55.1	0.95	45.9	3.42	0.50	12.8	25.6
Festive Treat	53.6	1.73	25.8	3.25	0.78	13.5	17.3
average	68.0	1.1	35.7	3.3	0.7	13.3	21.0
			Unreleased selections ('R's are from U of SK, 'SX's are from other program)				
R52T22	76.8	1.00	16.8	3.61	0.41	13.8	33.7
SX81-48	66.4	0.70	18.2	3.72	0.46	16.6	36.1
SX81-69	62.1	0.65	45.1	3.40	0.76	17.9	23.6
R76T31	59.7	1.19	45.9	3.37	0.56	13.2	23.6
R64T11	58.2	0.77	29.5	3.50	0.46	14.2	30.9
SX08-15	?	1.17	15.2	4.22	0.21	16.6	79.0
SX08-16	?	1.03	-	3.48	0.57	15.8	27.7
average	64.6	0.93	28.5	3.61	0.49	15.4	36.4

3.1 COMPARISON TO CIDER APPLES FROM BC

Table 5 below shows the acid, sugar and tannin contents of apple cultivars grown in BC specifically for use in fermented ciders. These apples were collected during a trip to the Okanagan in 2011. Partnerships with traditional cider apple producers were sought to discuss and investigate some of the topics addressed in this research project, and to gather apples which were to act as a 'baseline' against which we could measure the suitability of our apples for use in fermented beverages. The apples from Summerland were donated by Bob Thompson, and the apples from Keremeos were donated by Markus Keller. These apples have a wide range of tannin and acid contents. Juices from these apples are mixed to form a finished product with a moderate titrateable acidity and medium to high tannin content.

Tannins are complex molecules that break down over time during the fermentation process. The two tannin tests measure tannins in different ways and may not be measuring the exact same tannins. The prairie apples tested have similar tannin levels to BC cider apples using the L-T test. But BC cider apples tend to have higher tannin levels on the MCP tests. Apples have not been widely investigated for tannins compared to grapes. Sugar Acid ratios of BC apples are similar to our unreleased selections and a few of the seedlings preliminary screened in Appendix 3. On average, BC cider apples yield 10% less juice when extracted but the BC varieties 'Dabinett' and 'Kerrmerian' are excellent for this characteristic.

Table 5. Properties of apples grown in BC in 2011 that are used for fermented cider. None of these cultivars would be hardy in Saskatchewan but the evaluation of these may be useful for comparing to prairie grown apples or identifying varieties that could be used as parents in our breeding program.

Cultivar	Juice Yield % w/w	%Tannin L-P test	MCP Tannin	pH	Titrateable Acidity	Soluble Solids	Sugar Acid Ratio
Dabinett (S)	75.1	0.80	31.1	3.26	0.47	15.3	32.6
Kerrmerian (S)	73.4	0.90	58.9	3.15	0.64	14.8	23.1
Dabinett (K)	71.2	1.40	47.2	3.45	0.35	13.1	37.4
Brown's Apple (S)	65.4	2.00	33.8	3.12	0.69	15.1	21.9
Saurgrau (S)	54.0	1.40	83.8	3.33	0.50	13.3	26.6
Yarlinton Mill (S)	43.3	2.00	271.7**	3.70	0.21	12.9	61.4
Michelin (S)	41.7	1.30	150.2**	3.71	0.21	13.2	62.9
Michelin (K)	38.9	0.90	60.6	3.69	0.25	13.4	53.6
Blauacher (K)*	37.2	1.10	32.3	3.10	0.68	14.7	21.6
Kingston Black (S)	?	0.80	75.7	3.29	0.49	15.5	31.6
average	55.6	1.3	84.5	3.4	0.4	14.1	37.3

*(K) indicates apples obtained in Keremeos, (S) indicates apple obtained from Summerland

** these values seem unusually high, but no apples were available for retesting

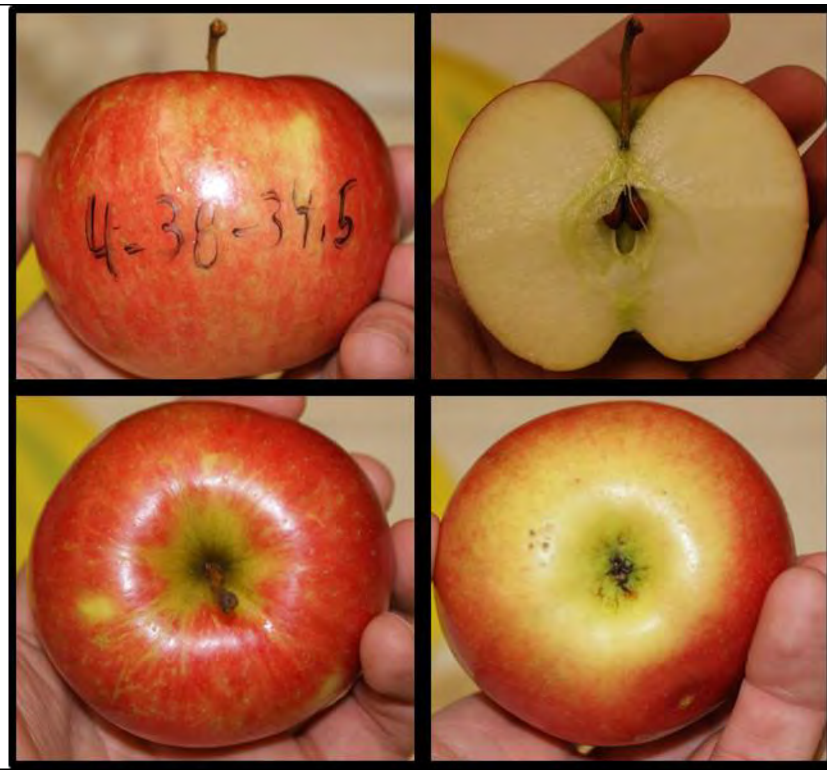
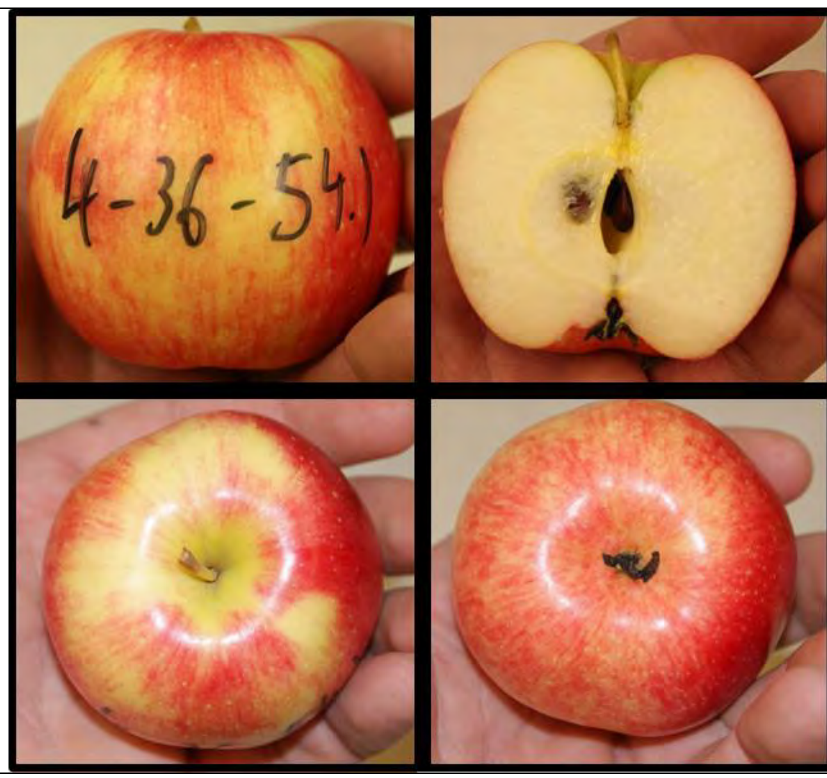
In Saskatchewan, apples containing high acid and low tannin are most common. In order to complement these existing hardy cultivars and create a balanced finished product, low acid, high tannin cultivars are most desirable. A pH below 3.8 is also desirable to prevent microbial spoilage, but this usually comes at the expense of a higher titratable acidity. Table 6 ranks ranks some advanced selections and cultivars based on their suitability in filling this role. While many of these apples have a lower than average acid content and higher than average tannin content, it should be noted that many could also be classified as dual use apples, that is, suitable for use in fermented products, and for dessert/fresh sale. However, it is noted that the 5 apples with the highest rankings for tannins were had undesirable notes regarding dessert qualities and had small fruit size. Perhaps varieties with a mid-range tannin content would be best to choose if desiring a variety to be used for both fresh and hard cider.

Table 6. Cultivars scored and ranked based on their suitability for hard cider in Saskatchewan. Tannin score is determined according to the Methyl Cellulose Precipitable (MCP) tannin values. MCP tannin values of all cultivars are distributed according to their value on a scale of 1-5 (1 being the lowest tannin content, and 5 being the highest). Tannin score is given according to where the MCP tannin content falls on that scale. Acid score is determined according to the cultivars titratable acidity (TA). TAs of all cultivars are distributed according to their values on a scale of 1 to 5 (5 being the lowest acid content and 1 being the highest). Acid score is given based on where the cultivar's TA falls on that scale.

Cultivar	Score (out of 5)		Score	Dessert Quality
	Tannin	Acid		
SX08-15	3	5	8	Fair.
Norkent	3	4	7	Good
R52T22	1	4	5	Poor. Small Fruit
Red Sparkle	2	4	6	Unique flavor
SX81-48	2	4	6	Poor. Sweet. Hard texture
Anna Gold*	3	3	6	Good.
Autumn Delight*	2	3	5	Good. Nice texture
Goodland	1	3	4	Good quality
Minn 447	3	3	6	Good. Unique flavor
Misty Rose	3	3	6	Fair. Tart.
R64T11*	4	3	7	Poor. Small fruit
R76T31	1	3	4	Fair. Juicy, crispy.
SX08-16	1	3	4	Good. Sweet
Trail	2	3	5	Poor. Small fruit
Carlos Queen	2	2	4	Good. Balanced acid
Festive Treat*	4	2	6	Fair. Tart
Granny Annie	3	2	5	Good
Prairie Sensation*	3	2	5	Good
Prolific	4	2	6	Poor. Small fruit
SX81-69	5	2	7	Poor. Small fruit. Unique flavor
Brightness	3	1	4	Poor. Fresh astringent flavor. Small fruit
Kerr	4	1	5	Poor. Small fruit. Unique flavor
Patience	2	1	3	Fair. Tart
Prairie Rose	3	1	4	Very tart

In addition to cultivars and advanced selections, seedlings were also screened. A complete list of seedlings and measurements is included in Appendix A. Below in Table 7 are listed some of the most promising seedlings for fresh eating. These apples have extremely long shelf life and crispness which they inherited from 'Honeycrisp'. The other parents ('Prairie Sensation' and '18-10-31') were some of the most productive, early fruiting and good quality varieties from our own program. As they all have sugar acid ratios higher than 30 they would be ideal for blending with other fruit juices with higher acidity for fresh juice and possibly blended & fermented fruit wines.

Table 7. Data and photos of some promising apples that may be desirable for fresh eating and cider production.

-#4-38-34.5 -Prairie Sensation x Honeycrisp -Crisp Honeycrisp texture with unique flavor reminiscent of MacIntosh. -pH: 3.25 -Titrateable Acidity: 0.32% Malic Acid -Soluble Solids: 13.2 brix 41.3 sugar/acid	
-#4-36-54.1 -Prairie Sensation x Honeycrisp - Breaking, sweet, sub-acid. Good keeper. -pH: 3.43 -Titrateable Acidity: 0.33% Malic Acid -Soluble Solids: 13.9 brix 42.1 sugar/acid	

-#4-36-59.4

-Prairie Sensation x Honeycrisp

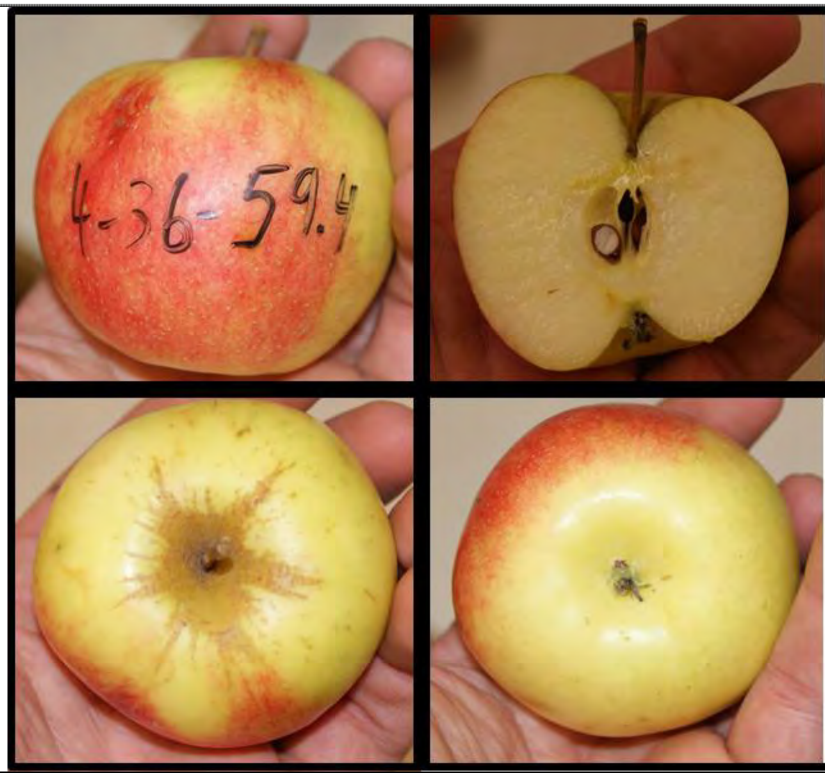
-Firm, coarse, tender crisp texture. Good aroma. Yellow flesh

-pH: 3.36

-Titrateable Acidity:
0.32% Malic Acid

-Soluble Solids: 13.7 brix

42.8 sugar/acid



-#4-38-28.1

-Prairie Sensation x Honeycrisp

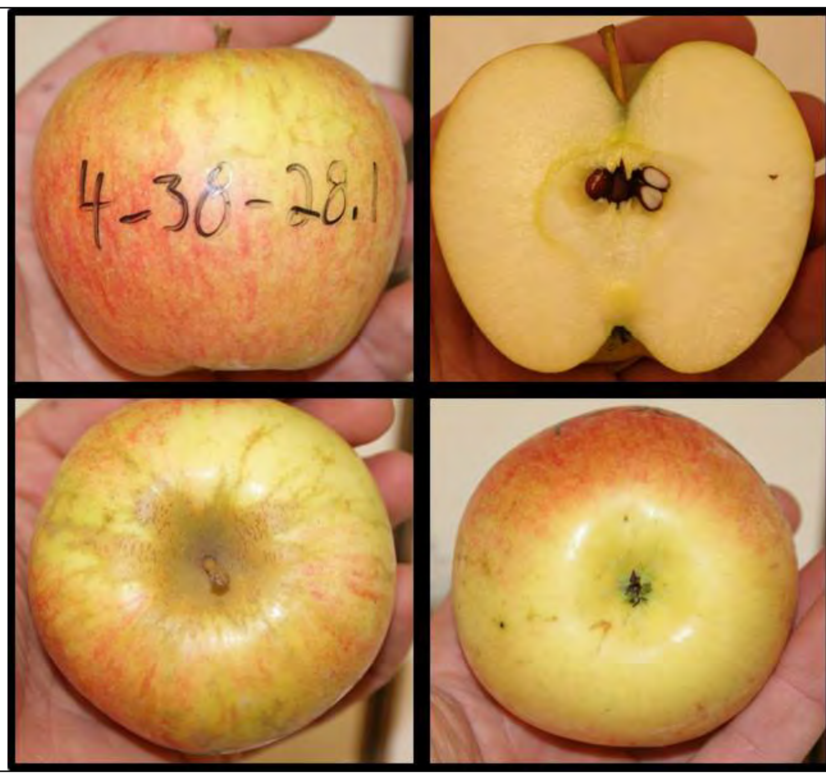
-Conic. Sweet. Very juicy.

pH: 3.36

-Titrateable Acidity:
0.41% Malic Acid

-Soluble Solids: 14.3 brix

34.9 sugar/acid



-#4-37-11.9 -18-10-32 x Honeycrisp -V. Attractive. 95% pink/red wash/stripe on yellow. -pH: 3.76 -Titrateable Acidity: 0.42% Malic Acid -Soluble Solids: 14.8 brix 35.2 sugar/acid	

3.2 CULTIVARS AND SEEDLINGS SUITABLE FOR FRESH CIDER

The characteristics of cultivars suitable for fresh cider are different than for hard cider. In fermented beverages, sugar is lost during the process of fermentation, while in fresh cider the sugar remains. The ratio of sugar to acid in fresh cider will determine how sweet or sour a particular juice is perceived. The ideal sugar / acid ratio is generally considered to be between 15 to 20 in Europe but in the USA the acceptable range goes up to 30. An internet search did not show and stats on a range specifically for Canada. It seems reasonable to consider that lower sugar levels would be advisable for a healthier product. This ratio can be achieved by blending the juices of sour and sweet apples or by adding acids to artificially modify the acidity. Table 7 lists the sugar to acid ratios of some prairie hardy cultivars and important germplasm used in our program.

Figures 12 and 13 depict population curves for seedlings evaluated in the lab for cider production. These were considered the best from among ~3000 seedlings based on field evaluations. Details for individuals are located in the appendix. About 60% of the field selections were found to be in the desirable range for fresh cider. Six selections were identified as having an unusually high sugar/acid ration and might be best used for blending with other fruits. Most of these seedlings have not been tested by growers.

Figure 12. Fresh cider. **The most important variable when considering fresh cider is the sugar to acid ratio. A low tannin content can also be a good trait for fresh cider.**

Table 8. Sugar acid ratios of named varieties and important germplasm for our breeding program. Based on measurements from the 2011 crop. 'SX' designations are breeding selections from other programs that are not available to the public but we are using them as breeding stock. 'R' designations are breeding selections being tested by growers but have not been named and released. A * indicates that the variety was bred at the University of Saskatchewan.

Cultivar	Sugar to Acid Ratio (Brix/%MA)
SX81-48	36.1
R52T22*	34
Norkent	31.3
Red Sparkle	29.8
Trail	28.2
R64T11	27.8
SX08-16	27.7
R76T31*	23.8
Sx81-69	23.6
Misty Rose*	23.4
Autumn Delight*	23.1
Prolific*	23
Goodland	22.7
Minn447	21.6
Anna Gold*	21.4
Carlos Queen	20.1
Prairie Sensation*	20
Brightness	19
Granny Annie*	17.9
Kerr	17.4
Festive Treat*	17.4
Patience*	16.2
Prairie Rose*	15.2

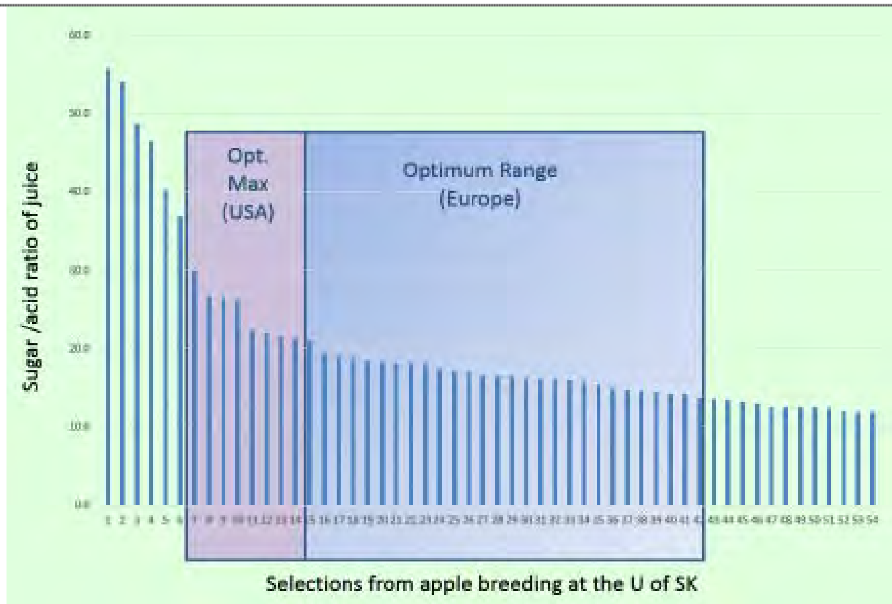


Figure 13. Sugar Acid ratios of apple seedlings from the U of SK breeding program depicting optimum ranges for cider production. A range between 15 and 20 is widely considered best in Europe while in the USA 15 to 30 is considered a good ratio.

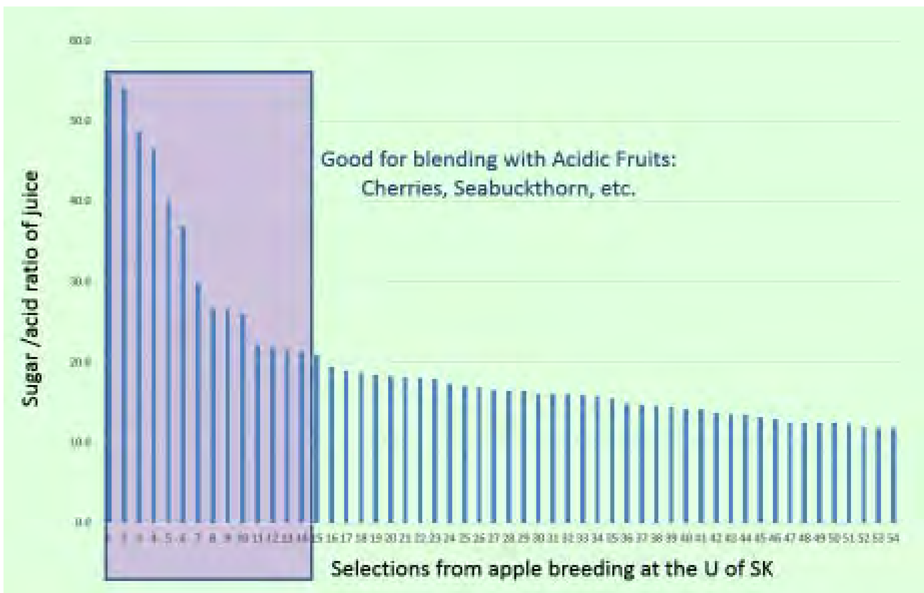


Figure 14. Selections from the U of SK apple breeding. Highlighted are selections that have potential for blending with acidic fruit. If these selections were used only by themselves the cider would be considered too sweet and perhaps lacking in character.

3.3 Crosses made for cider production.

Crosses were done in 2011 and 2012 between 'cider germplasm' obtained from B.C. and prairie hardy varieties. These crosses were done based solely on recommendations of others. Only two hundred seedlings were created and planted since the choice of parents was based largely on recommendations of others. Crosses done in 2013 were based on preliminary results of our own data and over 2000 seeds were obtained. Crosses done are summarized in table 9. The 2013 seeds are being germinated in the greenhouse. About ½ will be planted at the U of SK and ½ at cooperators orchards in Saskatchewan.

Apple pollen was ordered from Ag Canada's Canadian Clonal Genebank in Harrow Ontario. Officials at the genebank agreed to harvest and send pollen to us. As requested, we sent them our protocols for gathering and shipping pollen. [I believe that we sent them a kit of desiccant and micro centrifuge tubes, but I am not 100% sure of that aspect since Peter Reimer relocated to Alberta]. When we received our 'pollen' the instructions had been ignored. Instead, whole flowers were shipped which had rotted and were full of fungus. This disrupted our plans for crosses in 2012. We decided not to count on pollen from the genebank in the future. In fairness, they had never shipped pollen before, but why they requested but did not follow the protocols is a mystery.

Table 9: Controlled apple crosses performed from 2011-2013

Pollen Parent	Seed Parent
Tremletts Bitter	Autumn Delight
Tremletts Bitter	Patience
Brown's apple	Patience
Kingston Black	Exeter
Stoke Red	Exeter
Kerr	Kingston Black
Exeter	Muscadet de Dieppe
Kerr	Muscadet de Dieppe
Muscadet de Dieppe	Prairie Sensation
Michelin	Kerr
Tremletts Bitter	Exeter
Tremletts Bitter	Kerr
Yarlington Mill	Kerr
Autumn Delight	Kingston Black
Minn 447	Autumn Delight
SX81-48	Minn 447
Red Sparkle	4-38-34.5
Red Sparkle	4-38-52.0
Autumn Delight	SX81-48

9 Conclusions and Recommendations: *Highlight significant conclusions based on the previous sections, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project.*

Prospective cider producers in Saskatchewan can be confident that cultivars exist that have quality equal to or better than cider type cultivars grown elsewhere in Canada. Saskatchewan cider makers have the potential to grow varieties with good juice quality.

Data generated in this project has identified promising varieties suitable for fresh cider as well as varieties with higher tannin content (and other traits) that would be best for hard cider. This information is immediately useful for growers and processors wishing to establish orchards in the near future

Also identified are breeding selections that have high sugar content and low acidity that would be best for blending with other fruits. As these are seedlings in the breeding program, they will need to be propagated and tested further before being released.

Our apple rootstock collection was field screen and then the more promising selections were intensely screen for fireblight resistance under greenhouse conditions. That 2 rootstocks were found highly resistant will be a great advantage for our growers particularly since these rootstock have already proven themselves as very cold hardy. These rootstocks need to be better evaluated to determine their dwarfing ability so that growers can know how far apart to space their trees. But those 2 selections need to undergo large scale propagation.n

Preliminary data has been obtained for yield efficiency for our apple rootstocks on previously planted grafted trees. But this project has established a new large orchard that will allow us to fully study yield efficiency and dwarfing ability of our most promising rootstocks.

10 **Success stories/ practical implications for producers or industry:** *Identify new innovations and /or technologies developed through this project; and elaborate on how they might impact the producers /industry.*

The superior rootstocks identified by this project will likely become the standards for dwarf apple rootstocks in northern areas, assuming they have dwarfing capabilities similar to their parents. Many apple growers north of Saskatoon are using seedling crabapple rootstocks (full size trees) because they are hardier than 'Ottawa 3'. Those growers will have hardier rootstocks. In the southern part of the province, they will benefit by having an alternative rootstock or 2 with greater fireblight resistance. Dwarf apple orchards are not only easier to prune and pick, but they also producer more per acre and tend to have higher quality fruit.

Knowing that many of our apple seedlings are in an excellent range for cider production will mean that we do not have to concentrate on making more crosses but can emphasize selection criteria for productivity.

Several already named cultivars have been identified as suitable for cider production, and many of these were recently produced by our program. It will be possible for growers to start orchards with these varieties immediately.

11 **Patents/ IP generated/ commercialized products:** *List any products developed from this research.*

The apple rootstocks are very likely to receive PBR in the future. The trial established during this project will likely be old enough to inspect for PBR in 4 or 5 years.

Perhaps some of the cider apples will be trademarked or given COPF protection. We have already contacted a few nurseries willing to do trials which will later serve as propagation stock blocks.

12 **List technology transfer activities:** *Include presentations to conferences, producer groups or articles published in science journals or other magazines.*

1 Extension

Extension is a way to share the knowledge gained during this research. Extension has been one of the focuses of the U of SK fruit program under the direction of Dr. Bors. For the apple research two major strategies are used: 1) Presentations at fruit related events and 2) Putting information on the U of SK fruit program website (www.fruit.usask.ca). Both methods are effective at raising awareness of the research at the U of SK, and they also tend to complement one another. Events are better attended for having been advertised on the website, and after giving the URL at presentations, website traffic increases. Depending on the size of the audience, website traffic has been known to spike by a factor of 10 after a presentation.

1.1 Website

The goal for website development is to share as much information as possible regarding the apples at the U of SK and our research with these apples. Over 1000 links and images were added to the apple section of the website. These images were mostly taken of apple seedlings or advanced selections. Many of these apples were also analyzed chemically for acid and sugar content. There were 1.16 million requests in 2012 and 1.6 million requests in 2013 to our fruit program website.

1.2 Presentations

• Bruno Cherry Festival.	Peter Reimer	Apple Cider on the Prairies
• NAFEX Annual Conference	Peter Reimer	Prairie Apple Cider
• NAFEX Annual Conference	Harry Burton	Apples: Mother Nature's Champions of Diversity
• NAFEX Annual Conference	Rick Sawatzky	Field tour
• U of S Horticulture Extension	Rick Sawatzky	Grafting Workshop (2011)
• U of S Hort week	Bob Bors	Field tour
• Apple and Grape Day	Peter/Rick/Bob	Field Tour and Presentations
• Sask Fruit Grower annual meeting	Bob Bors	Research Updates (2012, 2013, 2014)
• Sask Fruit Grower annual meeting	Rick	Tree Fruit presentation

13 **List any industry contributions or support received.**

Several volunteers donated time to assist with pruning, harvesting, and evaluating fruit. In 2011 and 2012 in particular we had a retired volunteer who spent 20 hours a week, mostly on apples. Volunteers may have contributed 400 hours per year on this projects

Royalties for our fruit varieties sales funded about 30% of this project.

Mike Noel of Petrofka Orchards was notable for donating time to help evaluate fruit and advising us on cider production.

14 **Is there a need to conduct follow up research?** *Detail any further research, development and/or communication needs arising from this project.*

The rootstock trial will need evaluations in about 5 years, maybe 4.

It would be good to couple additional rootstock trials with nurseries interested in trying several cider selections.

Perhaps communication with the assistance of the Provincial Fruit Advisor, could help put together such trials.

15 **Acknowledgements**. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.*

The Saskatchewan Ministry of Agriculture is acknowledged in all our presentations, usually with ADF printed or spoken.

Our fruit website has a Saskatchewan Ministry of Agriculture symbol in the upper right hand corner which when clicked goes directly to the ADF website where research reports can be downloaded.

Students and growers interested in more details are urged to visit the ADF website.

16 **Appendices:** *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited*

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van der Zwet, T. and Beer, S.V. (1995). Fire Blight - Its Nature, Prevention and Control: A Practical Guide to Integrated Disease Management. *Agric. Info. Bull*, (631), United States Dept. of Agric.

2 EQUIPMENT

Homesteader Single-Tub Cider and Wine Press. Happy Valley Cider Presses. 1677 W 327th St. Paola, KS 66071

HI 84432 Titratable Acidity Mini Titrator and pH Meter for Fruit Juice. HANNA INSTRUMENTS CANADA INC. 3156, boul. Industriel, Laval, Québec (Canada) H7L 4P7

PAL-α ATAGO Digital Pocket Refractometer. Office : 32-10 Honcho, Itabashi-ku, Tokyo 173-0001, Japan

3 APPENDIXES

Appendix 1: Timeline of work in 2011

Month	Apple activities	Grapes activites
April	Budwood and scionwood ordered from Quebec and BC. Pollen ordered from Harrow Clonal Genebank. Apple trees pruned.	Day-long grape workshop held at the U of SK generated awareness, interest in grape breeding/growing and identified possible co-operators Seed of (DM 8521-1 x Cabernet), (Shiraz x MN 1094) and (C-16 x Cabernet) crosses germinated
May	Controlled crosses were performed in the field. Trees were bagged and immasculated before accepting pollen from the chosen parents	Cuttings were taken from the hardiest seedlings of 2009 plantings and rooted in the greenhouse Cuttings of 'Petite Pearl' and 'Frontenac blanc' rooted
June	Crosses continue. Weeding and maintenance of parent apple trees continue	Crosses made in winter 2010/2011 were planted out in the U of SK Hort field nursery plots (~2,000 seedlings)
July	Weeding and maintenance of breeding parents continues	Three vines of 'C-16' crossed with 'MN 1094' in the greenhouse
August	Weeding and maintenance of breeding parents continues. Harvest begins on early apple cultivars	Seedlings of (DM 8521-1 x Cabernet), (Shiraz x MN 1094) and (C-16 x Cabernet) crosses crosses cut back (flower induction)
September	Evaluation of apples in plots and harvesting of cultivars with high potential. Harvesting	C-16 fruit harvested, seed cleaned

	controlled crosses	
October	Harvesting fruit continues. Pressing begins	
November	Pressing continues. Chemical analysis of Apple juices begins. Maintenance of breeding parents continues, including weed control using herbicides.	Parent vines to be used in 2012 crosses placed in the cooler for vernalization Fruit of (DM 8521-1 x Cabernet), (Shiraz x MN 1094) and (C-16 x Cabernet) crosses progeny evaluated for flavour, earliness and fruit colour and inferior seedlings discarded
December	Tannin analysis of pressed apple juices continues. Data compilation and analysis begins	Fruit of (DM 8521-1 x Cabernet), (Shiraz x MN 1094) and (C-16 x Cabernet) crosses progeny evaluated for flavour, earliness and fruit colour and inferior seedlings discarded
January	Data analysis continues. Information organized for presentation.	Various tissue culture medias are being investigated for embryo rescue Delphinidin standards ordered for anthocyanin screening in March Planned crosses
February		Planned crosses

Appendix 2: Rootstock trial data. Yield efficiency and tree height are given.

Location	Selection No.	Yield Efficiency (% of Ott 3)	Tree Ht(m)
29-39	2-36.5	0	0
28-38	2-22.6	173	2.5
28-45	2-22.6	204	2.6
28-29	SX96-04	74	1.85
29-30	SX96-04	0	0.6
28-42	1-09.3	115	3.2
29-33	1-09.3	0	1.5
29-52	1-09.3	102	3
28-22	2-10.5	110	2.8
28-32	2-10.5	68	2.3
28-43	2-10.5	48	2.35
30-30	V3	76	2.25
30-29	V3	0	0
30-28	V3 yg	0	1.4
28-47	3-14.2	79	2.5
28-51	3-14.2	133	2.15
30-04	3-14.2	31	1.95
28-49	1-16.0	90	2.2
29-24	1-16.0	0	0
30-07	1-16.0	61	2.85
28-02	1-16.7	18	1.3
28-34	1-16.7	0	0
28-39	3-23.6	80	3.5
28-50	3-23.6	52	2.7
30-09	3-23.6	0	0
29-05	1-06.5	126	2.7
29-41	1-06.5	0	0.8
30-11	1-06.5	0	0
29-15	Ott 3	60	2.5
29-53	Ott 3	0	0
30-18	Ott 3 v yg	0	0
28-46	Ott 3 yg	98	2.3
28-52	Ott 3 yg	0	0
28-53	Ott 3 yg	142	2.15
29-38	Ott 3 yg	0	2.1
30-17	Ott 3 yg	0	0
29-31	3-22.2	113	3

29-43	3-22.2	0	1.6
30-06	3-22.2	91	2.95
28-20	1-09.0	89	2.6
28-40	1-09.0	147	2.95
30-02	1-09.0	83	2.8
29-07	2-41.2	0	0
30-10	2-41.2	176	2.3
28-12	2-35.8	97	2.6
29-44	2-35.8	90	2.7
28-13	3-19.2	61	2.8
28-48	3-19.2	81	2.9
30-12	3-19.2	43	2.7
29-09	3-24.3	51	2.3
29-42	3-24.3	111	2.6
28-03	2-41.8	155	2.5
28-36	2-41.8	138	2.6
29-34	2-41.8	126	2.1
28-04	3-40.4	106	2.5
28-35	3-40.4	36	2.5
29-22	3-40.4	84	2.9
28-06	1-07.9	79	2.5
29-19	1-07.9	76	2.5
29-23	1-07.9	120	2.8
28-16	3-37.2	20	2.1
29-10	3-37.2	0	0
29-51	3-37.2	166	2.4
28-21	Ott 8	40	2.5
28-44	Ott 8	199	2.8
29-18	1-1.1	0	0
29-40	1-1.1	0	0
29-11	2-07.8	79	2
29-50	2-07.8	90	2.7
29-54	2-07.8	97	2.65
30-26	V1	70	2.7
30-27	V1	48	2.9
28-14	2-07.5	126	3.4
28-41	2-07.5	60	2.6
29-45	2-07.5	75	2.85
28-30	2-21.9	91	3.4
28-37	2-21.9	71	3.05
29-08	2-21.9	7	1.8

29-12	2-27.0	91	2.8
29-21	2-27.0	43	2.7
30-16	2-27.0	50	2.9
28-28	2-39.2	75	2.85
29-13	1-03.8	62	2
29-01	2-39.2	0	0
29-32	2-39.2	0	0
29-27	1-03.8	110	2.6
28-09	3-33.1	63	2.8
29-20	3-33.1	97	3.25
28-05	3-39.1	60	2.35
28-26	3-39.1	6	2.2
28-33	3-39.1	89	2.85
30-19	T2	81	2.5
28-54	1-13.3	0	0
29-02	1-13.3	0	0
29-06	1-13.3	18	2.35
28-01	2-34.4	51	2.7
29-14	2-34.4	108	3.25
28-10	1-03.2	0	0
29-47	1-03.2	0	1.7
29-28	1-03.2	0	0
29-03	2-12.4	118	3.2
29-29	2-12.4	0	1.75
30-14	2-12.4	0	0
28-23	3-33.7	0	0
30-01	3-33.7	0	0
28-17	3-29.0	99	2.35
29-48	3-29.0	0	0
30-08	3-29.0	45	2.85
29-35	2-05.8	8	2.6
29-46	2-05.8	0	0
28-07	1-02.4	58	2.7
29-26	1-02.4	28	1.85
30-03	1-02.4	85	2.7
28-15	2-39.8	0	0
30-13	2-39.8	24	2.4
30-15	2-39.8	57	2.8
28-08	3-34.4	0	1.6
28-18	3-34.4	27	2.055
29-25	3-34.4	0	2.4

30-32	V4	0	0
30-23	T5	0	1.95
30-20	T3	3	2.9
30-21	T3	3	2.6
28-27	1-16.3	0	1.65
29-04	1-16.3	0	0

Appendix 3: Analysis of apple seedlings for juice quality.

Selection	Total Acidity	Sugar	pH	Sugar/Acid Ratio
10-1-39.7	0.33	18.40	3.90	55.8
10-1-9:19	0.31	16.80	3.78	54.2
4-41-3.2	0.32	15.60	3.88	48.8
10-1-40.4	0.40	18.60	3.82	46.5
4-41-26.7	0.40	16.10	3.99	40.3
4-41-10:25	0.39	14.40	3.79	36.9
4-34-62.1	0.50	15.00	3.44	30.0
10s-10-4:23	0.54	14.40	3.42	26.7
4-38-51.6	0.55	14.60	3.59	26.5
4-38-9.6TW	0.54	14.10	3.29	26.1
10-1-39.4	0.82	18.20	3.23	22.2
4-26-7.3	0.78	17.10	3.38	21.9
4-38-13.3	0.68	14.70	3.28	21.6
4-37-10:57	0.72	15.40	3.55	21.4
10-1-37.7	0.75	15.70	3.26	20.9
4-34-0.6	0.73	14.20	3.22	19.5
10s-11-27.7	0.72	13.70	3.36	19.0
4-25-1.4	0.79	14.90	3.30	18.9
10s-3-3:33	0.74	13.70	3.31	18.5
10s-12-44.9	0.83	15.20	3.30	18.3
4-25-10:58	0.85	15.50	3.15	18.2
10-1-29.15	0.85	15.40	3.24	18.1
10s-3-2:48	0.78	14.00	3.20	17.9
4-25-10:00	0.88	15.30	3.16	17.4
10-1-38.8	1.08	18.40	3.09	17.0
10-1A-12.5	0.96	16.30	3.26	17.0
10s-12-25.8	0.81	13.40	3.17	16.5
10s-10-4:18	0.92	15.20	3.18	16.5
10-1-34.8	1.09	17.90	3.28	16.4
4-38-13.3	0.91	14.70	3.07	16.2
10-12-11:33	0.89	14.30	3.22	16.1

10s-12-46.0	0.99	15.90	3.19	16.1
10s-2-47.2	0.90	14.40	3.23	16.0
10s-10-27.7	0.93	14.70	3.16	15.8
10s-10-3:58	0.92	14.20	3.19	15.4
10s-12-41.6	0.96	14.20	3.16	14.8
4-36-59.4	0.98	14.40	3.03	14.7
4-36-11.6tw	0.91	13.30	3.05	14.6
4-36-67.3	1.17	17.00	2.97	14.5
10s-12-27.8	1.03	14.70	3.15	14.3
10s-1-11:52	1.11	15.80	3.14	14.2
4-26-10:11	1.03	14.20	3.15	13.8
4-26-9:36	1.13	15.40	3.09	13.6
10s-12-39.3	0.96	13.00	3.20	13.5
10s-12-11:43	1.05	13.90	3.19	13.2
4-26-9:27	1.24	16.10	3.14	13.0
10s-2-1.4	1.00	12.50	3.14	12.5
10s-11-25.7	1.17	14.60	3.01	12.5
10-12-6.9	1.11	13.80	3.22	12.4
10-3A-9.7	1.03	12.80	3.14	12.4
10s-10-1.6	1.26	15.60	3.05	12.4
10s-10-24.8	1.26	15.10	3.03	12.0
10s-11-9:37	1.12	13.30	3.24	11.9
10s-10-23.7	1.26	14.90	3.12	11.8
Average	0.86	15.09	3.28	20.3

1. Project title and ADF file number.

Breeding of grapes and apples with emphasis on juice products and rootstocks-20100079 (Part 2 Grapes)

2. Name of the Principal Investigator and contact information.

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3. Name of the collaborators and contact information.

Tyler Kaban, Ellen Sawchuk, Peter Reimer, Rick Sawatzky are/ were technicians with Dr. Bors

4. Abstract/ Summary: *This must include project objectives, results, and conclusions for use in publications and in the Ministry database. Maximum of 300 words in lay language.*

A major collection of cold hardy wild grapes and worthwhile wine and eating grapes were brought to the U of Sk fruit program to be used in breeding. Cold- hardy *Vitis riparia* were intercrossed with important wine grapes and varieties from the Minnesota grape programs. 4200 seedling were planted that had resulted from 7500 seeds which were planted at 3 locations. The crosses were planned with the objectives of producing hardy red wine grapes, white wine grapes, table grapes and seedless grapes in future generations. About half of the crosses grape seedlings done have the potential for creating new varieties but all crosses could generate improved germplasm for breeding. Most likely worthwhile juice grapes will result in the short term while wine varieties will take longer to develop. Unexpectedly, 10% of the 2 year field planted grapes have already started blooming with most of those fruiting. Such precocious traits may be quite valuable in future varieties. Difficulties with the rootstock trial indicate that larger plants should be planted or that breeding should be done to improve adaptability or that site selection should be tested beforehand.

5. Introduction: *Brief project background and rationale.*

Grapes are the most valuable temperate fruit crop in the world, the North American grape and wine industry alone is estimated to be worth greater than 160 billion annually (ARS, 2007). At this present time, only a few growers attempt to grow grapes in Saskatchewan but the few that do have to use unusual overwintering techniques like mulching or burying them to survive. The coldest location in North America (other than the U of SK) where grapes are being bred is Minnesota, but cultivars bred in Minnesota by 3 different programs are not hardy here. However, wild grapes are often grown in the city of Saskatoon and two old varieties "Valiant' and 'Beta' have been grown in Saskatchewan for over 50 years. Unfortunately all these very hardy grapes have very poor fruit and juice quality. The 'riverbank' grape (V. riparia) which grows wild in Manitoba and Saskatchewan is the world's hardiest grape species and is the key source of adapted genetic variability for both a northern grape breeding program and in grafted vines. There is a chance that using this species as a rootstock might help borderline grape cultivars survive better, but breeding cold hardy cultivars is the most promising solution to this problem. A strategy of using wild grapes and hybridizing them with Minnesota grapes is most likely to give short term results, but crossing with European northern grapes might also be worthwhile.

Minnesota's grape industry model is of direct relevance to emerging viticultural regions such as the Canadian Prairies in that they have successfully developed it 'from the ground up' starting with locally adapted grape varieties. Neighbouring states such as North Dakota have taken notice of the success of the Minnesota grape growers and have opted to initiate a breeding program of their own (Pates 2010). With a population of ~700,000 North Dakotans foresee a strong local demand for grape products and a valuable alternative crop for producers (Pates 2009).

Grape breeding at the University of Saskatchewan was restarted (work had occurred in the 1980's) in 2006 by Horticulture student, Tyler Kaban, as part of his undergraduate thesis, and later continued when he did a master's degree focussing on grape breeding. Nearly 10,000 seedlings from controlled crosses were reared prior to the start of this project, although winter survival rate has been less than 1%.

The main grape breeding objectives of this project is to breed worthwhile germplasm into cold hardy germplasm and to select for improved juice quality. Juices could be used either directly as grape juice or blended with other fruits. Juices could also be used in making wine. Breeding for a quality red wine would be a long term objective, but along the way grapes for juice or eating grapes might be generated.

Grapes were included with apples in a single project because the methods for testing juice quality are the same. Also, protocols have been developed to do crosses in the greenhouse with grapes in the winter while apples can best be crossed outside. Seedlings would also be germinated at different times. By working with these two crops with different timelines throughout the year better use can be made of personnel. The U of Sask fruit program has a long history of breeding hardy apples but only recent experience with grapes. Most of our apples are hardy while most of our grapes are not. Combined, this project involves a crop almost certain to generate new varieties (apples) with a crop more likely to generate useful parents for future breeding (grapes).

6. Methodology: *Include approaches, experimental design, methodology, materials, sites, etc.*

Germplasm acquisition and Prep of Breeding parents

This project began with at least 50 varieties of grapes but throughout the project new varieties and seeds of wild types were obtained. Names of the various varieties are listed as they were used. At the end of this project there were an estimated 75 named cultivars obtained. We also obtained a large number of seeds from Russia that are currently being germinated. Some of this germplasm arrived too late to be utilized in breeding by the time of this report. Much of the activities in early spring involve propagating our existing collection varieties in our heated mistbed and growing them in 1 gallon pots during the summer. When acceptable size they would be cut back, leaves removed and put in coolers for 2 months or longer. This protocol is fully describe in Tyler Kaban's MSc thesis (2011). Plants with complete flowers needed to be emasculated and bagged. Female plants (no Pollen produced) were preferentially used in many crosses.

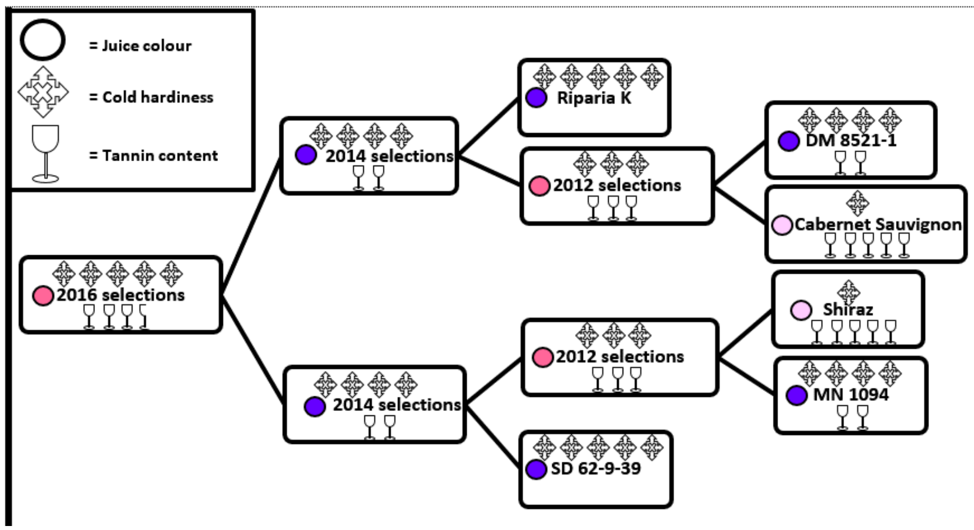
Figure 15. C-16 was used extensively in crosses. It was bred at the U of SK and is extremely hardy and a female variety. Shown is a C-16 plant in the field on a high trellis wire which had no dieback. This is very rare for a hybrid grape to be so cold hardy.

Crossing Strategy

A short-term goal is the selection of early-ripening white grapes for juice. Both neutral and aromatic types will arise. The primary breeding parent utilized in the development of such genotypes will be the hardy, ultra-early ripening selection 'C-16' (Figure 4). This vine has come through one of the coldest winters of the last decade ('08/'09) and ripens its fruit in Saskatoon by late August. Greenhouse experiments in 2011 confirmed that this genotype requires less than 750 growing degree days at base 10°C to fully ripen its fruit. Unfortunately, C-16 is a female plant that produces no pollen. Grapes are wind pollinated and are self-compatible, if they have complete flowers. We intend to release only grapes with complete flowers to make production easier for growers.

Many of the quality traits in grape are controlled by additive gene action so certain breeding strategies such as mass selection will be employed. As well, the phenomenon of transgressive segregation may be exploited in recovering moderate fruit acidities and tannins combined with extreme cold hardiness. Crossing strategies will initially combine hardy and high quality varieties. Wild hardy grapes often have black 'inky' juice which is an undesirable dominant trait. Figure 2 illustrates a set of crosses over several generations that could be done to create a hardy red grape suitable for wine. Wild grapes are usually black but there are a few white wild grapes that we are using for breeding. Currently the two varieties (Beta and Valiant) widely grown on the prairies are both black, so any red or white grape variety, even if for eating, would likely be more marketable as something different.

Figure 2: A theoretical crossing plan designed to use transgressive segregation for cold hardiness, juice colour and tannin content of grape seedlings. Long term objective is to recover *Vitis vinifera*-like quality with hardiness in progeny. While quality traits might be possible, it may be too simplistic to expect full hardiness by then as plants may need testing over several winters.



A few crosses were done with a goal of having seedless eating grapes. The seedless parent is used as a male in crosses. Seedlessness is multi-gene and usually requires extensive intercrossing to obtain seedless progeny after a few generations.

Evaluation of juice quality

The grape portion of this project mainly involved producing and planting controlled crosses. We assessed some of the parents used in breeding and some seedlings planted the 1st year of this project bore fruit by 2013. Sugar content (Brix), pH, total acidity, and tannins were assess using methods described in the Apple portion of this report. Notes were also made of grape colour. Suitable time of ripening was judged by observing that grape seeds had turned brown along with tasting and smelling a few grapes in cluster.

An important goal of this project was to become familiar with various techniques that are used in grape juice assessment. While more likely to be needed in future generations, a simple assessment for determining 'Inkyness' is illustrated in Figure 3.

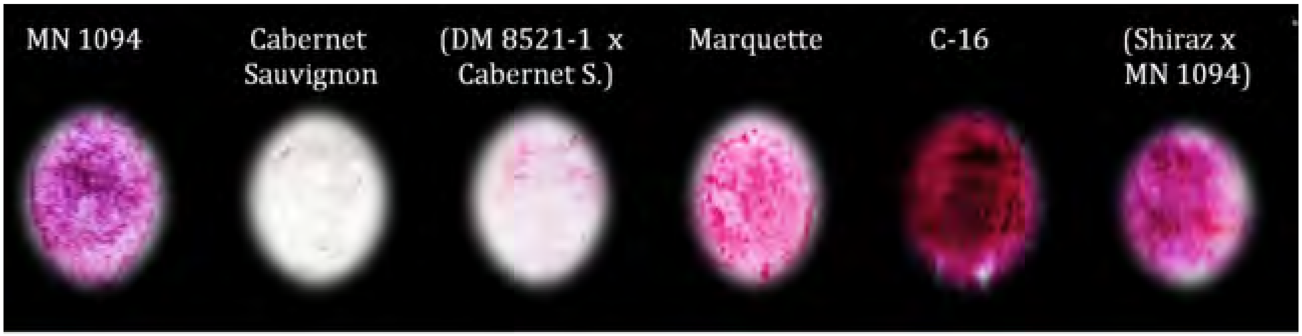


Figure 3. Qualitative assessment of anthocyanin colour and content ("inkyness") in the skins of select grapes used as breeding parents. Inner exocarps were rubbed on filter paper to release cell contents. Bluish hues as seen in 'MN 1094' and 'C-16' are undesirable in red wine grapes and attributed to the concentration of delphinidin present.

Rootstocks

The proper selection of adapted rootstocks has enabled the spread of viticulture to regions previously inappropriate for conventional grape growing. Classic European wine grapes (*Vitis vinifera* L) such as 'Cabernet Sauvignon' and 'Chardonnay' are grafted onto wild grape roots from North America (often *Vitis riparia* M.) because they are resistant to phylloxera, a small almost microscopic root aphid. Phylloxera heavily decimated European vineyards in the late 1880's wiping out 2/3rds of France's orchards in about 10 years. As we will be breeding with European grapes there could be a possibility that the natural resistance in wild Canadian grapes could be bred out of our selections and that we will need rootstocks. There is also the possibility that grape rootstocks could assist in cold hardiness, early ripening, drought tolerance, or possibly reduce or increased vigour to cultivars. If hardiness influence was strong enough, perhaps a cold hard grape rootstock could make borderline hardy grapes (like Minnesota varieties) survive Saskatchewan conditions. Breeding for grape rootstocks is common for gaining phylloxera resistance but breeding for increasing cold hardiness is virtually unknown.

In 2011 germplasm was gathered for potential use as rootstock breeding or use in rootstock trials. Grapes at our Hort field from the 1980's were evaluated for health and lack of winter damage. Three were selected (see Figure 4). It was also planned to use these as a source of hardiness for crossing with quality grapes.

Figure 4. Wild grapes planted 25 years earlier at the U of Saskatchewan Horticulture Field Lab were scrutinized for hardiness and the 3 best were propagated. They had been planted along the fence and against a building. Rick Sawatzky was involved in the previous breeding project and indicated that these were all pure V. riperia but that they had superior fruit quality compared to most wild grapes and that some may have green fruit.

It was planned to propagate and field plant an assortment of rootstocks in four reps. The following year the rootstock would have a Minnesota variety 'Marquette' grafted onto them at various heights as shown in Table 1. Marquette is a zone 4 variety and is the most widely grown of the Minnesota varieties. Saskatoon is located in Zone 2. It was also planned to graft 'Petite Pearl' and 'Frontenac blanc' on various hardy rootstock as these two varieties are reputed to be hardy to -38C.

Table 10. Plan for a rootstock trial to determine if rootstocks could convey hardiness with the Minnesota variety 'Marquette'. Numbers in parentheses indicate length of the rootstock vine in meters at which the scion would be grafted.

Block #1		Block #2	
Rip K (0.5')	Marquette (5')	Marquette (5')	Marquette (0.5')
C-16 (3')	Valiant (0.5')	C-16 (5')	Rip K (3')
Valiant (3')	Marquette (0.5')	C-16 (0.5')	Rip K (5')
Marquette (3')	Valiant (5')	C-16 (3')	Valiant (0.5')
C-16 (5')	Rip K (5')	Rip K (0.5')	Marquette (3')
C-16 (0.5')	Rip K (3')	Valiant (3')	Valiant (5')
Block #3		**Block #4**	
Valiant (0.5')	Marquette (3')	C-16 (0.5')	Marquette (0.5')
C-16 (3')	Marquette (5')	Marquette (5')	C-16 (5')
Marquette (0.5')	Valiant (5')	Rip K (3')	C-16 (3')
C-16 (0.5')	Rip K (3')	Rip K (0.5')	Valiant (5')
C-16 (5')	Valiant (3')	Marquette (3')	Valiant (0.5')
Rip K (5')	Rip K (0.5')	Valiant (3')	Rip K (5')

Grape rootstocks were propagated, greenhouse grown and then planted in August 2012 (Figure 5). This field had higher ground that was not prone to spring flooding and seemed similar to an area nearby where grapes were doing well.

Figure 5. Field planting of grape rootstocks in 2012. They were planted between rows of haskap which was would provide extra wind protection and hold more snow during winter for additional insulation.

Germinating and starting grape seeds

Seeds are soaked in water, a one part three percent hydrogen peroxide solution to 5 part water solution, and a 1000ppm gibberellin solution. Then the seeds are placed in damp peat moss in the cooler for 3 months. Peat moss is wetted more and the seeds and peat moss are placed under the lights in the greenhouse until they germinate. The germinated seeds are removed and planted into plug trays and are allowed to grow in the greenhouse for a few months. Commonly only half of the seeds will result in healthy seedlings either because of poor germination or poor vigour. In early June they are placed in the shade house outside to get them acclimated. After a few weeks they are relocated to receive full sunlight in a nursery area until they are planted. At the time of planting weak seedlings are culled and only healthy plants are planted.

Field planting of grape seedlings from controlled crosses

All seedlings in 2011 and 2013 were planted at the U of SK. In 2012 two groups of seedlings were divided up between Jefferies Tree Nursery in Manitoba and Terra del Sol which is located near Saskatoon (figure 6). Jeffries Nursery is one of the largest nurseries in Manitoba and has a long history of doing selection trials for ornamental crops, and has in the past tested some of our ornamental crab apples and cherries for us. The owner, Wilbur Ronald, was an Agriculture Canada breeder of ornamentals. Michel Touchette will be in charge of that trail at their location. This site has higher humidity and milder winters than the Saskatchewan sites. It is expected that leaf diseases and fruit rots could be more of a problem there. Since this site is in zone 3b/4a, it would be a good site for hybrids likely to be less hardy or F1 hybrids between *V. riparia* and *V. vinifera* might survive there. Theoretically we could intercross worthwhile plants from this site and then grow that next generation in colder locations. It would be a logical site to assist in the introgression of new genetics form warmer-adapted varieties. We had an abundance of seedlings from one specific cross so we decided to plant all of those seedlings at Terra Del Sol as it would be easy to maintain records that way.

Figure 6. Planting sites of U of SK grape seedlings in 2012. All three sites have dark brown soils but the University of Saskatchewan in Saskatoon is clay (A), Jeffries Nursery at Portage la Prairie MB is loam (B), while Terra del Sol Farm, near Saskatoon is sandy loam (C). Soils of the two cooperator sites are considered ideal for grape production but both are farther north than normal grape production.. Clay is less desirable since the soils warm up more slowly and grapes have a rather long growing season. Potentially more root problems could occur on clay soils if not well drained but the sites picked at the U of SK Horticulture Fields were chosen for have good drainage most years.

Evaluations of fruiting seedlings

In the spring of 2013 it was noted that some of the seedlings that were planted in 2011 were fruiting. In addition to that, some of the vines that were transplanted from one field at the Hort research plots to another more suitable area were also flowering. A coloured tag was tied to each vine which was flowering. In the fall the tagged plants were scrutinized and fruit was harvested from plants that had good fruit. The fruit was frozen and used and the juice later evaluated in the lab that winter. Tags were left on to identify these precocious plants. Lab analysis was conducted in November of 2013. The clusters were weighed in their entirety; the stems were not removed. This was done to evaluate precocious productivity. There were also assessed based on flavour, presence of seeds and colour. As shown in table 7 all of the grapes were purple. We did find two green grapes later in the season in a different location but those had been hit by a frost. Since the other seedlings had been picked before the frost the green grapes were omitted. The vines were marked and will be analyzed next year.

The acid and sugar content of the various seedlings was also evaluated. This was done using an automated titrator and a digital refractometer . From these measurements the sugar to acid ratio was determined.

7. Research accomplishments: *(Describe progress towards meeting objectives. Please use revised objectives if Ministry-approved revisions have been made to original objectives.)*

Objectives	Progress
1) Breeding and Selection of table grapes, juice grapes, wine grapes and rootstocks	Approximately 6000 seedlings were produced and planted out in the three years that this grant ran. We are beginning to see the fruits of our labours as the first batch of seedlings. Two white/green grapes were found and selected as well as another 25 red/purple grapes. More selections will be made every year as more vines come into bearing.
2) Test grape rootstocks for their ability to convey winter hardiness to varieties grafted onto it. Develop production practices around these new rootstocks	Potentially useful hardy rootstocks were obtained and propagated but many had winterkill and poor growth the following season. They did not make sufficient growth to carry out grafting experiment. The rootstocks that had not over wintered were re-propagated to be re-planted in spring 2014. Possible lessons from this may be that rootstocks need to be container grown to a much later size and/or crosses should be done amongst potential rootstocks to develp hardier offspring.
3) Analytical assessments of sugars and acids useful for wine makers	Lab analysis was conducted in November of 2013. The clusters were weighed in their entirety; the stems were not removed. This was done to evaluate precocious productivity. There were also assessed based on flavour, presence of seeds and colour. The acid and sugar content of the various seedlings was also evaluated. This was done using an automated titrator and a digital refractometer . From these measurements the sugar to acid ratio was determined. At this time a taste test was also performed. The majority of the grapes were very sour but a select few actually tasted quite pleasant. Valiant and C-16 were also evaluated at this time as a direct comparison for the seedlings.

8. Discussion: *Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

Rootstocks

The field trial was propagated and field planted but winterkill was too great and regrowth of the survivors too poor to continue with plans for grafting onto them. As a matter of record, snow arrived at the beginning of October 2012 and stayed until the beginning of May 2013 with is perhaps a month longer than normal. We often plant seedlings of many other fruits in early august, but perhaps with grapes rootstock this is not long enough. But these plants were planted in late August. The rootstocks that had not over wintered were re-propagated. Likely we will test these grape rootstocks in several locations. And may retry the plan designed in the future.
The stenting experiments planned which would have tried to simultaneously root a rootstock and graft on a scion variety were not done. This aspect of the project was perhaps too ambitious. It would have been better to have the rootstock already well-established before attempting such a project. Stents would be better done at a later stage of grape breeding. It seemed better to focus on the apple portion of this project seemed more attainable for worthwhile results for growers. More was done with the apples than originally planned. Visiting cider makers in BC, obtaining fruit for evaluations and trees for breeding were all much more than planned.

A positive result regarding grape rootstocks is that potentially useful germplasm was identified and gathered. A batch of about 300 seeds of the very cold hardy species *Vitis amurensis* came from Russia too late to be included in this project. It may be worthwhile to consider interbreeding this collection of hardy germplasm and growing seedling in various location.

Identifications of useful parents for tannin production

Knowing that V. riparia has not been known to have high levels of tannins, some varieties were screening in the greenhouse and evaluated for tannin levels (figure 7). Shiraz had the highest levels which it passed onto one of its descendants. These and a few others would be worthwhile to use in breeding for higher tannin content.

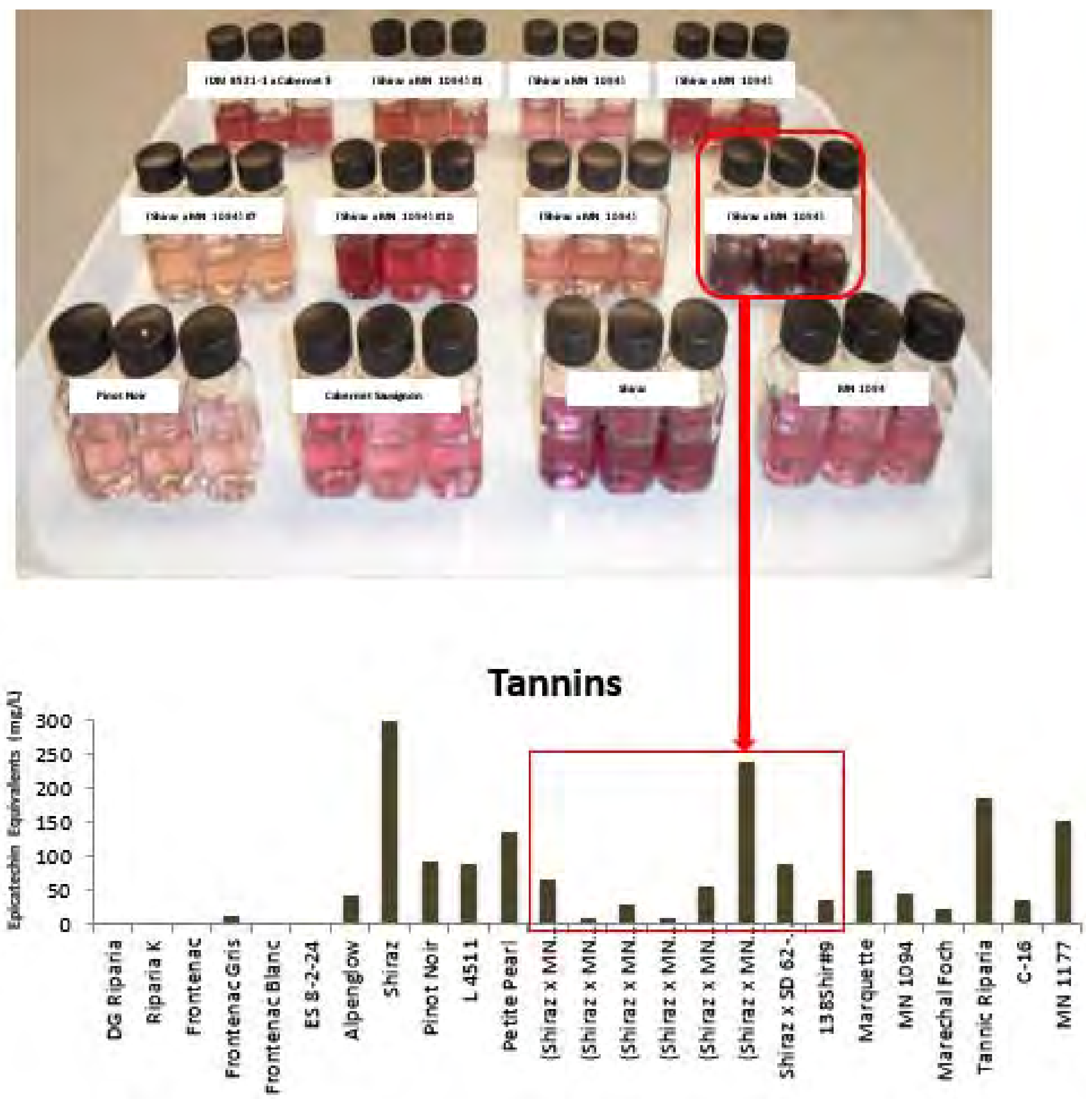


Figure 7. Tannin content of parent vines used in grape breeding, U of SK greenhouse selections are outlined in red box. Genotype 'Shir94#16' is an example of a selection with good tannin structure. Based on absorbance units at 280 nm, average of three replicates. Tannins are compounds is higher quality wines that improve as the wine is aged, imparting to the wine a desirable mouthfeel. Tannins are usually lacking in grapes breed in more northern locations.

Crosses, seeds, and plants in the field

Tables 2 through 6 give details about crosses, seeds produced and seedling planted during this project. About 7500 seeds were created which resulted in 4600 plants in the field. 1231 of those seeds are being germinated at the time of this report. Quite likely it will be 3 of 4 years before the last of these plants can be evaluated in the field. As the lead technician on this project, Tyler Kaban, left us for a higher paying job, he was asked to include notes for each cross regarding expectations for selection. The columns called "cross objectives" give us an idea of what colours and uses are expected to arise within the seedling population. The column called "Use in Program" indicate whether we might get selections both hardy and high quality that could be released as new varieties or whether we should just expect better germplasm that could be used in breeding.

Table 2. Parents used in crosses done in early 2011. This table provides useful information regarding whether a plant might be female or hermaphrodite (complete flowers). Notes were also made regarding potential end goals of using these parents for future breeding plans. The U of SK greenhouse selections were those hybrids with superior vigour and fruit qualities that had been fast tracked in a greenhouse but not evaluated outside. The field selected plants had superior winter survival but fruit has not yet been seen from these. All of these hybrids have a very hardy parent. It is expected that seedlings resulting from intercrossing these hybrids will have higher amounts of hardiness than the average F1 hybrid of V. vinifera x V. riparia. Some crosses have multiple selections; for example there are 8 selections from Valiant x Frontenac.

Germplasm Names & Codes	Used in crosses		Pollen Collected	Primary Use
	♀ parent	♂ Parent		
Vitis vinifera cultivars				
Cabernet Franc	√		√	Red Wine
Cabernet Sauvignon	√	√	√	Red Wine
Merlot	√		√	Red Wine
Pinot Noir	√	√	√	Red Wine
Riesling	√	√	√	White Wine
Shiraz	√	√	√	Red Wine
Vitis riparia germplasm				
DG Riparia	√			Wine/Table/Juice
Riparia K	√			Wine/Table/Juice
SD 62-7-2		√	√	Wine/Table/Juice
SD 62-8-138	√			Wine/Table/Juice
SD 62-9-39		√	√	Wine/Table/Juice
Hybrid cultivars				
72B		√	√	Table/Juice
Amurski #1		√	√	Red Wine
Aris		√	√	White Wine
DM 8521-1	√		√	Red Wine
ES 10-18-14		√	√	White Wine
ES 2-8-1	√	√	√	White Wine
ES 3-22-16		√	√	Table/Seedless
ES 417	√			White Wine
ES 4-23-60	√			White Wine
ES 8-2-43		√	√	White Wine
Frontenac Blanc	√	√	√	White Wine
Kay Gray			√	Table/Juice
LaCrescent		√	√	White Wine
Landot 4511	√			Red Wine
Louise Swenson		√	√	White Wine
Marquette	√	√	√	Red Wine
MN 1094		√	√	Red Wine
Petite Pearl	√	√	√	Red Wine
Prairie Star			√	White Wine
Ripinot #1		√	√	Wine/Table/Juice
Valiant			√	Table/Juice

Table 2 Continued.

Germplasm Names & Codes	Used in crosses		Pollen Collected	Primary Use
	♀ parent	♂ Parent		
U of SK hybrids, greenhouse selected				
CCab #8		√	√	Red Wine
CShir #20		√	√	Red Wine
CShir #5		√	√	Red Wine
DMCab #1		√	√	Red Wine
DMCab #3	√	√	√	Red Wine
DMCab #7	√			Red Wine
DMCab #8	√			Red Wine
Shir138 #4		√	√	Red Wine
Shir138 #6		√	√	Red Wine
Shir138 #7		√	√	Red Wine
Shir94 #1	√	√	√	Red Wine
Shir94 #11	√	√	√	Red Wine
Shir94 #13		√	√	Red Wine
Shir94 #15		√	√	Red Wine
Shir94 #3		√	√	Red Wine
Shir94 #5		√	√	Red Wine
Shir94 #6		√	√	Red Wine
Shir94 #9		√	√	Red Wine
U of Sk hybrids, field selected				
B-102		√	√	Table/Seedless
C-16	√			Wine
Valiant x Frontenac #1-8		√	√	Table/Juice
Valiant x Suffolk		√	√	Table/Juice
Valiant x V. rip #1-3		√	√	Table/Juice

Table 3. Lineage of successful crosses done in early in 2011 and field planted later that year. All of these seedling were planted at the U. of SK.

Pedigree	# seedlings in field	Cross objective	Use in program
U of SK Plots Block 11 2011 planting			
(DG Rip x MN 1094)	89	Red wine	Parent*
(Rip K x MN 1094)	145	Red wine	Parent
(Marquette x SD 62-9-39)	11	Red wine	Parent
(DG Rip x Marquette)	162	Red wine	Parent
(Rip K x Marquette)	104	Red wine	Parent
(Frontenac Blanc x ES 8-2-43)	20	White wine	Parent
(C-16 x Petite Pearl)	39	Red wine	Parent/selection**
(C-16 x Marquette)	84	Red wine	Parent/selection
(C-16 x Alpenglow)	190	White wine	Parent/selection
Total	844		

*Parents will be used in future breeding, **Parents/selections have the potential for future breeding a cultivar development in the short-term.

Table 4. Lineage of successful crosses done in winter of 2011/2012 and field planted in 2012. Only about a thirds of the plants went to the U of SK. The other 2/3rds went to a farm having sand soil just outside of Saskatoon and to a cooperator in Manitoba with a warmer hardiness zone. By using these remote locations we had hoped for better survival.

Pedigree	# seedlings in field	Cross objective	Use in program
U of SK Plots. Block 18. 2012 Planting			
(C-16 x LaCrescent)	229	White wine	Parent/selection
(C-16 x Kay Gray)	97	White wine	Parent/selection
(C-16 x ES 8-2-43)	99	White wine	Parent/selection
(C-16 x Valiant)	29	Table	Parent/selection
(C-16 x Marquette)	9	Red wine	Parent/selection
(C-16 x Petite Pearl)	106	Red wine	Parent/selection
(ES 4-23-60 x SD 62-9-39)	154	White wine	Parent
(Shir94#1 x SD 62-9-39)	50	Red wine	Parent
(Rip J x ES 2-8-1)	94	White wine	Parent
(Rip K x Kandiyohi)	113	Table	Parent
(Rip I x Petite Jewel)	67	Table	Parent
(Rip L x ES 2-8-1)	5	White wine	Parent
(Rip L x Somerset)	11	Table	Parent
(Rip L x Petite Jewel)	12	Table	Parent
(Rip K x ES 2-8-1)	30	White wine	Parent
(DG Riparia x Kandiyohi)	24	Table	Parent
(Rip A x Kandiyohi)	10	Table	Parent
(DG Riparia x Petite Jewel)	21	Table	Parent
(Rip A x ES 8-2-43)	27	White wine	Parent
(Rip A x ES 2-8-1)	17	White wine	Parent
(Rip A x Petite Jewel)	22	Table	Parent
(Rip K x Petite Jewel)	131	Table	Parent
(Shir94#1 x SD 62-9-39)	17	Red wine	Parent
(Shir94#1 x SD 62-7-2)	48	Red wine	Parent
(Shir94#3 x SD 62-9-39)	84	Red wine	Parent
(Shir94#11 x SD 62-7-2)	17	Red wine	Parent
(Shir94#1 x DMCab#3)	6	Red wine	Parent
(Petite Pearl x Ripinot)	5	Red wine	Parent
(Shir94#3 x DMCab#1)	3	Red wine	Parent
(ES 417 x SD 62-9-39)	75	White wine	Parent
Total	1168		
Jefferies Nursery 2012 Planting			
(C-16 x MN 1094)	505	Red wine	Parent/selection
(Frontenac Blanc x ES 10-18-14)	78	White wine	Parent
(ES 4-23-60 x Frontenac Blanc)	109	White wine	Parent
(ES 417 x Frontenac Blanc)	56	White wine	Parent
(Petite Pearl x Ripinot)		Red wine	Parent
(DMCab#3 x Marquette)		Red wine	Parent
(ES 4-23-60 x SD 69-9-39)		White wine	Parent
(Shir94#1 x SD 62-9-39)		Red wine	Parent
(C-16 x Petite Pearl)	336	Red wine	Parent/selection
Total	1144		
2012 Tierra del Sol Planting			
(C-16 x MN 1094)	1200	Red wine	Parent/selection
Total	3512		

Table 5. Crosses done in winter of 2012/2013 and number of seedling that were field planted in 2013. Germination was very poor and many seedlings died in the greenhouse. It is unknown what caused such an unusually high death rate.

Pedigree Info	# seeds	Plants in Field	Cross objective	Use in program
Crosses completed for spring 2013 planting:				
(C-16 x ES 10-18-14)	417	37	White wine	Parent/ selection
(C-16 x B-102)	276	38	Table	Parent/ selection
(C-16 x Frontenac Blanc)	15	105	White wine	Parent/ selection
(C-16 x DMCab#3)	252	34	Red wine	Parent/ selection
(C-16 x Shir94#1)	200	11	Red wine	Parent/ selection
(C-16 x Shir94#11)	45	4	Red wine	Parent/ selection
(Shir94#16 x SD 62-9-39)	195	26	Red wine	Parent
Total	1400	255		

Table 6. Crosses done during the summer of 2013 that are being germinated in early 2014.

Crosses completed for spring 2014 planting:				
C-16 x (Shiraz x Mn1094 #15)	170			Parent/ selection
C-16 x (Shiraz x Mn1094 #7)	4			Parent/ selection
C-16 x (Shiraz x Mn1094 #9)	102			Parent/ selection
C-16 x (SD62-8-138 x Shiraz #3)	52			Parent/ selection
C-16 x (SD62-8-138 x Shiraz #6)	163			Parent/ selection
C-16 x (Shiraz x Mn1094 #11)	50			Parent/ selection
C-16 x (Shiraz x Mn1094 #5)	90			Parent/ selection
C-16 x DmCab #3	200			Parent/ selection
C-16 x (SD62-8-138 x Shiraz #4)	400			Parent/ selection
Total	**1231**			

2 Field Evaluations 2013

Out of a planting of over 800 seedlings in 2011 only 85 flowered in 2013 (figure 8). Of these only 48 set fruit. Since female plants were used in breeding perhaps some or most of the plants were females that had failed to set. With only 10% of the plants in bloom pollen was at low density. Plants producing their own pollen were much more likely to self. The winter of 2012/2013 had heavy snow which insulated the grape seedling in that field very well as almost all seedlings were alive. Thus, it was not a good test winter for hardiness. The row closest to the shelterbelt bushes seemed to have less dieback and each row farther out had progressively more dieback.

Evaluations of these first plants to fruit are compiled in table 7. Noteworthy is that most selections are at the 20 Brix level which is a high enough sugar level for wine making. Only 8 selections have are within the optimum ranges of 15 to 20 for a sugar Acid Ratio, indicating that most of this generation is too acidic (figure 8). Lower acidity may be an important trait to focus in in future breeding.

Tannin analysis was not done on these seedlings as there were very few that had sufficient material to do all three tests (brix, acidity and tannins). Tannin analysis will be done next year on the seedlings that had a good sugar:acid ratio this year, and are hardy enough to get through the winter.

Promising seedlings that were productive, hardy and had a fairly good flavour were flagged and cuttings were taken in late fall. This were rooted in the greenhouse and will be allowed to grow and will be used for future breeding. There were approximately 25 flagged for rooting.

Figure 8. One of the rows of grape seedlings planted in 2011. Plants showed vigorous growth in that location with about 10% of them blooming.

Table 7. Evaluations of the first seedling to produce fruit from the seedlings planted in 2011. Seedlings are named by their location in the field.

Variety	Brix	Acidity	Sugar:Acid Ratio	Weight (g)	Seeds	Colour	Flavour
BLK 10 #27	23.1	NOT ENOUGH JUICE	N/A	7.22	Yes	Red	semi sweet
C-16	24.9	0.45	55	N/A	Yes	Red	sweet
BLK 10 #30	16.8	NOT ENOUGH JUICE	N/A	6.52	Yes	Red	semi sweet
BLK 10 #28	21.3	NOT ENOUGH JUICE	N/A	3.93	Yes	Red	semi sweet
BLK 11 #3	19.6	1.64	12	9.85	Yes	Red	sour
BLK 11 #11	18.6	1.76	11	8.49	Yes	Red	sour
BLK 11 #43	17.3	1.82	10	24.49	Yes	Red	sour
BLK 11 #22	20.4	1.67	12	41.00	Yes	Red	sour
BLK 11 #9	20.8	1.58	13	9.52	Yes	Red	sour
BLK 11 #7	20.5	1.41	15	57.36	Yes	Red	sour
BLK 11 #10	20.6	1.58	13	17.42	Yes	Red	sour
BLK 11 #2	18.3	1.60	11	8.19	Yes	Red	sour
BLK 11 #6	19.4	1.58	12	26.09	Yes	Red	sour
VALIANT	19.7	0.36	55	N/A	Yes	Red	sweet
BLK 11 #4	20.8	1.18	18	14.84	Yes	Red	sour
BLK 11 #8	22.0	1.71	13	38.65	Yes	Red	sour
BLK 11 #1	23.0	1.27	18	35.51	Yes	Red	sour
BLK 11 #5	20.0	1.44	14	19.09	Yes	Red	sour
BLK 11 #32	18.5	1.71	11	18.45	Yes	Red	sour
BLK 11 #15	17.7	1.56	11	18.02	Yes	Red	sour
BLK 11 #34	18.9	1.92	10	13.38	Yes	Red	sour
BLK 11 #41	20.1	1.75	11	45.01	Yes	Red	sour
BLK 11 #29	18.7	1.62	12	7.79	Yes	Red	sour
BLK 11 #44	19.6	1.98	10	21.83	Yes	Red	sour
BLK 11 #19	21.1	1.40	15	5.69	Yes	Red	sour
BLK 11 #14	18.5	1.45	13	31.93	Yes	Red	sour
BLK 11 #39	22.8	1.64	14	58.36	Yes	Red	sour
BLK 11 #30	22.5	0.88	26	12.37	Yes	Red	semi sweet
BLK 11 #26	21.2	1.37	15	14.67	Yes	Red	sour
BLK 11 #20	16.8	2.22	8	15.18	Yes	Red	sour
BLK 11 #37	16.3	1.61	10	15.76	Yes	Red	sour
BLK 11 #45	20.4	1.11	18	61.84	Yes	Red	sour
BLK 11 #42	21.3	2.22	10	17.54	Yes	Red	sour
BLK 11 #13	20.6	1.81	11	25.54	Yes	Red	sour
BLK 11 #36	18.6	1.67	11	29.53	Yes	Red	sour
BLK 11 #27	18.3	1.22	15	103.85	Yes	Red	sour
BLK 11 #31	16.6	2.22	7	96.22	Yes	Red	sour
BLK 11 #38	19.4	1.90	10	38.55	Yes	Red	sour
BLK 11 #35	19.3	1.33	15	13.51	Yes	Red	sour
BLK 11 #40	19.1	2.03	9	22.33	Yes	Red	sour
BLK 11 #18	20.1	1.96	10	9.00	Yes	Red	sour
BLK 11 #16	22.8	1.63	14	22.44	Yes	Red	sour
BLK 11 #24	15.4	1.72	9	46.37	Yes	Red	sour
BLK 11 #25	22.0	1.14	19	12.79	Yes	Red	sour
BLK 11 #12	20.7	1.30	16	17.16	Yes	Red	sour
BLK 11 #17	18.0	1.16	16	3.60	Yes	Red	sour
BLK 11 #23	21.1	1.47	14	17.75	Yes	Red	sour
BLK 11 #28	20.9	1.62	13	155.33	Yes	Red	sour
BLK 11 #33	22.2	1.72	13	15.11	Yes	Red	sour

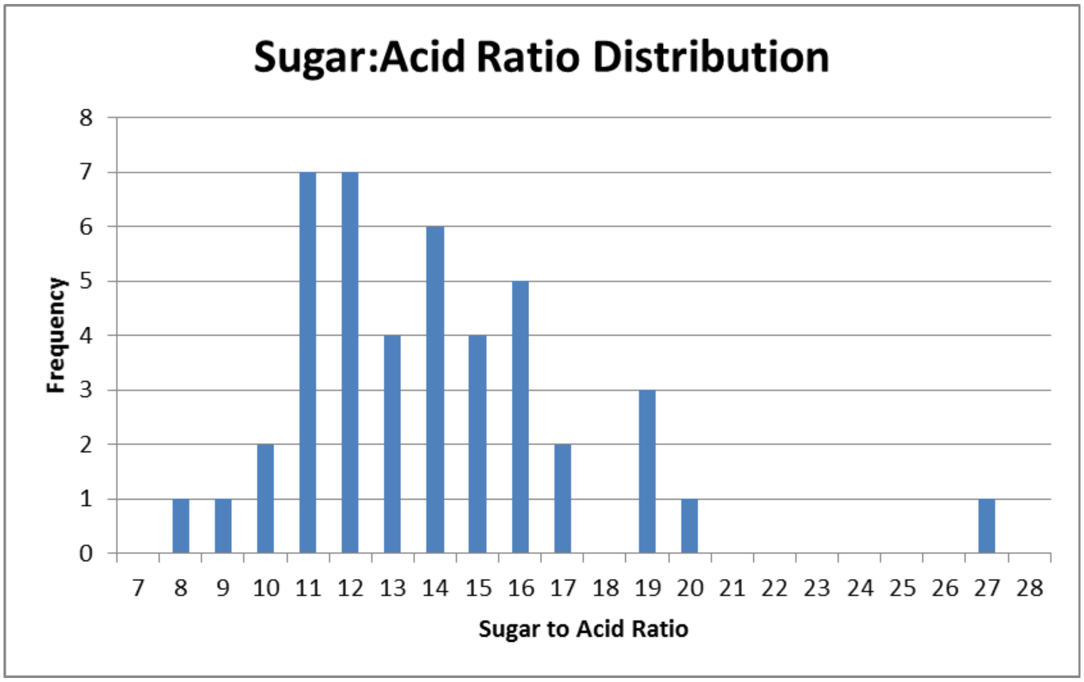


Figure 9. Frequency of seedlings for sugar acid ratios.

Breeding Notes and observations

In greenhouse selection experiments (June 2011 to January 2012), 'C-16' has proven to readily pass on the early ripening trait as well as Muscat-type flavours and white fruit to progeny. With this knowledge attained through rigorous greenhouse screening, 'C-16' served as the core in our strategy to breed white wine grapes for the Prairies. When crossed with the highest quality, white-fruited interspecific cultivars available, 'C-16' should produce progeny with black, red and white fruit. Fifty percent of the seedlings will be black fruited, 25% will be red and 25% will be the desired whites

A semi-hardy seedless genotype 'B-102' has been identified in our field trials. "Seedlessness" is a loose term that describes under-developed seeds that can range in size from non-existent to very small and un-noticeable as in most hybrid seedless cultivars. In 'B-102', seed traces are small and soft (Figure 13) so are easily consumed like in other berries such as strawberry or blueberry.

Figure 10. Seedless B-102 shown with its parents. Seedlessness was not expected to occur in this generation.

A few crosses were made with more than 1000 seeds that were treated with Treflan to make tetraploid grapes. Treflan has been reported for use in grapes as a substitute for colchicine to double ploidy levels. Tetraploids can be used in crosses to diploids to make seedless triploids grapes. The triploid strategy is used in seedless watermelon and banana breeding. Success in creating polyploids can be as high as 5% but often is 1% in some crops. Poor results were seen from this trial.

Some seedlings that were planted at the U of S plots in 2009 were relocated near where the 2011 planting was done. This was done because the 2011 seedlings put out a massive amount of growth in the 2012 growing season so we knew that location was conducive to growing grapes. It was suprizing to see such differences on our own plots which we had previously considered rather similar.

All three of the locations as previously mentioned had not only different weather conditions but very different soil profiles as well. This is shown below in Figure 11.

3.3 GROWER-ASSISTED SELECTION

Due to the very large seedling populations that were generated as a result of the highly efficient greenhouse breeding, co-operator growers throughout the Prairies assisted us in the selection process. Having early testing sites throughout the Prairies will contribute greatly in the selection of genotypes that have broad adaptability. In addition, co-operators gain value experience in grape culture which will be instrumental when cultivars are eventually released. As previously mentioned, co-operators for the 2012 plantings included Jeffries Nursery in Portage la Prairie, Manitoba and Terra del Sol (John Cote) of Saskatoon, Saskatchewan.

For the 2013 only a small number of seedlings survived the planting and hardening off so no co-operators were required. We did hear back from the people at Jefferies Tree Nursery in Manitoba that the seedlings were doing well there and only a few had died outright. Plans to travel back to Portage la Prairie in the fall of 2014 to collect any fruit are in the works.

3.4.1 Additional Co-operators

Many additional growers were willing to take on future seedlings or test advanced selections arising from the seedlings planted during this project. Olds college experience cutbacks and decided not to be involved in trials.

1. **Southern Saskatchewan, Cypress Hills.** This site is in cooperation with Marty and Marie Bohnet of 'Cypress Hills Vineyard and winery' Located in zone 3 on a higher elevation, it is expected that this will be the easiest site to grow grapes. Under drier conditions, fewer leaf diseases are expected. Because the cooperators are wine makers we expect to have more insight into fruit quality at this location.
2. **Southern Saskatchewan, Lumsden.** This site is in cooperation with Dean and Sylvia Krutzer of 'Over the Hill Orchards'. The site has clay soil but is very well drained on a steep slope by the river. They are interested in grape juice production which will be a worthwhile contrast to other Saskatchewan sites where owners are more interested in wine.
3. **Central Saskatchewan, Purdue**. This site is in cooperation with Vance Lester and Sue Echlin of the 'Living Sky Winery'. A similar site to the U of SK but in cooperation with an active winery where there is much interest in selecting superior grapes for wine.
4. **Northern Saskatchewan, Birch Hills/Prince Albert Area.** This site is in cooperation with Curtis Braaten and the 'Conservation and Learning Centre'. This site has the lowest temperatures of the major locations. This site will be a major site for breeding rootstocks for cold hardiness and improving fruit quality of *Vitis riparia.* It can also be used at a secondary testing site for cold hardiness of advanced selections.

9. Conclusions and Recommendations: *Highlight significant conclusions based on the previous sections, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project.*

Much germplasm was brought together and a large number of crosses were done with plants created and planted. Some of the first seedlings planted were beginning to come into production but it will be a few years before these seedlings can be evaluated. Many parents were also evaluated and some were particularly noted for tannin content. It is expected that some new varieties could be produce from these seedling and that improved parents for future breeding will result.

The rootstock trial had difficulties and could not be established during the timeframe due to winterkill. Alternative strategies for growing and breeding grape rootstock have been proposed. The rootstock portion of the project is not as important as having good fruiting varieties.

10. Success stories/ practical implications for producers or industry: *Identify new innovations and /or technologies developed through this project; and elaborate on how they might impact the producers /industry.*

Improved germplasm has been created that will benefit the breeding of grapes varieties for Saskatchewan. The poor quality grapes currently available in Saskatchewan are over 50 years old, and have 'inky' colour and poor quality. Grapes arising from this improved germplasm will be much superior.

11. Patents/ IP generated/ commercialized products: *List any products developed from this research.*

If superior selections arise from the seedling created in this project, they will likely undergo PBR certification. But it is most likely that superior parents for future breeding will be the result of this project.

12. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines.*

We had a small workshop at the U of SK greenhouse for those interested in grapes in April 2011 which was attended by about 15 people. We went over the goals of this grant and discussed with growers how they would like to participate.

ADF-funded research in grape breeding at the University of Saskatchewan has generated valuable knowledge shared at two major conferences in 2012. The annual meeting for the North American fruit enthusiast group NAFEX was hosted by the U of SK this past summer and the International conference on northern grape growing Vitinord was held in Neubrandenburg Germany in November/December. Both conferences were attended by Tyler Kaban and data from our grape breeding program was presented. These opportunities broaden interest and knowledge of our unique program and Saskatchewan agriculture in general. Tyler was an invited speaker at Vitinord, and the conference paid for most of his expenses.

In September of 2013 Apple and Grape day was hosted by the fruit program. During this day we spent the morning touring around the apple and grape orchards tasting and checking out all of our germplasm and the afternoon was spent listening to talks from various employees and staff of the fruit program. This field day was tailored to both home and commercial growers.

Conferences in Grand Rapids, Michigan and in Vancouver, British Columbia were attended by members of the fruit program. These conferences were attended in order to learn about how apples and grapes are grown in other regions, what cultivars are grown and attempt to network and find prospective co-operators. It was also a good way to promote the work that we do hear and introduce people to the concept of prairie grown fruit. Travel was funded by the Fruit Program and by individual staff members who worked the trips into a vacation.

Figure 11. An Apple and grape day were held in Sept 2013 at the U of SK. Participants were told about our reseach and visited the apple and grape plantings.

13. **List any industry contributions or support received.**

Major in-kind support was given by Jeffries Nursery in Portage la Prairie, Manitoba and Terra del Sol (John Cote) of Saskatoon, Saskatchewan by allowing us to plant large blocks of seedlings.

The Alberta Horticulture Conference had funded grape research immediately prior to this grant by funding Tyler Kaban's MSc research for $32,000.

Royalties from the sale of fruit varieties funded equipment purchases and about 1/3rd of the salaries for this project.

14. **Is there a need to conduct follow up research?** *Detail any further research, development and/or communication needs arising from this project.*

In a few years these seedlings will mature and can be evaluated for fruit quality and hardiness.

15. **Acknowledgements**. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.*

The Saskatchewan Ministry of Agriculture is acknowledged in all our presentations, usually with ADF printed or spoken.

Our fruit website has a Saskatchewan Ministry of Agriculture symbol in the upper right hand corner which when clicked goes directly to the ADF website where research reports can be downloaded.

Students and growers interested in more details are urged to visit the ADF website.

16. **Appendices:** *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited*

1 References

Sarneckis, C. J., Dambergs, R. G., Jones, P., Mercurio, M., Herderich, M. J., & Smith, P. A. (2006). Quantification of condensed tannins by precipitation with methyl cellulose: Development and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research, 12*(1), 39-49. Retrieved from www.scopus.com

Statistics Canada. (2006). *From the Vine to the Glass: Canada's Grape and Wine Industry*. (Catalogue No: 11-621-MIE2006049) Published by authority of the Minister responsible for Statistics Canada

2 Equipment

HI 84432 Titratable Acidity Mini Titrator and pH Meter for Fruit Juice. HANNA INSTRUMENTS CANADA INC. 3156, boul. Industriel, Laval, Québec (Canada) H7L 4P7

PAL-α ATAGO Digital Pocket Refractometer. Office : 32-10 Honcho, Itabashi-ku, Tokyo 173-0001, Japan

3 Appendices

The following article was written by Tyler Kaban. It will be edited and place on our website with proper acknowledgements

HOME TIES & PRODIGAL VINES: *A brief history of Canada's wild prairie grapes and their contribution to northern viticulture*
By Tyler Kaban

My interest in viticulture (the growing of grapes) goes back to my childhood when my grandmother presented me with a gift from one of her weekly shopping trips- it was a bareroot vine of the popular juice grape 'Niagara'. This white-fruited grape along with many other cultivars (cultivated varieties), are common items at garden centres throughout the Canadian Prairies in early spring. Many eager gardeners will plant these tender varieties in their Zone 2 or 3 sites only to find dead vines the following spring. This was the case with my new Niagara vine which I planted on the south side of my parents' house. Even though the vine died, my passion for grape growing was born and has led to nearly 20 years of interest into everything grape.

Later I was to learn that the reason for the Niagara's demise had nothing to do with my ability to care for it but rather, to the vine's genetic make-up. This particular vine belonged to the North American species *Vitis labrusca* of which the quintessential juice grape 'Concord' is also a member. This grape species is not hardy on the Canadian prairies as it has evolved in a much different environment which does not experience our near-arctic winters. The possibility of viticulture on the Canadian prairies seemed bleak until the early 1990's introduction of the super-hardy cultivar 'Valiant' which was a hybrid vine developed at South Dakota State University. Valiant combines the quality of a *V. labrusca* grape named 'Fredonia' with the extreme hardiness of another North American grape species, *Vitis riparia*, the 'Riverbank grape'. This hardy grape species has been grown for decades throughout Alberta, Saskatchewan and Manitoba as a climbing ornamental vine.

Figure 1 – Ornamental Manitoba *Vitis riparia* growing in a Saskatoon backyard.

Upon reading Lon Rombough's 2002 book 'The Grape Grower' I was introduced to the idea of using the riverbank grape in breeding to create ultra-hardy juice and wine grapes for climates like those on the Canadian Prairies.

World's Hardiest Grapes

Vitis riparia is the most widespread grape species in North America. Its northernmost range extends into the Canadian prairie provinces of Saskatchewan and Manitoba. The 'Manitoba Riparias' have been utilized the most as ornamental vines and one nursery in particular, 'Boughen Nurseries' of Dauphin Manitoba, was instrumental in spreading this species throughout the Canadian prairies in the form of seedlings. Dauphin is close to what is believed the most northern of this vine's habitat, the Riding Mountain National Park. This wild population arguably represents the hardiest grapevines in the world; the Montana Riparias of which Valiant is descended may also share in that claim as both 'ecotypes' annually live through temperatures of nearly -50°C! The major difference between these two wild grape populations is the latitude in which they evolved.

Figure 2 – Typical clusters of *Vitis riparia.*

Figure 3. Location of wild grapes on the Canadian Prairie.

From Manitoba to Minnesota

My hometown of Yorkton Saskatchewan lies approximately 90kmfrom the Manitoba provincial border and 200km straight west of the Riding Mountain National Park. The significance of this federal wildlife preserve to grape breeding goes back many decades when the staff at the AAFC research station at Morden Manitoba gathered seed from the park and grew out the resulting vines in their horticulture plots. It wasn't until the late 1970's that an enthusiastic young graduate student from the University of Minnesota would evaluate and select the best of these 'Manitoba Riparias' for use in grape breeding.

Patrick Pierquet was a graduate student at the University of Minnesota from the fall of 1975 through to the spring of 1978. His work was supervised by University of Saskatchewan alumnus, Dr. Cecil Stushnoff. Patrick's work had direct implications to breeding hardy grapes as he explains,

"My project had two parts: Documenting fruit quality variability in wild Vitis riparia populations, and exploring the mechanisms of cold hardiness in this species."

In search of the hardiest *V. riparia* populations, Patrick visited the Agriculture Canada Morden Research station and evaluated their seedlings from the Riding Mountain Park. Fruit evaluations were carried out in August of 1976 and cuttings of superior vines were collected that winter and brought back to the U of Minn. Patrick's evaluations were

"based primarily on fruit chemistry….good sugar content and low (relatively) acid levels….the Manitoba population exhibited near zero disease (at Morden)….Manitoba riparia did seem to require few[er] degree-days to ripen their fruit, and this was the main reason I thought they would be useful for breeding in Minnesota."

Patrick's instincts about using superior Riparias in breeding proved correct. His original cross (while still a graduate student) of a large-clustered Minnesota *V. riparia, '*Riparia#89' with a French Hybrid wine grape called 'Landot 4511' produced the immensely popular northern red wine grape MN 1047, later named and released as 'Frontenac'. A pink-fruited mutant of Frontenac, would later be named 'Frontenac gris'; these two selections represent two of the four wine grapes so far released by the University of Minnesota. Now a third sport of Frontenac has been identified (and soon to be released by the Minnesota program), the 'blanc' with pure white fruit. Three cultivars from a first generation cross to *V. riparia*- not bad!

Figure 4 - Comparison of cluster size of Riparia #89 (left) and its offspring 'Frontenac' (right). Cluster of #89 measures aprox. 12cm (4.7").*Photo courtesy Tom Plocher*

Mr. Pierquet would also use the superior Morden Riparias in crosses to produce some of the foundation material for the future U of Minnesota's wine grape breeding program. One of these notable Manitoba crosses was used to produce the superior breeding parent 'MN 1094' which would be further used at the U of Minn to create their newest red wine grape release 'Marquette'. MN 1094 has been used by other breeders and has proven to be very good at passing on good tannins and hardiness to its offspring, as is the case with Tom Plocher's new high quality red wine grape 'Petite Pearl'. Patrick recalls,

"MN 1094 is (MN 1019 x MN 1016). Both of these were selections from my early breeding work (1978-80), 1019 being Morden riparia X Carmine (pure vinifera)."

Of the Morden selections brought to Minnesota, Patrick primarily used three as parents in his breeding work, "#37, 39 and 64."Some other notable University of Minnesota selections based on these riparias (there are many more) include: MN 1095 (Riparia #37 x Veeblanc), MN 1177 (Riparia #39 x S 11701), MN 1051 (Riparia #64 x Aurore) and MN 1023 (Riparia #37 x SV 18-283).

Distinguished Wisconsin grape breeder Elmer Swenson also used the Morden-Pierquet Riparias in his breeding work. He was particularly fond of Riparia #37, and in a 1985 article for the Minnesota Grape Growers Association Annual Report he writes,

"I thought the #37 to be the best of them (Morden Riparias) – great health & vigour, with quite large clusters, the leaves having a distinct glossiness. It was the earliest to ripen, the fruit coloring in late July (in Minnesota) … I consider the 37 an outstanding Riparia."

Figure 5 - Elmer Swenson in his vineyard. ***Photo courtesy Patrick Pierquet***

Elmer thought that one of Patrick's Riparia #37 crosses (then numbered 1-16-126) later numbered MN 1023, was "one of the finest F1 hybrids I have seen." Swenson also had the three Morden Riparias in his private collection.

Back to Saskatchewan and the U of S

Cecil Stushnoff completed his undergraduate degree in Agriculture and graduate degree in Horticulture from the University of Saskatchewan in 1963 and 1964. He would go on to complete his PhD in Horticulture from Rutgers University, NJ in 1967. From 1967-1980 Dr. Stushnoff served as a professor in the Department of Horticultural Science at the University of Minnesota. During this time, he would pioneer research in the cold hardiness physiology of woody perennials as well as supervising the graduate work of Patrick Pierquet. In 1981, Stushnoff returned to the University of Saskatchewan as department head of Horticulture Science. With him came some of the elite grapevine selections of Elmer Swenson and some of the best *Vitis riparia* selections by Patrick Pierquet.

Having learned about these early selections and their role in the University of Minnesota's grape breeding program, I was amazed when the U of S Domestic Fruit Program's current and long term field technician, Rick Sawatzky, handed me his original field notes from 1981. They read,

"Vitis...grape from Minn. via Dr. C Stushnoff...23 Feb 81. (scions)",

and the list of now very famous grape cultivars included:

Beta', 'Edelweiss', 'Mandan', 'Suelter', 'Swenson Red', 'ES 1-63, 193, 282, 283, 294 & 642' (ES 1-63, 282 and 294 would later be named 'Kay Gray', 'St. Pepin' and 'LaCrosse'). Wild grape cuttings included Patrick's superior *Vitis riparia* selections 'Riparia#37, 39, 55 & 89'.

Below the line listing Riparia #37 was a special note, "Selection made by Dr. C. Stushnoff."To learn that the U of S had access to such germplasm nearly 30 years ago (even before the Minnesota wine grape breeding program was formally initiated) was astonishing but also a bit disappointing when considering the decades of breeding that could have been- if only we had a breeder like Patrick Pierquet back then!

Rick would have another surprise for me.   In mentioning that I was interested in crossing superior Manitoba Riparias with some of the best Swenson selections (ironically, some of the very selections that were in the plots 30 years previously!), he pointed to the large wall-mounted plot map identifying a large planting of wild grapes along the north fence line. These vines were all the seedlings of a single female grape growing on campus in the early 1980's. Rick explained that he himself had collected the seed after Dr. Stushnoff's favourable reaction to tasting the berries of the 'mother vine'. In Rick's words,

"I think it was the fall of 1981, we (the fruit program) had been given lab space at the POS plant on campus for Dr. Stushnoff to carry out cryopreservation research. I had previously noticed many wild grapes growing along the building and on this particular day Cecil happened to be walking with me to the lab so I quickly grabbed some ripe fruit off the largest vine. He tasted the berries and noted that this vine had very good flavour characteristics for a wild grape so he suggested I grow out the seedlings. I germinated the seeds and passed the young vines on to the campus grounds crew and they planted them along the north fence of the field plots. I went back many years later but the mother vine had been removed."

Figure 6 – Two very large sibling vines of Sawatzky's*V. riparia* seedlings growing up a metal machine shed, U of S grounds crew.

Sawatzky's seedlings, now 25 years old, have survived through some of the harshest winters on record in Saskatoon. I knew that when I was searching for superior *Vitis riparias* to use in breeding only the oldest, hardiest vines would do, and these definitely fit the bill. Rick led me to a remote location running along the north east corner of the Horticulture field plots now overgrown with pines, poplars, lindens, hawthorns and Mongolian cherries. Most astonishing was that the grapes were behind this thick 'forest' so they were heavily shaded from the south which seemed to have greatly reduced their vigour; in addition, Manitoba *V. riparias* are known for their particularly low vigour. The vines were old and gnarled, growing through the chain link. Only open field and Circle Drive Freeway are north of the fence so the grapes would have to endure the full brunt of the Zone 2 winter's extremely harsh, desiccating north winds.

Figure 7 – North fence line 'Sawatzky' *V. riparias* with Circle Drive Freeway/ College Drive in the background, U of S Horticulture field plots.

Not only were these vines extremely hardy but they were also some of the best tasting Riparia grapes I'd found; they possessed very little of the 'herbaceous' off-flavour usually associated with the fruit of this species. Given the history of these seedlings and based on the well qualified assessment of Dr. Stushnoff as well as my own experience with awful-tasting Riparias, I was confident in using these vines in breeding.

Beginning in the fall of 2007, I tagged every female vine growing along the north fence line. The first female was named 'Riparia A' and so forth until I reached 'Riparia L'. After three years assessment, I have found Riparias 'J', 'K' and 'L' to be the lowest in herbaceousness of this seedling population. Riparia J seems to have the largest clusters measuring up to 5" when grown in a pot in the greenhouse. Out of the three 'sisters', I have used Riparia K the most in breeding to date.

A number of other superior female *Vitis riparia* selections have been added to the U of S collection since I began my graduate work with the fruit program in the fall of 2008. As well, I have used male Manitoba Riparias in breeding. One vine I found outside the main campus library is the pollen parent of the hardy selection 'C-16'.

A Precious Genetic Resource

As previously mentioned, Boughen's Nurseries of Dauphin Manitoba has distributed *Vitis riparia* seedlings for decades. As a result, many of the superior-tasting wild grapes that I have collected for the U of S Domestic Fruit Program are most likely 'Boughen seedlings' and most certainly Manitoba Riparias. Boughen's has also selected a rare white-fruited form of *Vitis riparia* that has found its way into many North American breeders' collections. Listed as 'Boughen's White Riparia', this selection can also be found in the USDA hardy Vitis Gene Bank Collection in Geneva New York.

Figure 8 - 'Boughen's White Riparia' used in breeding at the University of Saskatchewan.

The current head of the U of S Domestic Fruit Program, Dr. Bob Bors, is an experienced 'Plant Explorer' who has collected wild plants of Blue Honeysuckle/Haskap from sites throughout Canada. The U of S horticulture plots now boast the world's largest wild *Lonicera caerulea* collection with plants from nearly every Canadian province. It is the intention of Dr. Bors that the wild riverbank grapes of Manitoba and Saskatchewan be equally represented in our plots.

Under the leadership of Dr. Bors, the Domestic Fruit Program is in a timely position to select, utilize and conserve these valuable wild grapes. With the initiation of our 'Prairie Fruit Gene bank', future goals will include plant collection at various sites throughout western Manitoba and extreme south eastern Saskatchewan. Collection of the Saskatchewan *Vitis riparias* is of particular importance as this population has never been fully characterized or utilized in any breeding efforts. It is possible that some novel characteristics exist in Saskatchewan's wild grapes that are not present in other *Vitis riparia* populations.

Conventional viticulture based on growing classic *Vitis vinifera* cultivars like 'Cabernet' and 'Chardonnay' etc. can also benefit immensely from breeding work done with these vines as new superior disease, pest and stress resistant rootstocks could be created for traditional Canadian wine growing regions like B.C.'s Okanagan and Ontario's Niagara.

The wild grapes of the Canadian Prairies are an extremely valuable genetic resource. In the past they have been utilized by breeding programs passing on their extreme hardiness and disease resistance to offspring. The future contributions of these unique populations to northern viticulture are yet to be tapped but they will no doubt play a central role in new cultivar development.