SERVICEBERRY: POTENTIAL NORTH DAKOTA ACCESSIONS FOR THE NURSERY INDUSTRY

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Jorden Renae-lyn Hinrichsen

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Harlene Hatterman-Valenti
Chair

Dr. Richard Horsley

Dr. Todd West

Dr. Sean Brotherson

Approved:

17 November 2017
Date

Dr. Richard Horsley
Department Chair

ABSTRACT

Saskatoon serviceberry (*Amelanchier alnifolia* Nutt.) is an ornamental Rosaceous shrub producing delicate white flowers that yield fruit similar in appearance and nutrition to blueberry (*Vaccinium* spp.). Most serviceberry are propagated in Canada and, as imported stocks are often expensive, clones were accessed from 70 locations in North Dakota. Following establishment, a replicated field trial of wild biotypes of serviceberry was initiated at the North Dakota State University Horticulture Research Farm (NDSU HRF) near Absaraka, ND and at the Williston Research and Extension Center (WREC) in Williston, ND. Yield data was taken upon harvest in summers 2014-2017 at NDSU HRF and 2016 at WREC. ND 1-2, ND 1-4, ND 1-6, ND 1-7, ND 48-2 often out-yielded market genotypes. ND 15-2 was high in sugar content and gelling ability, ideal for processing. Through continued selection, North Dakota growers may have quality serviceberry from a local source.

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Introduction

The small fruits industry enjoys diversity in color, flavor, nutrition, and fruit type. Brambles, ever- and june-bearing strawberries, blueberries, gooseberries, black and white currants, jostaberries, grapes, and loganberries have given rise to nurseries specializing in small fruit alone. This allows growers to diversify their orchards, manage risk, and increase profitability of operations. Additionally, marketing strategy allows different opportunities for each small fruit. The convention in commercial production for high-coverage suppliers has become that good management and quality fruit remain top tier; however, these qualities are adjusted to meet high volume demand. With a recent heightened appreciation for food traceability, fruits that may yield lower can make a profit at higher prices, selling to conscientious consumers who want local food. In this case, a grower might consider Saskatoon serviceberry, a niche fruit of the Midwest prairies. Raspberry, blackberry, and strawberry accrued $102,572,000; $86,939,000; and $291,463,000 in exports in 2017, respectively (USDA, 2017). Saskatoon serviceberry commercial production is popular in Canada, with a net value of $1.2 million in 2011 (Statistics Canada, 2012). Although accepted locally, the United States has yet to introduce Saskatoon serviceberry on a commercial scale.

Saskatoon serviceberry (*Amelanchier alnifolia* Nutt., *Rosaceae*) has many names: juneberry, Saskatoon, and alderleaf serviceberry. For all intents and purposes, in this document, it is referred to as serviceberry. Native to the continental US, Canada, and Alaska it is hardy to zone 2, tolerates a variety of soil conditions, withstands drought, and grows in various light conditions. A pH range of 5.5 to 8.0 is sufficient for growth (Barney et al., 2009). Adapted to

low-elevation conifer forests up into mountain juniper communities, it handles high natural disturbance areas such as those prone to fire or insect outbreak (USDA, 2006).

Serviceberry ranges from a mid-sized shrub to a multistem tree, upright to compact in habit, with shallow roots, a massive root crown, and rhizomes. Its bark is grey brown with red new growth. In May, delicate white flowers with strap-like petals and pink sepals emerge, closely or concurrently with ovate, blue green leaves. Flower clusters in May yield to clusters of 5 to 15 purple black pomes, waxy in appearance, soft, and nutritious, which ripen in late June or early July. The flavor has been described as almond-like, blueberry-like, and apple-like (Barney et al., 2009).

Historically, these pomes were prized by Native Americans. They were often used by European settlers analogous to the blueberry. In addition, the fruit was used to stave off diseases such as scurvy (Ritz, 1991; Mazza, 2005). From the shrub itself, the Native Americans used branches to brew tea for colds and stomach problems and medicine for recovery after childbirth (USDA, 2006). The wood was used to make arrows, spear shafts, tool handles, and rope.

Health benefits of the fruit include the production of antioxidants like pelargonidin, which counteracts hyperglycemia and reduces the severity of diabetic reactions (Vinayagam and Xu, 2015). Other antioxidants found in serviceberry fruits have the effect of scavenging reactive oxygen species associated with oxidative stress and free radicals known to cause the development of tumorous diseases (Rop et al., 2012; Mazza, 2005). Serviceberry fruit may prove superior in nutraceutical benefit to other known super fruits like blueberry (Juríková et al., 2013).

Despite its uses, serviceberry remains relatively obscure in the U.S. horticulture industry. Most genotypes on the market are selections from the nursery plantings in Canada (Spencer et al., 2013; Harris, 1972).

Cultivars

‘Buffalo’ is slower-growing with moderate-sized pomes that ripen over a two-week period in Canada (St.-Pierre et al., 2005). No reports of ‘Buffalo’ have yet been made in North Dakota.

‘Honeywood’ is an early-ripening, high-yielding cultivar in Sasketchawan and Alberta (St.-Pierre et al., 2005; Spencer et al., 2013). It has not given high yields in North Dakota (Ardayfio, 2012; Ardayfio and Hatterman-Valenti, 2015). It also is reported to handle frost better because of its characteristic late bloom time.

‘Martin’ yielded high in 2010-2011 years in North Dakota, but the yields from year to year were dissimilar and more information is needed to confirm performance (Ardayfio, 2012). It is reported to have more uniform ripening (Spencer et al., 2013).

‘Northline’ has been one of the lower yielding cultivars in North Dakota (Ardayfio, 2012); but moderately productive in Saskatchewan, out-producing 'Pembina' and 'Nelson' (St.-Pierre et al., 2005).

‘Parkhill’ and ‘Regent’ are less commonly grown. ‘Regent’ is noted for high ornamental value, but also produces a sweet, mild pome. ‘Parkhill’ is reportedly smaller, low-yielding, but with good *Entomosporium* resistance (Spencer et al., 2013).

'Success' is low-yielding with smaller pomes (St.-Pierre et al., 2005; Ardayfio, 2012), which is useful for processing. Along with ‘Parkhill’, it is possibly a hybrid of *Amelanchier stolonifera* and *A. alnifolia*, whereas most named genotypes are hybrids of *A. alnifolia* (Spencer et al., 2013).

Plant Qualities

Several genotypes of serviceberry have been evaluated for commercial and ornamental purposes in North Dakota and similar climates (Ardayfio and Hatterman-Valenti, 2015; St.-Pierre et al., 2005; Rop et al., 2012). Important traits to consider are plant height and width, disease resistance, and cold tolerance; pome diameter, individual pome weight, fruit soluble sugar content, fruit pH, ripening uniformity, yield, pectin content, and storage ability.

Height and Width

In serviceberry, sexually produced seedlings are genotypically and phenotypically variable. Mazza and Davidson (1991) found that seedlings from open-pollinated and controlled cross populations did not differ in height. Conversely, Ardayfio and Hatterman-Valenti (2015) found differences in height and width among mature plants of genotypes planted in North Dakota. This suggests a shift in growth rate among genotypes from youth to maturity. Such variability in height and width affect harvesting options. Plants 1-2 m high and 1 m wide may be harvested with a sideways mechanical harvester. This makes it feasible to plant orchards in hedge form but damage plants and increases disease pressure. Fruit from cultivars 2-2.5 m high and 46 cm wide may be collected by upright mechanical harvesters. These may be harvested as individual plants; however, due to the height from which fruits are dropping, more will be damaged (Spencer et al., 2013).

Disease

While larger birds, mammals, insects, bacteria, and viruses are observable in serviceberry, fungal diseases are the predominant inhibitor of plant success and fruit production. *Entomosporium* leaf and berry spot (*Entomosporium mespili*), Saskatoon-juniper rust (*Gymnosporangium nelsonii*), *Cytospora* canker (*Cytospora leucostoma*), black leaf, brown fruit

rot (*Monilinia amelanchieris*), powdery mildew (*Podosphaera clandestine*), and fireblight (*Erwinia amylovora*) are prevalent diseases in serviceberry orchards. Saskatoon-juniper rust and *Entomosporium* leaf and berry spot occur during fruit development and harvest months and pose the greatest threat to commercial fruit production (St.-Pierre, 2006).

As spores from the previous year are released onto young flowers, brown rot symptoms manifest. The disease affects flowers, young fruit, and leaves, especially in humid conditions. It causes flowers to brown prematurely, brown spots on fruit that develop into grey-brown tufts, and fruit drop, or mummification. *Cytospora* canker affects the stem, and manifests as a black stain or splitting bark. Infection causes eventual dieback. Fireblight is a bacterial pathogen that affects leaves and twigs, killing both, and displaying persistent dead leaves on a crook-shaped stem. Stunted new shoots, distorted new leaves, and powdery white growth are symptoms of powdery mildew.

In early stages, Saskatoon-juniper rust appears as dense patches of small, yellow spots with orange edges on leaves and fruit. As pycnidia form, their brown spikes render the fruit unmarketable (Spencer et al., 2013).

Entomosporium leaf and berry spot has high incidence in the wild and is the most important disease in serviceberry orchards (Spencer et al., 2013). Angular lesions form on leaves and fruit, begin to yellow, then brown. Fruit becomes woody and inedible.

It is recommended that orchardists assume the *Entomosporium* pathogen is present and take measures to control it upon establishment. Protective fungicides chlorothanlonil, imazalil, propiconazole, and throphanate-methyl inhibited conidiospore germination at 1 ppm in vitro (Lange et al., 1998). In addition, chlorothalonil and propiconazole controlled *E. mespili* best at three sites in Alberta, Canada; while benomyl controlled infection in two out of three of these

sites. The best control is resistant cultivars, but the pathogen can be treated preventatively and post-infection.

Cultivars Buffalo and Success are benchmarks for high and low susceptibility to *Entomosporium* infection, respectively (Ronald and St.-Pierre, 2002). Two days following inoculation, 'Buffalo' was 43% infected by germinating conidiospores and 12% of germinating spores had penetrated infected leaves of 'Success'. In his dissertation, Ronald (2002) evaluated 17 genotypes after controlled and natural infection. Significant differences in disease response occurred among genotypes under both circumstances. 'Parkhill', 'Regent', and 'Success' had consistently low incidence and severity on leaves and fruit. These genotypes have also displayed the ability to restrict fungal sporulation on leaves and fruit (Ronald et al., 2001).

Leaf age and thickness have been shown as factors affecting the ability of spores to penetrate, with older and thicker leaves showing lower susceptibility to infection.

In addition to fungicides, defense gene inducers have been examined (Wolski et al., 2010). Jasmonic acid and Canada milkvetch extract were shown to prompt synthesis and accumulation of genes encoding for PR-1, PR-2, PR-5, LOX, and PAL. Treatment with these compounds reduced disease levels in 'Martin'. This study recommended using defense enhancers as an integrated management component to control *Entomosporium mespili* infection in serviceberry.

Fungal diseases may occur in years of higher precipitation and cannot be eliminated once plants become symptomatic; the solution is to isolate infected from non-infected tissue. This can be done by pruning, removal of leaf litter, treatment with fungicides, or removing diseased shrubs from the orchard. It is important that growers select resistant genotypes and that future breeding endeavors make resistance a priority.

Fruit Qualities

Harvest

Ripening is the primary factor used to determine harvest date. As such, a 9-level maturity class index was developed (Rogiers and Knowles, 1997). The latter stages (5-9) are characterized by an increase in soluble solid content and decrease in organic acids which make the fruit palatable. Excluding climatic factors, serviceberry pomes characteristically ripen unevenly. Hot weather may accelerate ripening and can reduce the harvest window from the standard 14 days to 5 days (Spencer et al., 2013).

For mechanical and commercial production, cultivars with uniform ripening patterns are recommended. Uniform ripening reduces collection of premature pomes which must be culled. In Saskatchewan, the number of days to ripen fruit from 10 to 90% did not differ among 15 genotypes, but the day on which 50% fruit were ripe varied by as much as 13 days between ‘Pembina’ and ‘Par90’ (St.-Pierre et al., 2005). The amount of time to ripening fruit is similar but onset may require different climatic requirements.

Variability in ripening can occur in pomes on the same plant, even within a raceme, and can range from green to ripe. In North Dakota, ‘Parkhill’ was reported to have the least uniform ripening (Ardayfio and Hatterman-Valenti, 2015). Discarded fruit were either red (under ripe) or shriveled (past ripe). A comparison of total to marketable weight may be used to infer ripening uniformity; however, it cannot be strictly applied without consideration of diseased and damaged fruit. Non-uniform ripening is valuable for you-pick farms, where an extended harvest is desirable.

Harvest yield has been the basis for most genotype selections for serviceberry fruit production. In 1998-2003, total harvest for 15 genotypes at two sites in Saskatchewan ranged

from 1.32 kg plant^{-1} to 4.7 kg plant^{-1}, with the highest yields from ‘Pearson II’, ‘Bluff’, ‘Buffalo’, ‘Forestburg’, Honeywood, ‘JB30’, ‘Martin’, ‘Northline’, ‘Smoky’, and ‘Thiessen’. ‘Success’, ‘Pembina’, and ‘Nelson’ produced the lowest yields. Marketable harvest ranged from 0.72 kg plant^{-1} to 4.16 kg plant^{-1} (St.-Pierre et al., 2005). Similarly, Ardayfio and Hatterman-Valenti (2015) evaluated fruit production for 10 cultivars and a native biotype at a southeastern North Dakota site. Yields drastically increased for all genotypes from 2010 to 2011, reflective of alternate bearing trends characteristic of *Rosaceae*. ‘Martin’, ‘Parkhill’, ‘Thiessen’, ‘Regent’, and the native biotype were most productive in 2010; in 2011, ‘Parkhill’ outperformed all genotypes. However, ‘Parkhill’ had the highest total mean to marketable yield ratio. Two years is not sufficient data to draw strong conclusions and the variability between years renders the yield information inconclusive.

Fruit Yield and Grading

To consumers, pome size is paramount. Serviceberry is often compared to blueberry for color and size, although blueberry is under more scrutiny as a high-volume crop. An individual blueberry weigh as much as 3-4 g (Scalzo et al., 2013; Williamson et al., 2014). Serviceberry pomes range from 6-14 mm in equatorial diameter and 0.43-1.10 g per pome (Ardayfio and Hatterman-Valenti, 2015; St.-Pierre et al., 2005). There are notable differences among genotypes in pome equatorial diameter and weight. The studies by Ardayfio and Hatterman-Valenti (2015) and St.-Pierre et al. (2005) show that ‘Honeywood’, ‘Martin’, ‘Northline’, ‘Parkhill’, and ‘Pembina’ have similar equatorial diameters in the three locations; from these data, it may be inferred that maximum pome size is genetically controlled. Ardayfio and Hatterman-Valenti (2015) also noted that 50-pome weight and equatorial diameter were not always consistent

within a genotype, suggesting that pomes may vary in dry matter and water content. Irrigation was not provided in the North Dakota trials, but the trials in Saskatchewan were irrigated.

Numerous factors contribute to the flavor, texture, and storage ability of serviceberry pomes; including soluble solids, pH, soluble solid to titratable acidity ratio (SS:TA), and pectin content.

Ripening is characterized by an increase in soluble solid content (SSC). While consumers make initial fruit purchase decisions based on outward qualities such as shape, color, aroma, and size, continued purchase depends largely on sweetness and flavor. Based on this convention, soluble solid content may be used as an indicator of fruit maturity. The terms total soluble solids (TSS), SSC, and degree Brix (°Brix) have been used synonymously. TSS and SSC account for sugars, acids, and small amounts of vitamins, fructans, proteins, pigments, phenolics, and minerals; °Brix refers to sucrose, glucose, fructose and sugar alcohols found in juice (Magwaza and Opara, 2015).

In serviceberry, the pome will generally consist of 80% water and 18% sugar (Spencer et. al., 2013), with variability among genotypes. As shown below), even the same genotypes vary in soluble solids content by environment and season. Among 11 genotypes ranging from 15.3 to 18.8 °Brix over two seasons, Ardayfio (2012) found no significant differences. Conversely, field trials in the Czech Republic ranged in soluble sugar content from 14.17 to 18.78 °Brix and did show differences among 10 genotypes (Rop et. al., 2012). The seeming contradiction between trials is likely due to differences in experimental design. The Brix values corroborate Spencer et al.

Rogiers and Knowles (1997) showed a general downward trend in pH from maturity classes 1-7, while in the latter stages ‘Northline’ remained steady and ‘Smoky’ increased slightly. At maturity, pome pH ranged from 3.65 to 4.10 in Saskatoon, SK (Zaytlny et al., 2005).

Objectives

No named cultivars have resulted from deliberate crossing, as serviceberry is a smaller niche in the horticulture industry. Thus, opportunities for improvement lie first in selection of natural crosses. If proven adequate, natural germplasm may be released to the nursery industry or may become part of a breeding program.

To identify plants that perform well in North Dakota, natural accessions were collected across the state in 2007 and 2008 for quality characteristics, including perceived disease resistance, form, yield, pome size, and fruit flavor. The objective of this research was to evaluate native accessions to standard genotypes available in the industry.

Materials and Methods

Field Design

Serviceberry plants were propagated in tissue culture in 2010 and planted outdoors in 2012. Field trials were planted as a randomized complete block design (RCBD) with 10 replications in the North Dakota State University Horticulture Research Farm (NDSU HRF) near Absaraka, ND (Latitude: 46º 59’ 22.0986”, Longitude: -97º 21’ 22.2222”) and at the Williston Research Extension Center (WREC) in Williston, ND (Latitude: 48.133 º, Longitude: -103.739 º). Named standards included in this report are Honeywood, Northline, Parkhill, and Regent. Accessions taken from North Dakota locations, hereafter denoted “ND [location number]-[accession number]”, include those determined valuable for either ornamental or edible value or

Table 1. Soluble solid content of serviceberry fruit varies according to genotype and environment.

Cultivar	Soluble Solids Content (SSC)	Location	Year(s)
Bluff	20.9	Moon Lake, SK, Canada	1998-2000
Brnensky	14.2	Žabčice, Brno, Czech Republic	2009-2011
Buffalo	22.6	Moon Lake, SK, Canada	1998-2000
Forestburg	20.6	Moon Lake, SK, Canada	1998-2000
Honeywood	21.7	Moon Lake, SK, Canada	1998-2000
Honeywood	18.2	Absaraka, ND, United States	2010 & 2011
JB30	19.9	Moon Lake, SK, Canada	1998-2000
Lee II	17.1	Absaraka, ND, United States	2010 & 2011
Martin	20.8	Moon Lake, SK, Canada	1998-2000
Martin	18.7	Žabčice, Brno, Czech Republic	2009-2011
Martin	16.4	Absaraka, ND, United States	2010 & 2011
Native	17.9	Absaraka, ND, United States	2010 & 2011
Nelson	25.1	Moon Lake, SK, Canada	1998-2000
Northline	23.0	Moon Lake, SK, Canada	1998-2000
Northline	17.7	Absaraka, ND, United States	2010 & 2011
NS-1	14.5	Žabčice, Brno, Czech Republic	2009-2011
NS-2	14.1	Žabčice, Brno, Czech Republic	2009-2011
Ostravsky	19.0	Žabčice, Brno, Czech Republic	2009-2011
PAR 90	20.9	Moon Lake, SK, Canada	1998-2000
Parkhill	22.6	Moon Lake, SK, Canada	1998-2000
Parkhill	16.5	Absaraka, ND, United States	2010 & 2011
Pearson II	21.4	Moon Lake, SK, Canada	1998-2000
Pembina	27.9	Moon Lake, SK, Canada	1998-2000
Pembina	18.8	Absaraka, ND, United States	2010 & 2011
Regent	17.3	Absaraka, ND, United States	2010 & 2011
Skolsky	16.2	Žabčice, Brno, Czech Republic	2009-2011
Smoky	21.5	Moon Lake, SK, Canada	1998-2000
Smoky	18.1	Absaraka, ND, United States	2010 & 2011
Smoky	15.1	Žabčice, Brno, Czech Republic	2009-2011
Success	21.9	Moon Lake, SK, Canada	1998-2000
Success	15.3	Absaraka, ND, United States	2010 & 2011
Thiessen	20.4	Moon Lake, SK, Canada	1998-2000
Thiessen	16.5	Absaraka, ND, United States	2010 & 2011
Thiessen	15.5	Žabčice, Brno, Czech Republic	2009-2011
Tisnovky	16.2	Žabčice, Brno, Czech Republic	2009-2011

both. This trial examined the cultivars mentioned and 15 accessions. For our intents and purposes, these will be referred to as genotypes in this document.

Plant Dimensions

Measurements of plant height and width were done at NDSU HRF and WREC in 2016 and 2017 at the beginning of the growing season using a tape ruler. Height was measured to the highest point from the base of the plant. Width was measured twice across the plant and averaged.

Disease

Visual observation of disease was recorded when symptoms noticeably manifested. For both locations, it was not until after harvest of 2016. NDSU HRF notes were taken 1-2 September and WREC notes were taken 31 August to 2 September. Severity was recorded as percentage of plant covered, average percent leaf area covered for 10 leaves infected, and an index of the percentages multiplied.

Harvest

Yield and pome qualities were recorded for NDSU HRF in 2014, 2015, 2016, and 2017; and for WREC in 2016. Harvests at NDSU HRF were done on 17 July 2014; 8-9 July 2015; 27-29 June (first) and 6-7 July (second) 2016; and 5 July 2017. Harvests at WREC were done 20-29 June (first) and 6-11 July (second), 2016. Yields were harvested when most fruit turned dark purple before drop. Fruit were collected in 13 x 13 x 8 cm disposable boxes, full sheet cake containers, or zip-closed plastic bags (Dacotah Paper Co. 3940 15th Avenue NW, Fargo ND 58103); and stored in a cooler at 3° C until grading was completed. After initial grading, fruit was quiescently frozen at 0° C until pectin testing could be performed.

Fruit Yield and Grading

Pome properties measured within two weeks of harvest included soluble sugars, pH, diameter, weight of 50 pomes, marketable weight, and total weight.

Total weight was measured using a digital scale. Marketable weight was recorded after diseased, damaged, shriveled, underdeveloped, or otherwise unacceptable pomes were culled. The weight of 50 pomes was measured using the same. A sample of 10 pomes from each genotype was measured and averaged for diameter from each replicate.

Pomes for juice testing or other measurements were taken from the marketable yield. Juice was pressed from about 10 g fresh pomes into small plastic cups and used for soluble sugar and pH measurements. For pH, a pH meter was used by placing the probe in the juice enough to submerge the glass head and held until the reader stabilized. Between readings, the probe was cleaned with deionized water and wiped with Kimwipes (Kimtech Science, Kimberly-Clark Global Sales, Inc., Roswell, GA 30076-2199), and periodic calibration was performed using 4.0, 7.0, and 10.0 pH buffers. For soluble sugar content, 2-3 drops of fluid were placed on the stage of a refractometer (Refractometer Pal-1, ATAGO U.S.A. Inc., WA, USA) for analysis. Between samples, juice was rinsed away with deionized water and the stage wiped thoroughly using Kimwipes.

Pectic substances were quantified using a rapid determination protocol for select genotypes (Shelukhina & Fedichkina, 1994). Frozen pomes were weighed to 20 g in glass columns and homogenized using a blending probe. In a 100-ml graduated cylinder, fruit homogenate was combined with 50 ml 0.1 N HCl and allowed to sit for 5 minutes to precipitate the pectin. The mixture was suctioned with a water-pressurized tube through Whatman #1 filter paper into a beaker. Twenty milliliters of the filtrate were placed in a 50-ml beaker. The

remaining residue and filtrate were mixed again in a 100-ml beaker. Each mixture was then titrated separately. This was done by placing each beaker on a stir plate with a magnetic stir bar and releasing 0.1 N NaOH slowly from a burette, which was clamped slightly above the beaker. In their titration, the original authors used Hinton's indicator; however, this was not feasible in serviceberry, as the juice itself was dark and changed hue naturally during titration between the mixtures and NaOH. Each liquid was titrated to a pH endpoint of 8.2. Using the total volume of NaOH applied to the residue-filtrate mixture and the calculated volume of HCl filtrate, and applying the following differential equation, the amount of polygalacturonic acid was calculated:

$$P\% = [(V2-V1)*176*0.1*K/1000*W]*100$$

Where V1 is the calculated volume of 0.1 N NaOH needed to neutralize the HCl in the entire mixture; V2 is the volume of 0.1 N NaOH neaeded to bring the entire mixture to endpoint; 176 is the gram-equivalent of polygalacturonic acid; 0.1 is the concentration of the titrant; K is the coefficient of NaOH concentration; and W is the weight of the fruit used.

Data Analysis

For plant dimensions, disease index, fruit total weight, fruit marketable weight, and fruit quality factors, each environment was individually analyzed using analysis of variance by PROC GLM in Statistical Analysis Software (SAS). O'Brien's homogeneity of variance test determined whether the environments could be combined at a confidence level of $P \geq 0.01$. Each quality found to have significance was subject to mean separation using Tukey's test at a confidence level of $P \geq 0.05$.

Results and Discussion

Plant Dimensions

Per O'Brien's test of homogeneity of variance, serviceberry plant height could not be combined among environments. Individual ANOVAs revealed genotype as significant, except for WREC 2017 (Table A1-4). The different result is likely attributable to smaller sampling; all other environments had five replicates where WREC 2017 had three.

Individual environments subject to mean separation revealed superior genotypes for compact habit (Table 2). Among environments, ND 18-1 and ND 71-1 were consistently tall. 'Regent' was the shortest cultivar and similar to 'Parkhill', ND 48-2, ND 41-1, ND 5-1, ND 17-2, ND 16-1, ND 12-1, ND 1-7, ND 1-6, ND 1-5, ND 1-4, and ND 1-2. In a study by Ardayfio and Hatterman-Valenti (2015) 'Parkhill' and 'Regent' were among the tallest while 'Honeywood' and 'Northline' were shorter. However, the comparisons were among named cultivars in said study, contrary to this study, which focused on accessed genotypes; and do not reflect comparisons of plant dimensions among all serviceberry genotypes.

O'Brien's test for homogeneity of variance revealed homogeneity among environments for serviceberry plant width. According to combined ANOVA, an interaction between environment and genotype exists (Table A5). This is likely due to differences in orchard maintenance. For example: the WREC uses fabric in between rows and the NDSU HRF has grass alleys. Fabric between rows may suppress suckering. Mechanical damage and limb breakage may also be a cause for variable width among environments. WREC plants were pruned at the end of the season, while NDSU HRF plants were not. In future studies, coordinated orchard practices and plant stem counts would better inform width measurements.

ANOVAs for serviceberry plant width in each environment revealed significance among genotypes (Table 3). However, most were within the width limit for mechanical harvest. It may be noted that stem count would inform width more precisely.

For fruit production, a compact habit makes mechanical harvest feasible. Height and width affect harvesting options. Plants 1-2 m high and 1 m wide may be harvested with a sideways mechanical harvester (Spencer et al., 2013). Orchards may be planted in hedges, which accelerates harvest, but harvesters damage plants and foliage density increases

Table 2. Serviceberry plant heights were not combinable among environments in the NDSU HRF and WREC for years 2016-2017.

	HRF 2016		WREC 2017		HRF 2017	
Genotype	Height					
	----------m----------					
ND 1-2	1.33	bcd	1.27	abc	1.54	cde
ND 1-4	1.31	bcd	1.16	bc	1.55	cde
ND 1-5	1.27	bcd	1.20	abc	1.35	ef
ND 1-6	1.37	bcd	1.25	abc	1.60	cde
ND 1-7	1.22	cd	1.02	c	1.46	def
ND 12-1	1.32	bcd	1.15	bc	1.55	cde
ND 15-2	1.35	bcd	1.30	abc	1.81	bc
ND 16-1	1.32	bcd	1.10	c	1.52	cde
ND 17-2	1.19	cd	1.05	c	2.54	cde
ND 18-1	2.03	a	1.47	a	1.43	a
ND 41-1	1.26	bcd	1.19	abc	1.47	ef
ND 48-2	1.26	bcd	1.30	abc	1.48	cdef
ND 5-1	1.29	bcd	1.28	abc	1.96	cde
ND 71-1	1.59	b	1.46	a	1.98	b
ND 9-1	1.51	bc	1.21	abc	1.94	b
Honeywood	1.23	cd	1.48	a	1.75	bcd
Northline	1.21	cd	1.41	ab	1.57	cde
Parkhill	1.22	cd	1.26	abc	1.17	f
Regent	1.12	d	1.10	c	1.32	ef

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

disease pressure. Fruit from cultivars 2-2.5 m high and 46 cm wide may be collected by upright mechanical harvesters. These may be harvested as individual plants. This is convenient for trials; however, due to the height from which fruits are dropping, more will be damaged.

The most compact serviceberry plants in this collection appear to be include ND 17-2 and ND 5-1. However, plants that meet the height minimum for hedge planting include all except ND17-2 in NDSU HRF in 2017 and ND 18-1 in NDSU HRF 2016. With proper pruning, all cultivars qualify for further trial, commercial production and mechanical harvest regarding harvest dimensions.

Table 3. Combined serviceberry plant width at NDSU HRF and WREC in 2016 and 2017.

Genotype	Width	
	---------m---------	
ND 1-2	1.50	ab
ND 1-4	1.42	abcde
ND 1-5	1.60	a
ND 1-6	1.54	ab
ND 1-7	1.44	abcde
ND 12-1	1.14	gh
ND 15-2	1.14	gh
ND 16-1	1.29	defg
ND 17-2	1.18	fgh
ND 18-1	1.37	bcdef
ND 41-1	1.31	cdefg
ND 48-2	1.60	a
ND 5-1	1.08	h
ND 71-1	1.43	abcde
ND 9-1	1.46	abcd
Honeywood	1.24	efgh
Northline	1.50	abc
Parkhill	1.38	abcdef
Regent	1.43	abcde

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Disease

Animals, insects, and microbial pathogens are observable in serviceberry and inhibit plant success and fruit production. *Entomosporium* leaf and berry spot, Saskatoon-juniper rust, *Cytospora* canker, brown fruit rot, powdery mildew, and fireblight are common serviceberry orchards. Saskatoon-juniper rust and *Entomosporium* leaf and berry spot have been some of the greatest prohibiting factors in fruit production (St.-Pierre, 2006). As such, it is important to screen potential cultivars for disease resistance, especially in family farms or organic operations which might serve a CSA or local food vendor.

Entomosporium leaf and berry spot has been the most prohibitive factor in berry production (Spencer et al., 2013). Humidity and warm temperatures from May through July cause the fungus to flourish, especially in dense plantings. *Entomosporium* overwinters in fallen leaves and soil beneath its host plants. A preventative regimen includes selection of resistant genotypes, proper pruning, good orchard hygiene, and timely fungicide applications.

Entomosporium leaf and berry spot (Table A6-11) and powdery mildew (Table A12-17) were present in 2016 in both locations. Additionally, Saskatoon-juniper rust was recorded at WREC (Table A18-21); this disease recurred in the field in 2017 and, combined with bird damage, caused enough damage for caretakers to forego harvest. Saskatoon-juniper rust was observed at NDSU HRF in negligible amounts.

O'Brien's homogeneity of variance test revealed non-homogeneity and precluded combination of locations for *Entomosporium* and powdery mildew evaluations and are listed with mean separation individually (Table 4-7). For these data, it is important to note that the severity index is based on the product of two percentages and will converge on 1.0 the higher the severity and zero when symptoms are negligible.

ND 5-1, ND 16-1, ND 15-2 were listed in both locations as statistically similar and least adapted to resist *Entomosporium* infection. ND 1-7, ND 18-1, ND 1-2, ND 48-2, 'Regent', ND 71-1, ND 1-5, ND 1-4 were better adapted to resist infection in both locations. For our intents and purposes, the

Observation of 'Regent' and 'Parkhill' by Ronald et al. (2001) suggested the genotypes' ability to restrict sporulation on leaves. The current study also has implications of superior resistance for these and similarly grouped genotypes, although 'Parkhill' is less consistent.

Table 4. *Entomosporium* leaf and berry spot severity for serviceberry plants at NDSU HRF in 2016.

	Bush coverage		Leaf coverage		Severity index	
Genotype	----------%----------					
ND 5-1	95	a	47	ab	0.40	ab
ND 16-1	91	ab	56	a	0.51	a
ND 12-1	88	abc	41	abcde	0.36	bc
Parkhill	79	abcd	42	abcd	0.34	bc
Honeywood	79	abcd	34	bcdef	0.27	bcde
ND 41-1	72	abcde	35	bcdef	0.22	cdef
ND 17-2	70	abcde	45	abc	0.30	bcd
ND 9-1	69	bcde	26	def	0.18	def
ND 1-6	67	bcde	22	f	0.14	def
Northline	65	cde	46	ab	0.29	bcd
ND 15-2	63	cdef	59	a	0.37	abc
ND 48-2	62	def	32	bcdef	0.18	def
ND 18-1	60	def	23	ef	0.14	ef
ND 1-5	59	def	22	f	0.16	def
ND 1-4	59	def	21	f	0.13	ef
Regent	55	def	25	def	0.09	f
ND 1-2	53	ef	27	cdef	0.10	f
ND 1-7	52	ef	25	def	0.16	def
ND 71-1	39	f	24	def	0.10	f

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Table 5. *Entomosporium* leaf and berry spot severity of serviceberry orchard at WREC in 2016.

	Bush coverage		Leaf coverage		Severity index	
Genotype	----------%----------					
ND 12-1	76	a	57	abcd	0.43	a
ND 15-2	54	ab	73	a	0.42	a
ND 5-1	51	abc	64	abc	0.45	a
Honeywood	51	abc	63	abcd	0.36	abc
ND 41-1	49	abc	58	abcd	0.37	ab
ND 16-1	44	abcd	43	abcd	0.24	abcd
ND 17-2	44	abcd	68	ab	0.33	abc
ND 1-7	35	bcde	45	abcd	0.26	abcd
ND 18-1	34	bcde	54	abcd	0.23	abcd
ND 1-2	30	bcde	60	abcd	0.18	abcd
ND 48-2	20	bcde	53	abcd	0.15	abcd
Regent	19	cde	41	abcd	0.10	bcd
ND 71-1	18	cde	36	bcd	0.10	bcd
ND 1-5	17	cde	54	abcd	0.12	bcd
ND 1-6	13	de	47	abcd	0.07	cd
Northline	12	de	34	bcd	0.08	bcd
ND 9-1	11	de	34	bcd	0.06	cd
Parkhill	8	de	25	cd	0.04	cd
ND 1-4	8	e	25	d	0.03	d

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Powdery mildew causes stunted and disfigured new growth and infects fruit, giving it an undesirable waxy coating. Powdery mildew was observed mainly on the upper or outer foliage of the plant. ND 48-2, ND 1-5, ND 1-4, 'Regent', ND 1-2, ND 1-7, ND 71-1 were heavily infected in both locations. ND 18-1, 'Parkhill', ND 5-1, ND 12-1, ND 9-1, 'Honeywood', ND 71-1, ND 16-1, and 'Northline' were not highly infected. However, 'Parkhill' and ND 12-1 were also listed as statistically like observations of highly infected for one environment.

Table 6. Powdery mildew severity for serviceberry orchard at NDSU HRF in 2016.

	Bush coverage		Leaf coverage		Severity index	
Genotype	----------%----------					
ND 1-6	33.0	a	71.9	abc	0.24	a
ND 1-2	27.0	ab	76.9	a	0.20	ab
Regent	26.0	abc	75.6	ab	0.19	abc
ND 1-4	26.0	abc	71.8	abc	0.21	a
ND 1-7	22.2	abcd	70.0	abc	0.17	abcd
ND 15-2	22.0	abcd	54.0	cd	0.15	abcde
ND 1-5	21.2	abcd	75.5	ab	0.17	abcd
ND 18-1	17.2	abcde	49.3	d	0.07	bcdef
ND 48-2	16.0	cbcdef	81.6	a	0.13	abcdef
Parkhill	14.0	bcdef	83.7	a	0.11	abcdef
ND 41-1	12.0	bcdef	8.1	f	0.03	ef
ND 5-1	10.0	cdef	52.5	cd	0.06	cdef
ND 12-1	9.2	cdef	53.7	cd	0.04	def
ND 9-1	9.0	def	56.7	bcd	0.05	def
ND 17-2	4.2	ef	16.7	ef	0.01	f
Honeywood	1.2	ef	3.2	f	0.00	f
ND 71-1	0.8	ef	8.1	f	0.00	f
ND 16-1	0.0	f	0.0	f	0.00	f
Northline	0.0	f	0.0	f	0.00	f

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Harvest

O'Brien's test for homogeneity of variance precluded combination of environments for fruit total weight, fruit marketable weight, soluble sugar content, and pH (Table A22-25). The change from environments was notable for these traits (Table 8). As such, individual ANOVA were performed for each environment and reviewed for fruit total and marketable weight for consistently high yielding genotypes (Table 9 & 10). Ardayfio and Hatterman-Valenti (2015) saw a similar interaction between yield and year in a genotype trial performed at NDSU HRF and attributed it to differences in rainfall. In Canada, trials were irrigated and appeared to yield more consistently (St.-Pierre et al., 2005). In future trials, irrigation is recommended.

Table 7. Powdery mildew severity for serviceberry orchard at WREC in 2016.

	Bush coverage		Leaf coverage		Severity index	
Genotype	----------%----------					
Regent	80.0	a	73.0	abc	0.58	a
ND 1-7	69.0	ab	84.0	a	0.61	a
ND 41-1	67.0	ab	55.0	abcde	0.45	abcd
ND 1-2	65.0	ab	73.0	abc	0.46	abc
ND 17-2	63.8	ab	57.0	abcde	0.36	abcde
ND 1-4	62.0	ab	80.0	ab	0.51	ab
ND 1-6	54.0	abc	50.0	bcdef	0.36	abcde
ND 1-5	54.0	abc	78.0	ab	0.44	abcde
ND 48-2	52.2	abcd	89.7	a	0.45	abcde
ND 9-1	49.2	abcd	48.0	bcdefg	0.25	bcdef
ND 12-1	46.0	abcd	70.0	abcd	0.32	abcdef
Parkhill	42.8	abcde	35.2	defg	0.28	abcdef
ND 16-1	33.0	bcde	45.0	cdefg	0.18	cdef
ND 18-1	29.0	bcde	66.0	abcd	0.22	bcdef
ND 71-1	21.6	cde	25.2	efg	0.15	cdef
ND 15-2	20.0	cde	40.0	defg	0.13	def
ND 5-1	18.7	cde	52.6	abcdef	0.10	ef
Northline	11.6	de	18.0	fg	0.00	f
Honeywood	1.6	e	15.0	g	0.00	f

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Total fruit yield was consistently highest in genotypes ND 1-2, ND 1-4, ND 1-6, ND 1-7, ND 48-2 for each environment, except NDSU HRF 2014. ND 1-2, ND 1-4, ND 1-6, and ND 48-2 consistently gave higher marketable yields. Variability in serviceberry yield can be caused by several things bird predation, insect and disease infestation, alternate bearing tendencies, and the combination of manual harvesting and uneven ripening. It is possible that alternate bearing is occurring, as it is common in *Rosaceae* fruit.

Accumulated growing degree days for NDSU HRF 2014-2016, WREC 2016, and NDSU HRF 2017 were 1073, 760, 758, 572, and 765, respectively, upon harvest. The advanced accumulation of growing degrees and low overall harvest at NDSU HRF 2014 suggest that

genotypes were past peak harvest. As the accumulation of this heat unit appears related to ripening of serviceberry, it is recommended that genotypes advanced to further trials are monitored for correlation between growing degree and ripeness factors. Rainfall did not appear to have as great an impact on fruit ripening as growing degrees.

Table 8. Serviceberry fruit was variable among five North Dakota environments for fruit mean total weight, marketable weight, soluble sugars, and acidity.

Environment	Fruit Total Weight	Fruit Marketable Weight	Soluble sugar content	pH
	----------g----------		--°Brix--	
HRF 2014	84±121	49±64	16.7±2.8	4.29±0.16
HRF 2015	544±602	503±563	15.5±2.4	3.79±0.26
HRF 2016	2335±1711	2247±1635	12.8±2.3	4.00±0.24
WREC 2016	2313±2250	1995±1739	14.0±1.8	4.28±0.23
HRF 2017	1484±968	1413±929	16.5±2.1	3.99±0.21

O'Brien's test for homogeneity of variance for fifty-pome weight and diameter showed homogeneity of variance for environment. Therefore, fifty-pome weight and diameter were combined across environments (Table 11). A single pome usually weighs 1 g; between genotypes, pome weight has ranged from 0.79 to 1.66 in Saskatoon, SK (Zatylny et al., 2005) and 0.43 and 1.10 at NDSU HRF, ND (Ardayfio and Hatterman-Valenti, 2015). Pome size parameters in this study were similar among environments, despite statistical differences in yield and flavor qualities. This suggests that maximum pome size is genetically predetermined. 'Northline' is the largest in pome diameter. ND 48-2 and 'Northline' are highest for fifty-pome weight, a single pome weighing over 1 g.

Table 9. Mean total serviceberry fruit yield among the five North Dakota environments.

	HRF 2014		HRF 2015		HRF 2016		WREC 2016		HRF 2017	
Genotype	----------g----------									
ND 1-2	293	a	1151	a	4060	a	3395	abcd	2096	abcd
ND 1-4	168	ab	1152	a	3735	ab	3383	abcd	2700	a
ND 1-6	140	b	942	abc	3769	ab	4402	ab	2272	ab
ND 1-7	125	bc	912	abc	4101	a	3553	abcd	2062	abcd
Parkhill	116	bc	678	abcd	2527	abcde	1541	cdef	1271	def
ND 71-1	106	bc	460	bcde	1914	cdef	1505	cdef	2450	a
ND 17-2	89	bc	72	de	553	f	1511	cdef	63	g
ND 9-1	83	bc	733	abcd	3219	abcd	1500	cdef	1330	cdef
ND 41-1	76	bc	58	de	715	ef	1846	cdef	477	fg
ND 18-1	70	bc	509	abcde	1468	def	1382	def	2234	abc
ND 1-5	68	bc	1062	ab	3509	abc	4632	a	1281	def
Regent	61	bc	727	abcd	2219	bcdef	3155	abcde	1487	bcde
ND 48-2	49	bc	778	abcd	3912	ab	3919	abc	2719	a
ND 16-1	32	bc	66	de	1117	ef	1346	def	467	fg
ND 5-1	25	bc	99	de	1501	def	654	f	570	efg
ND 12-1	23	bc	244	de	1290	ef	1099	def	1462	bcde
Honeywood	21	bc	69	de	1313	ef	2271	abcdef	1792	abcd
ND 15-2	20	bc	321	cde	1140	ef	757	ef	490	efg
Northline	0	c	2	e	753	ef	2101	bcdef	404	fg

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Flavor components for pomes include soluble sugar content and pH. Here we have included pectin, as it affects the mouthfeel and process ability of pomes.

The pH of fruits is important for flavor and storability, more specifically. As shown by Plagge and Gerhardt (1930), there is a close relationship between carbohydrates and available hydrogen ions that fluctuates with storage time and temperature in pome fruits such as apple. In serviceberry, genotype had a significant effect on pome pH (Table A43-47). Upon analysis, a bimodal pattern was revealed in the distribution of pH. The shift in acidity may be attributed to uneven ripening and coloration or fluctuation of post-storage acid to carbohydrate ratios. The last 3 stages of ripening consist of deep coloration and rapid decrease in pH, followed by relatively constant pH in the final stage (Rogiers and Knowles, 1997). The bimodal pattern seen in our data may reflect the difference in pH in purple stages of ripeness.

Mean separation of genotypes revealed that ‘Regent’, ND 1-2, ND 48-2, ND 1-4, ND 1-5, ND 1-7, ‘Parkhill’ had consistently high pH (Table 12). ND 15-2, ND 71-1, ‘Northline’, and ND 12-1 were consistently low in pH, excepting NDSU HRF 2014.

The mean soluble sugar content (SSC) for each environment varied among genotypes (Table A37-41). Water intake to fruit cells is an important part of fruit development. A major event in ripening near harvest is the swelling of cells before accumulation of anthocyanins. The environments, in the same order, received 25.5, 27.8, 11.9, 12.0, and 7.9 cm of rainfall prior to harvest. In future studies, it may be useful to monitor a potential correlation between rainfall and SSC (Table 13). If there is a compelling case for this, then flavor parameters for the NDSU HRF 2014 juice tests might be skewed.

No cultivar was consistently high among the five environments for SSC. ND 5-1, ND 16-1 were ranked high for at least 3 environments and are worth consideration. ND 17-2 appeared to have low yields, but these may be attributed to a much slower ripening period than other accessions, which was disregarded in once-over harvesting. ND 15-2 may be recommended on soluble sugar content alone; it repeatedly ranked among the highest and in 2 of the environments obtained a mean °Brix higher than 20.

Two accessions and one named genotype were selected for apparent gelling ability in juice form, and three were selected as comparisons. 'Regent,' ND 1-7, and ND 15-2; and Honeywood, ND 12-1, and ND 71-1 were these comparisons, respectively. Despite appearances, there were no significant differences among genotypes. The mean pectin content for each was 3.62, 3.57, 3.80, 3.72, 3.94, and 3.75%, respectively. The agreement of these samples contradicts the variability seen in a study of Czech genotypes, in which mean pectin content ranged from 0.8-1.3% (Rop et al., 2012). The mean pectin content for the North American plants was more than twice the amount of those in the Czech Republic. This result may cast doubt upon quantification protocols or reflect significant genotype differences. In any case, the amount of pectin is not the only factor affecting gelling ability. In cherry and apricot jams were found a mere 0.37 and 0.76% polygalacturonic acid, the base for all pectic compounds (Shelukhina and Fedichkina, 1994).

Table 10. Mean marketable serviceberry fruit yield among five North Dakota environments.

	HRF 2014		HRF 2015		HRF 2016		WREC 2016		HRF 2017	
Genotype	----------g----------									
ND 1-2	149	a	1104	a	3889	a	2643	abcde	1968	abcd
ND 1-4	84	ab	898	abc	3519	ab	2856	abcde	2574	a
ND 1-6	82	ab	913	abc	3568	ab	3604	ab	2184	ab
ND 71-1	81	ab	452	bcde	1834	cdef	1107	efg	2360	a
ND 1-7	76	abc	893	abc	3900	a	2546	abcdef	1989	abcd
Parkhill	74	abc	646	abcd	2412	abcde	1440	defg	1202	def
ND 18-1	48	bc	503	abcde	1395	def	1298	defg	2093	abc
ND 17-2	47	bc	72	de	505	f	1400	defg	52	g
ND 9-1	41	bc	695	abcd	3079	abcd	1333	defg	1259	cdef
ND 41-1	41	bc	57	de	1100	ef	1681	cdefg	467	efg
ND 1-5	34	bc	1021	ab	3289	abc	4037	a	1203	def
Regent	29	bc	546	abcde	2115	bcdef	3133	abcd	1400	bcde
ND 48-2	29	bc	729	abcd	3750	ab	3348	abc	2618	a
ND 16-1	24	bc	71	de	1056	ef	1231	efg	459	fg
ND 12-1	23	bc	240	de	1290	ef	1064	efg	1523	abcde
Honeywood	20	bc	56	de	1289	ef	1906	bcdefg	1670	abcd
ND 15-2	19	bc	293	cde	1067	ef	747	fg	465	efg
ND 5-1	18	bc	97	de	1461	def	637	g	562	efg
Northline	0	c	2	e	734	ef	1903	bcdefg	387	fg

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Table 11. Combined serviceberry pome size parameters combined for five North Dakota environments.

Genotype	50 Pome Weight		Mean Diameter	
	---g---		---mm---	
ND 48-2	74	a	11.1	bcd
Northline	69	a	13.0	a
ND 1-2	44	b	11.5	b
ND 1-4	43	b	11.2	bc
Honeywood	43	b	11.5	b
ND 1-5	42	b	10.7	cd
ND 9-1	42	b	11.1	bcd
ND 71-1	41	b	11.1	bcd
ND 1-6	41	b	11.1	cbd
ND 1-7	41	b	11.0	cbd
Parkhill	38	b	10.8	cbd
Regent	37	b	10.9	cbd
ND 16-1	35	b	10.5	de
ND 41-1	33	b	10.8	cbd
ND 5-1	30	b	9.9	ef
ND 15-2	27	b	9.5	f
ND 18-1	27	b	9.3	f
ND 12-1	26	b	9.5	f
ND 17-2	25	b	9.5	f

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Conclusions

These plants were collected from all corners of North Dakota for their virtues as ornamental edibles. In conclusion, we recommend genotypes for continued trials.

ND 15-2 may be a viable candidate for nursery production and commercial processing. It has a small, sweet pome. If evaluated more closely, it may have better resistance to Saskatoon-juniper rust than genotypes currently on the market. As a smaller pome (Table 10), there is more skin surface area per volume. The pectic chain responsible for formation of gel is found in the middle lamella between plant cells, and is responsible for holding cells together. It is found in

high abundance in the outer layer of such fruits as citrus and in apples. It may be argued that a smaller pome with high sugar is better suited for processing for these reasons.

ND 48-2 has a large pome and consistently high yields. It also appeared moderate in size. It will, however, need to be monitored for fungal disease continually. Further trials may reveal that infection rates are not as severe as they were in 2016.

ND 71-1 was noted as having broken bud in November 2015 before hard frosts. The following spring, there appeared to be no damage to the flower buds. The pome has been consistently high in sugar content and moderate in yield.

While these three genotypes stand apart for yield and processing qualities, depending upon the niche the others may fit into, they may also be considered. Additionally, several traits would be beneficial to continue to study, including bud development, flower display, ripening periods, storage stability, mechanical harvesting, and food processing. When these efforts have been made, this relatively obscure super fruit may become a common household item.

Table 12. Serviceberry fruit mean pH among five North Dakota environments.

	HRF 2014		HRF 2015		HRF 2016		WREC 2016		HRF 2017	
Genotype	----------pH----------									
ND 1-6	4.43	a	3.87	bc	4.28	a	4.42	ab	4.04	ab
ND 15-2	4.42	a	3.47	f	3.68	fg	4.06	f	3.76	cd
Regent	4.41	a	4.01	ab	4.16	ab	4.41	abc	4.01	abc
ND 9-1	4.41	a	3.77	cd	3.97	cd	4.24	bcdef	4.10	ab
ND 48-2	4.40	ab	4.02	ab	4.24	a	4.41	abc	4.11	ab
ND 1-2	4.40	ab	3.94	abc	4.25	a	4.42	abc	4.15	a
ND 1-5	4.37	ab	4.03	ab	4.24	a	4.39	abc	4.21	a
ND 1-4	4.34	ab	4.09	a	4.22	a	4.51	a	4.10	ab
ND 41-1	4.33	ab	3.56	def	3.85	def	4.21	bcdef	3.68	d
ND 1-7	4.32	ab	3.96	abc	4.17	ab	4.43	ab	3.99	abc
Honeywood	4.29	ab	3.95	abc	4.00	bcd	4.37	abcd	4.01	abc
Parkhill	4.29	ab	3.89	abc	4.14	abc	4.37	abcd	4.04	ab
ND 16-1	4.25	ab	3.74	cde	3.92	de	4.41	abc	4.07	ab
ND 5-1	4.25	ab	3.55	ef	3.77	efg	4.02	f	3.93	bc
ND 18-1	4.19	b	3.53	ef	3.78	efg	4.14	def	4.02	ab
ND 12-1	4.17	b	3.64	def	3.76	efg	4.08	ef	3.74	cd
ND 17-2	4.17	b	3.56	def	3.90	de	4.31	abcde	NA	
ND 71-1	3.98	c	3.54	ef	3.77	efg	4.19	cdef	3.75	cd
Northline	NA		3.28	f	3.66	g	4.03	f	3.9	bcd

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

Table 13. Serviceberry fruit mean soluble solid content among five North Dakota environments.

	HRF 2014		HRF 2015		HRF 2016		WREC 2016		HRF 2017	
Genotype	----------°Brix----------									
ND 71-1	20.3	a	16.5	cd	15.2	abc	14.5	abcd	16.5	bcd
ND 17-2	19.3	ab	19.4	ab	16.5	a	15.6	ab	NA	
ND 12-1	19.0	abc	15.7	cdef	15.3	abc	13.9	bcd	16.9	bcd
ND 5-1	18.8	abc	18.9	ab	13.8	cde	14.9	abc	17.9	b
ND 16-1	18.5	abc	15.1	cdefg	14.7	abcd	15.8	ab	18.1	b
Honeywood	18.3	abcd	13.8	efg	14.7	abcd	12.8	cd	15.0	de
ND 9-1	17.6	bcd	16.5	cd	12.2	fg	14.2	bcd	15.5	cde
ND 15-2	17.3	bcd	20.8	a	16.2	ab	14.7	abc	23.7	a
ND 1-6	16.6	cd	14.7	defg	10.5	h	12.7	cd	15.3	cde
ND 18-1	16.4	cde	13.6	g	13.5	def	12.5	d	16.5	bcd
ND 1-7	16.2	cde	13.6	fg	10.4	h	14.0	bcd	16.4	bcd
ND 1-5	15.8	cdef	13.6	g	10.8	gh	13.7	bcd	15.7	cde
ND 1-4	15.5	cdef	13.4	g	11.5	gh	12.4	d	17.5	bc
Parkhill	15.1	def	13.6	g	11.5	gh	16.7	a	16.3	bcd
ND 48-2	14.3	def	14.4	defg	10.5	gh	13.8	bcd	15.1	cde
ND 1-2	14.1	ef	14.3	efg	10.7	gh	12.9	cd	17.6	bc
ND 41-1	13.2	f	14.8	cdefg	12.3	efg	14.3	bcd	13.9	e
Regent	12.6	f	16.1	cde	11.2	gh	12.8	cd	15.1	cde
Northline	NA		17.2	bc	14.5	abcd	14.0	bcd	16.0	bcde

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).

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APPENDIX A: TABLES

Table A1. ANOVA for serviceberry plant height at NDSU HRF in 2016 revealed genotype as a significant factor.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.04	0.64
Genotype	18	0.20	2.91**
Error	72	0.06	

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A2. ANOVA for serviceberry plant height at WREC in 2016 revealed genotype as a significant factor.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.12	2.49
Genotype	18	0.08	1.82*
Error	68	0.04	

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A3. ANOVA for serviceberry plant height at NDSU HRF in 2017 revealed genotype as a significant factor.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.05	0.96
Genotype	18	0.45	7.73**
Error	70	0.05	

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A4. ANOVA for serviceberry plant width combined for 2016 and 2017 at NDSU HRF and WREC.

Source of variation	Degree of freedom	Mean square	F-value
Env	3	4.12	51.7**
Rep(env)	14	0.07	0.94
Genotype	18	0.39	5.01**
Genotype*Env	54	0.18	2.33**
Error	243	0.07	

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A5. ANOVA of *Entomosporium* bush infection at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	2341	5.91**
Genotype	18	1026	2.59**
Error	72	396	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A6. ANOVA of *Entomosporium* leaf infection at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	92	0.43
Genotype	18	733	3.45**
Error	72	212	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A7. ANOVA of *Entomosporium* index at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.036	0.04*
Genotype	18	0.067	4.86**
Error	65	0.013	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A8. ANOVA of *Entomosporium* bush infection at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	261	0.36
Genotype	18	1790	2.46**
Error	68	726	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A9. ANOVA of *Entomosporium* leaf infection at WREC in 2016.

Source of variation[NS]	Degree of freedom	Mean square	F-value
Rep	4	1326	0.19
Genotype	18	954	1.12
Error	68	726	-

[NS]No source of variation was significant at 0.05 level of confidence.

Table A10. ANOVA of *Entomosporium* infection index at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.037	0.59
Genotype	18	0.096	1.77*
Error	68	0.054	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A11. ANOVA for powdery mildew bush infection at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	69	0.38
Genotype	18	530	2.94**
Error	72	180	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A12. ANOVA for powdery mildew leaf infection at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	835	3.46*
Genotype	18	4466	18.5**
Error	72	241	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A13. ANOVA for powdery mildew infection index at NDSU HRF in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.010	0.93
Genotype	18	0.036	3.09**
Error	72	0.011	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A14. ANOVA for powdery mildew bush infection at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	261	0.36
Genotype	18	1790	2.46**
Error	68	726	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A15. ANOVA for powdery mildew leaf infection at WREC in 2016.

Source of variation [NS]	Degree of freedom	Mean square	F-value
Rep	4	1326	0.19
Genotype	18	954	1.12
Error	68	726	-

[NS]No source of variation was significant at 0.05 level of confidence.

Table A16. ANOVA for powdery mildew infection index at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.037	0.59
Genotype	18	0.096	1.77*
Error	68	0.054	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A17. ANOVA for Saskatoon-juniper rust bush infection at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	1152	1.57
Genotype	18	1752	2.39**
Error	68	731	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A18. ANOVA for Saskatoon-juniper rust leaf infection at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	756	2.34
Genotype	18	1067	3.30**
Error	68	323	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A19. ANOVA for Saskatoon-juniper rust infection index at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.059	2.79*
Genotype	18	0.039	1.84*
Error	68	0.021	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A20. Mean separation of bush and leaf percentages and severity index for Saskatoon-juniper rust at WREC orchard in 2016.

	Bush coverage		Leaf coverage		Severity index	
Genotype	----------%----------					
ND 16-1	61.2	a	26.0	bcdef	0.18	abc
ND 71-1	56.0	a	33.4	abc	0.22	ab
Honeywood	54.5	ab	55.1	a	0.30	a
ND 9-1	45.2	abc	28.4	bcd	0.15	abcd
ND 5-1	43.8	abcd	26.3	bcdef	0.17	abcd
ND 18-1	42.2	abcd	27.6	bcde	0.16	abcd
Northline	37.0	abcde	43.0	ab	0.17	abcd
ND 12-1	34.0	abcdef	38.0	ab	0.18	abcd
ND 1-7	26.6	abcdef	23.0	bcdefg	0.14	abcd
Parkhill	26.0	abcdef	9.1	defg	0.04	bcd
ND 41-1	20.4	bcdef	13.4	cdefg	0.09	bcd
ND 15-2	19.4	bcdef	15.0	cdefg	0.10	bcd
ND 17-2	18.0	cdef	14.2	cdefg	0.04	bcd
ND 48-2	11.8	cdef	7.4	defg	0.01	cd
ND 1-6	9.4	def	4.0	fg	0.01	cd
ND 1-4	7.0	ef	5.6	efg	0.01	cd
ND 1-2	6.4	ef	6.6	defg	0.01	cd
ND 1-5	3.4	ef	2.4	fg	0.00	d
Regent	0.4	f	1.2	g	0.00	d

Different letters within each column indicate significant differences according to the Tukey's test ($P < 0.05$).This test was not included in the main body as one environment is not representative of a trend.

Table A21. O'Brien's homogeneity of variance test for total fruit weight from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation	Degree of freedom	Mean square	F-value
Environment	4	3.99	20.0**
Error	408	1.99E13	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A22. O'Brien's homogeneity of variance test for fruit marketable weight from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation	Degree of freedom	Mean square	F-value
Environment	4	1.60E14	22**
Error	407	7.50E12	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A23. O'Brien's homogeneity of variance test for soluble sugar content from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation	Degree of freedom	Mean square	F-value
Environment	4	217	2.8*
Error	371	74	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A24. O'Brien's homogeneity of variance test for 50-pome weight from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation [NS]	Degree of freedom	Mean square	F-value
Environment	4	7.88E07	1.20
Error	357	6.54E07	-

[NS] No source of variation was significant at 0.05 level of confidence.

Table A25. ANOVA for total fruit weight at NDSU HRF 2014.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	37213	2.36*
Genotype	18	21742	1.91*
Error	60	11399	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A26. ANOVA for total fruit weight at NDSU HRF 2015.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	4.31E+05	1.78
Genotype	18	7.73E+05	3.19**
Error	63	2.42E+05	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A27. ANOVA for total fruit weight at NDSU HRF 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	1.80E+06	1.01
Genotype	18	7.17E+06	4.03**
Error	61	1.78E+06	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A28. ANOVA for total fruit weight WREC 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	1.48E+08	3.86**
Genotype	18	7.82E+06	2.04*
Error	72	3.84E+06	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A29. ANOVA for total fruit weight at NDSU HRF 2017.

Source of variation	Degree of freedom	Mean square	F-value
Rep	3	7.59E+05	2.19
Genotype	18	2.35E+05	6.79**
Error	43	3.46E+05	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A30. ANOVA for marketable weight at NDSU HRF 2014.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	9691	2.89*
Genotype	18	5764	1.72
Error	60	3349	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A31. ANOVA for marketable weight at NDSU HRF 2015.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	3.72E+05	1.68
Genotype	18	6.47E+05	2.93**
Error	63	2.21E+05	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A32. ANOVA for marketable weight at NDSU HRF 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	1.79E+06	1.04
Genotype	18	6.18E+06	3.61**
Error	61	1.71E+06	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A33. ANOVA for marketable weight at WREC in 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	7.46E+06	3.33*
Genotype	18	5.19E+06	2.32**
Error	72	2.24E+06	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A34. ANOVA for marketable weight at NDSU HRF 2017.

Source of variation	Degree of freedom	Mean square	F-value
Rep	3	6.90E+05	2.17
Genotype	18	2.13E+06	6.71**
Error	42	3.18E+05	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A35. O'Brien's homogeneity of variance test for mean diameter from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation [NS]	Degree of freedom	Mean square	F-value
Environment	4	16.4	0.92
Error	372	17.8	-

No source of variation was significant at 0.05 level of confidence.

Table A36. ANOVA for soluble sugar content at NDSU HRF 2014.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	18	4.87**
Genotype	17	15	4.01**
Error	40	3.9	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A37. ANOVA for soluble sugar content at NDSU HRF 2015.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.93	0.44
Genotype	18	18	8.45**
Error	57	2.1	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A38. ANOVA for soluble sugar content at NDSU HRF 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	5.3	3.32*
Genotype	18	18	11.3**
Error	59	1.6	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A39. ANOVA for soluble sugar content at WREC 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	3.6	1.28
Genotype	18	6.5	2.31**
Error	68	2.8	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A40. ANOVA for soluble sugar content at NDSU HRF 2017.

Source of variation	Degree of freedom	Mean square	F-value
Rep	3	4.5	2.06
Genotype	17	10.6	4.84**
Error	40	2.2	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A41. O'Brien's homogeneity of variance test for acidity from harvests during 2014-2017 at NDSU HRF and 2016 at WREC.

Source of variation	Degree of freedom	Mean square	F-value
Environment	4	0.01	3.65**
Error	356	0.003	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A42. ANOVA for acidity at NDSU HRF 2014.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.02	2.10
Genotype	17	0.05	4.59**
Error	31	0.01	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A43. ANOVA for acidity at NDSU HRF 2015.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.08	2.71*
Genotype	18	0.17	6.43**
Error	51	0.02	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A44. ANOVA for acidity at NDSU HRF 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.02	1.66
Genotype	18	0.19	11.9**
Error	59	0.01	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A45. ANOVA for acidity at WREC 2016.

Source of variation	Degree of freedom	Mean square	F-value
Rep	4	0.12	3.69**
Genotype	18	0.11	3.54**
Error	68	0.03	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A46. ANOVA for acidity at NDSU HRF 2017.

Source of variation	Degree of freedom	Mean square	F-value
Rep	3	0.16	7.46**
Genotype	17	0.07	3.67**
Error	40	0.02	-

*, **Significant at the 0.05 and 0.01 levels of significance, respectively.

Table A47. O'Brien's homogeneity of variance test for serviceberry fruit pectin content from harvests at NDSU HRF in 2017 and at WREC in 2016.

Source of variation [NS]	Degree of freedom	Mean square	F-value
Environment	1	0.04	1.98
Error	34	0.02	-

No source of variation was significant at 0.05 level of confidence.

Table A48. Combined ANOVA for fruit pectin content of selected serviceberry genotypes from the NDSU HRF in 2017 and WREC in 2016.

Source of variation [NS]	Degree of freedom	Mean square	F-value
Environment	1	0.09	0.98
Rep(env)	4	0.06	0.69
Genotype	5	0.10	1.10
Environment*Genotype	5	0.05	0.57
Error	34	0.09	-

[NS]No source of variation was significant at 0.05 level of confidence.

APPENDIX B: TISSUE CULTURE

Summary

There are sufficient systems in place for *Amelanchier* spp. regeneration from shoot tips and dormant buds (Lineberger, 1981; Pruski et al., 1990). Cited materials include the use of Murashige-Skoog-based (MS) medium supplemented with agar and plant growth hormones (PGRs) (Hajela et al., 1993; Pruski et al., 1990). A treatment of continuous agar medium produced an average 6.8 shoots in 73 days. In most other studies, however, semi-solid medium was more efficient, propagating as much as 100 shoots within six to 10 weeks. In shoot tips, MS medium has established serviceberry with a variety of PGRs. BA consistently gave positive results in serviceberry (Pruski et al., 1990; Lineberger, 1981; Hajela et al., 1993).

Shoot tips are convenient for culture establishment and multiplication of Allegheny serviceberry (Lineberger, 1981) and Saskatoon serviceberry Northline, 'Pembina', 'Smoky' and 'Thiessen' (Pruski et al, 1990). Additionally, introduction of leaf laminar tissue in culture has been applied to various species and was successful for regeneration of pear (Javadi et al., 2013), blackberry (Vujović et al., 2010), and crabapple (Lu et al., 2015); while Saskatoon serviceberry has systems sufficient for clonal propagation, adventitious regeneration from leaf is desirable for recalcitrant genotypes like 'Northline'.

Leaf initiated tissue culture for four varieties, ND 48-2, ND 9-1, Northline and 'Thiessen,' were attempted. These genotypes represent difficult, easy, and unknown initiation ability.

Serviceberry is a member of *Rosaceae*. Other fruiting woody plants in this family include crabapple, pear (*Pyrus communis* L.) and blackberry (*Rubus fruticosus* L.). Several factors of medium composition and culture were examined for leaf initiation in 'Flame', 'Strawberry

Parfait', and 'Royalty' crabapples (Lu et al., 2015). Explants inoculated onto MS medium containing various combinations of thidiazuron (TDZ) and naphthalene acetic acid (NAA) regenerated. Pear genotypes 'Harrow Delight', 'Bartlett', and 'Dargazi,' were indirectly regenerated to accelerate the breeding process in pears (Javadi et al., 2013) by inoculating young leaves on modified MS mediums with four TDZ concentrations and three NAA concentrations. The greatest shoot-forming callus was 128 mm3, taken from 'Harrow Delight' on medium amended with 4 µM TDZ and 1 µM NAA. Blackberry genotype Ĉaĉanska Bernstra leaves from in vitro shoots were inoculated onto 30 media with varying PGRs; 12 of these induced indirect shoot formation (Vujović et al., 2010). Callus formation rates (100%) occurred on media containing 2.0 mg L-1 BA with each of three rates (0.1, 1.0, and 2.0 mg L-1) of IBA, NAA, or 2,4-D; and TDZ at 1.0 mg L-1 with the same auxin combinations. Treatments with highest shoot regeneration rates were 1.0 mg L-1 TDZ, alone and with all 3 rates of IBA. After that, the top treatments were 2.0 mg L-1 BA plus 0.1 mg L-1 IBA and 2.0 mg L-1 BA plus 0.1 mg L-1 NAA, 1 mg L-1 TDZ plus 0.1 mg L-1 NAA. This indicates that the genotype Ĉaĉanska Bernstra responds consistently to TDZ.

In May 2016, an initiation experiment was undertaken in which 'Northline', 'Thiessen', ND 48-2, and ND 9-1 were applied to different media containing 0, 0.5, 1, 2, and 4 µM TDZ; and 0, 1, and 2 µM NAA. There were 3 replications and 3 light treatments: 24-hour, 16-hour, and 0 hour. Five leaf discs were inoculated onto petri dishes with 30 ml of medium. These were labeled, sealed and placed in an incubator at 27/25°C day and night; and were left for 4 weeks.

In August 2016, a similar experiment was performed. Three replications of the same media with combinations of TDZ and NAA were inoculated with the four genotypes. The photoperiods were 24-hour and 16-hour, without a dark option.

Callus formed on many of the treatments in various colors, textures, and quantities; however, no shoots formed. Results were inconclusive.

Attempts to extend tissue culture season were made by forcing branches in December, January, and February. However, none of these forced vigorously enough to produce the leaf material needed for experimentation.

Based on observations, attempts to initiate tissue culture from leaf tissue has been unsuccessful because of contamination and intensive labor; that is, establishment from leaf requires more phases to promote leaf callus, shoot initiation, and eventual root initiation. In addition, there is increased risk of losing clonal properties and testing must be performed to ascertain genetic semblance to the parent (Vujović et al., 2010). If further studies are to be pursued, it is recommended that attempts be directed at establishing mutants.

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