JUNEBERRY (AMELANCHIER ALNIFOLIA) MICROPROPAGATION AND CULTIVAR EVALUATION IN NORTH DAKOTA

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MASTER OF SCIENCE

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ABSTRACT

A growth chamber experiment was carried out for ten weeks to reduce post-rooting dormancy in juneberry micropropagation. An RCBD with a split plot arrangement and three replicates were used. Plantlets subjected to 750 mg/L GA, 100 mg/L BA, and 250 mg/L GA + 100 mg/L BA recorded the greatest leaf number. Pre-rooted 'Thiessen' plantlets recorded the greatest biomass (fresh and dry weight) and root volume.

In a second study, a cultivar evaluation was conducted in Absaraka, ND, where ten juneberry cultivars and a native biotype planted were evaluated for plant and fruit characteristics. An RCBD with four replicates was used. The high yielding cultivars for total yield were 'Thiessen', 'Martin', 'Parkhill', 'Pembina', 'Regent' and Native. 'Thiessen', 'Martin', and 'Parkhill' maintained a significant higher marketable yield. 'Thiessen', 'Regent', 'Martin', 'Parkhill' and 'Northline' had the largest fruits, while 'Thiessen' and 'Martin' fruit had the greatest mass.

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GENERAL INTRODUCTION

Juneberry, also known as Saskatoon or service berry and scientifically called *Amelanchier alnifolia* NUTT., is a shrub native to the North America, and specifically, Alaska, most of north-western Canada, and western and the north-central United States. The fruit is a small purple pome, sweet in taste and can be added to dried meat as flavor and preservative. An individual bush is capable of bearing fruits for up to 30 or more years (Schooley, 2003).

Juneberry has historically been known for its significance as food, medicine and wood uses (Harris, 1976). Juneberry is also known to contain significant amounts of dietary fiber, vitamins B2 (riboflavin) and biotin, and the essential minerals iron and manganese. Its nutrient profile is similar to the profile of blueberry, *Vaccinium corymbosum* (Mazza, 2005). Juneberry, however, belongs to the Rosacea family just like apple (St-Pierre, 2005). The release of recent data by the USDA indicates that the average juneberry antioxidant levels, specifically flavonoids are higher when compared to *Vaccinium corymbosum* (blueberry), *Fragaria ananassa* (strawberry) and *Rubus spp.* (raspberry) (Bhagwa et al., 2011).

Juneberry has significant potential for jam, juice, pastry, wine, jellies, sauces, and dried and frozen berry (St-Pierre, 2005). Income can be generated for farmers and in due course the state, when large scale production of juneberry becomes a reality in North Dakota. Canada has taken the lead in its commercialization where demand for juneberry has surpassed supply (St-Pierre, 2005).

In the North Dakota State University (NDSU) Plant Science Department there are at present fourteen cultivars and thirty one lines in culture. The fruit quality and yield differences of cultivars are as varied as the number of existing cultivars. There has been the

need to further research the ease of juneberry micropropagation after the most productive cultivars in the region of North Dakota have been identified.

This thesis consists of an overall abstract, a general introduction, literature review, literature cited, two distinct chapters, overall conclusion and an appendix. Each distinct chapter consists of an abstract, introduction, objectives, materials and methods, results and discussion, conclusions and literature cited. Each category is listed in the table of contents along with page numbers.

LITERATURE REVIEW

Propagation Methods

Juneberry plants can be propagated through sexual propagation methods which utilize seed, or by asexual propagation, which uses different vegetative parts of the plant. These asexual propagation methods include cuttings, crown divisions, suckers, and micropropagation (Nelson, 1987). Asexual reproduction is convenient when traits in the parent plant needs to be preserved. This is because the progeny are true to type, containing all the traits of the mother plant. Production of uniform, high quality juneberry plant material has been a challenge to juneberry producers (St-Pierre, 2005). Juneberry plants which were propagated by softwood cuttings exhibit rooting and post-rooting summer dormancy. Hard wood cuttings were found to be difficult to root and incompatibility symptoms were seen in grafted stocks. Sexual reproduction may not be a good propagation method as approximately 30% of the seedlings propagated from seeds can exhibit variation from the mother plant (Pruski et al., 1990).

The most convenient and successful propagation methods include seed germination and micropropagation (St-Pierre, 2005). Micropropagation techniques utilize small plant parts, such as pieces of leaves and stems or entire buds, which are cultured under sterile conditions on artificial growing medium. These techniques ultimately produce thousands of new plants. Micropropagation allows for the quick multiplication of what may be very limited parent material (St-Pierre, 2005).

Most juneberry cultivars do not aggressively spread, but they produce suckers beyond what was needed for economic fruit production (Pheh, 2004). These suckers are often removed during yearly dormant pruning. Dormant divisions were the most widely used

method of obtaining propagating materials until the 1960s. Obtaining suckers through yearly dormant pruning was not the cheapest method, since it is labor intensive and time consuming. Another disadvantage was that extra precaution needed to be taken to prevent roots from drying excessively. In addition, a long establishing period was generally required and damage to donor plants was possible if extra care was not taken when removing the suckers.

Juneberry plants do not transplant easily and divided suckers take a long time to recover from the transplanting shock and often died before recovering (Pheh, 2004). As much as 60% mortality of juneberry was experienced by early growers with the use of suckers as propagating materials. The availability of planting material with suckers was limited to the age of the plant, cultivar, suckers production and cultural practices. Suckers serve as a good source of identical material to the parent plant irrespective of the limiting factors of obtaining planting materials. This is a convenient asexual propagation method when limited planting material is required to replace dead plants in the juneberry orchard (St-Pierre, 2005).

Rhizomes are below ground stems that grow horizontally beneath the soil surface (Pheh, 2004). Vegetative buds protrude through the soil to the surface at the nodes and roots develop downwards into the soil at the same node. It is very important that the upward facing ends with node and axillary bud are planted vertically for rooting success. Without accurate identification, determining the proximal or distal rhizome ends can be very time consuming at planting. The disadvantages of rhizomes as a propagation method include labor intensiveness and time requirements making the procedures involved in rhizome propagation costly (Pheh, 2004).

The pruning of mature stalks of shrubs at ground level initiates the sprouting of stems with juvenile characteristics (Pheh, 2004). Young cuttings root more easily in comparison to mature cuttings. In addition, cuttings placed in a dark environment have the potential to improved rooting. Nelson and Sawatzky (1987) reported that 90% rooting success was obtained when juneberry and other woody plant shoots were rooted from juvenile materials. Better rooting was also observed when cuttings were made from plants that had been properly over-wintered. Research at Crop Diversification Centre North (CDCN), in 2000 and 2001, has also shown that non-etiolated young shoots have the possibility of increased rooting success (Pheh, 2004). Unreliable rooting has been experienced *in vitro* with juneberry as establishing plants either in soil or artificial medium has been difficult (St-Pierre and Shen, 2004).

Juneberry is a self-pollinating fruit crop. Although cross pollination is not necessary for seed production, seeds often contain genetic material from two parent plants (cross pollination). As a result, seedlings are not necessarily identical to their parents and desirable characteristics are likely to be lost (Pruski et al., 1990). Also, plants grown from seed require a longer period to produce fruits than vegetatively propagated plants (Wen-Quan et al., 1991).

The micropropagation technique uses different plant parts including small pieces of leaves, stems, buds, which are cultured under aseptic conditions on artificial growing medium. These techniques result in the production of many new plants identical to the parent plant. According to St-Pierre (2005) micropropagation is possibly the best method currently available for mass propagation of large quantities of juneberry plants. (Harris, 1980) reported on micropropagation of juneberry and even though he did not encounter any

major problems, he suggested that shoot multiplication could be manipulated with cytokinins, and rooting was dependent on the addition of auxins. Nevertheless, post-rooting dormancy (the cessation of growth that occurs after plantlets have successfully rooted) has been reported as a major problem during transplanting of juneberry which ultimately causes transplant losses (Hatterman-Valent, personal communication).

Dormancy: Cause and Effect

Over the many years of exposure to conditions of local climate in the temperate zones, trees have developed populations that are limited in their growth and adapted to certain light and temperature conditions (Olsen et al., 1997). The cessation of growth is usually physiological and not very well understood. Dormancy, as defined by (Lang, 1987), is the temporal cessation of visible growth in the meristem of a plant structure. Many plant parts are affected by dormancy and include seeds, bulbs and buds (Weaver, 1972). Responses of plant growth, which include germination and dormancy, are most likely influenced by the balance and presence of growth promoters and inhibitors.

Under dormant conditions the capacity for bud growth is limited to a very narrow environmental condition range, even though the buds are alive (Pallardy, 2008). This can occur to the extent that elongation of shoot apices cease even though environmental conditions are favorable. Exposure to cold conditions has been used to break the dormancy in buds so that active growth can be resumed (Pallardy, 2008). Dormancy for vegetative growth has been classified into three types: ecodormancy, which is influenced by environmental factors; paradormancy, which is influenced by physiological factors found outside the structures affected (i.e. apical dominance); and endodormancy, which is influence by physiological factors found inside the structures affected (Pallardy, 2008).

Drought and low temperatures are known to have the potential to maintain apical bud dormancy in a term referred to as ecodormancy. When the length of day light begins to decrease, as usually seen during the fall season, dormancy in plants can be sustained in a process called paradormancy. Endodormancy is a term which refers to the period of dormancy through a chilling condition. These conditions are known to overlap (Lang et al., 1987). Dormancy characteristics have been shown to vary with the dormancy type relative to dormancy initiation, sustenance and release. Plant hormones are known to play a major role with regards to dormancy (Pallardy, 2008).

Influence of Plant Growth Hormones

Plant hormones are classified into five basic groups, namely, gibberellins, cytokinins, auxins, abscisic acid and ethylene (Pallardy, 2008). Plant growth hormones are substances that occur naturally in plants, are effective in small quantities, and act to suppress or initiate growth in the form of signals. Developmental processes in plants are also regulated by these hormones (Pallardy, 2008). “Hormones are chemical signals that facilitate intercellular communication” (Fosket, 1994).

Gibberellic acid (GA) has been shown to stimulate growth in plant stems and leaves (Fosket, 1994). Gibberellic acid is also known to trigger the germination of seeds and to break bud dormancy. Gibberellins are mainly associated with dormancy release and increasing of bud activity. This bud activity is known to decrease at bud set in accordance with reduced GA production. Bud set refers to the process that a bud undergoes prior to dormancy (Fosket, 1994). Walser et al. (1981) concluded that the application of GA to break dormancy and induce stem elongation could substitute for the effects of cold chilling in dormant plants. In gymnosperm and angiosperm woody plants, investigations have revealed

that synthetic GA elongates internodes and increases plant height. However, exogenous GA causes a wide range of plant responses based on the type of GA, concentration, frequency, and application method. The effect of exogenous GA may also be affected by the age of the plant and the specific species (Pallardy, 2008).

Plant growth has been observed to be regulated by GAs at molecular, cellular, organ, and whole plant levels (Pallardy, 2008). Cell wall loosening, synthesis of membrane phospholipids, and hydrolysis of protein and sucrose are processes influenced at the molecular level. At the cellular level, GAs regulates cell differentiation, cell division and cell elongation (Pallardy, 2008). Leaf expansion, and stem elongation are processes activated by GAs at whole plant and organ levels. Specific GAs have been identified and are denoted with subscripts, (i.e. GA_3, GA_4, and GA_7). Noggle and Fritze (1983) showed that GAs could be used to break bud and seed dormancy. The regulation of internode growth is another important function of GAs. Dwarf plants are likely to grow to normal lengths after GA application (Pallardy, 2008).

Cytokinins, one of the classes of plant hormones, are known to be involved in plant biological activities (Cleland, 1999). Cytokinins are present in higher quantities in developing embryos, apical buds and root apices. N6-Benzyladenine is an example of a synthetic cytokinin that is used to enhance the growth and developments of plants (Cleland, 1999). The main effect of cytokinins is the promotion of cell division. In association with auxins, cytokinins are also known to inhibit apical dominance. In the presence of a high cytokinin to auxin ratio, shoot primordia formation is enhanced. Therefore, increased cytokinins influence lateral bud growth, which result in increased branching in plants (Cleland, 1999).

The effects of plant growth regulator applications of Benzyladenine (BA) and GA were studied by Pruski et al. (1990) to determine how these plant growth regulators could overcome post-rooting dormancy in micropropagated juneberry plantlets. Shoots were obtained from four juneberry cultivars ('Pembina', 'Smoky', 'Thiessen', and 'Northline'), and rooted in equal amounts of sand, soil, and vermiculite. Plant growth regulators and 0.1% Tween-20 surfactant were applied five weeks after planting to the newly rooted plantlets. The treatments contained 100 ppm GA, 400 ppm BA, 100 ppm GA + 400 ppm BA, and a control, which was sprayed with water and the surfactant. Ten weeks after the application, evaluations were made. All four cultivar plantlets sprayed with the GA treatments maintained a single stem but with elongated internodes, whereas the BA treated plantlets had multiple branched compact plants. 'Northline', 'Pembina' and 'Smoky' plantlets that were subjected to the BA treatment showed a significant increase in the length of the stems as well as an increase in the number of stems. In contrast, 'Thiessen' only demonstrated an increase in the number of branches. The authors concluded that the BA + GA treatment was best for all the cultivars because the plantlets subjected to the combined plant growth regulators treatment demonstrated an increase in the length and number of stems compared to the control group that were given no plant growth regulator.

Unfortunately, the BA + GA treatment studied by Pruski et al. (1990) did not measure root growth in response to the increased shoot growth. Teng and Timmer (1993) reported that GA stimulated hybrid poplar (*populous* x *euramericana*) shoot growth, but inhibited root growth. Similarly, preliminary research observed that when juneberry plantlets received BA + GA treatments shoot growth was also stimulate but root growth was inhibited (Hatterman-Valenti, personal communication).

Temperature and Light Effects on Dormancy and Plant Growth

Cold temperatures during the winter period is known to be the environmental condition that breaks dormancy in many plant species (Ross, 1996). When temperatures increase in the spring, germination resumes. Research with hazelnut (*Corylus avellana*) revealed that when subjected to a temperature of 5°C for approximately thirty days, seeds readily germinated as temperatures increased (Ross, 1996). Temperate wood species commonly have specific winter chilling requirements that must be satisfied in order to break dormant buds and facilitate growth with warmer temperatures in the spring (Schwartz and Hanes, 2010). Sufficient chilling is required to effectively break bud dormancy and initiate the onset of budburst for most temperate trees (Murray et al., 1989). When chilling requirements are not adequately met, budburst is delayed until sufficient warmth is received.

Generally, day-length during winter months is shorter than summer months. The constant yearly changes in photoperiod and temperature influence the growth cycles (growth and dormancy) of trees (Vegis, 1964). The length of night is also known to play an important role in the initiation of active or inactive bud formation. Night length also influences frost hardiness as well as dormancy in the fall (Olsen, 2010). Falusi and Calamassi (1990) observed in their experiment with *Fagus sylvatica* (beech), that growth of apical buds increased significantly with chilling. However, chilling did not increase the growth of lateral buds. Photoperiod also had little effect on lateral or apical bud growth. Beeches that were not chilled took more time to grow when compared to those that were chilled and the longer photoperiod resulted in internode elongation but not necessarily an increase in cell division within the internode.

Temperature and Root Formation

Soil temperature has been shown to have significant influence on root initiation and elongation. Andersen et al. (1986), reported that red pine (*Pinus resinosa* Ait.) grown in soil at a temperature of 20ºC had significantly more root growth than plants grown in soil at temperatures of 8, 12, or 16ºC. A temperature of 27ºC was recorded to produce longer and more lateral roots than a temperature of 15ºC for *Pinus radiata* (Monterey pine) (Bowen, 1970). Root length at 27ºC was increased by 70% compared to the 15ºC soil treatment. Seedlings of *Alnus viridis* (mountain alder), *Alnus glutinosa* (black alder), *Picea abies* (Norway spruce), *Pinus sylvestris*(Scots pine), and *Pinus cembra* (Swiss stone pine) when exposed to temperatures below 6ºC produced less than 3% new roots (Alvarez-Uria and Körner, 2007).

Juneberry Cultivars

'Honeywood' was discovered by A. J Porter in 1955 at Parkside, Saskatchewan in his nursery named 'Honeywood'. 'Martin' was a selected and introduced by D. Martin in his nursery block of 'Theissen' at Langham, Saskatchewan. Similarly, J. A. Wallace, in his nursery at Beaverlodge, Alberta, selected 'Northline' from the wild in 1958 and introduced it in 1965. In 1974, Parkhill nursery at Bismarck, North Dakota introduced 'Parkhill' which was a hybrid from Michigan. 'Pembina', as a wild plant, was selected by J. A Wallace in Barrhead, Alberta in 1932 and reselected in 1950 and introduced in 1956. A hybrid which was named 'Regent' was selected by J. Candrain in Regent, North Dakota. 'Regent' was introduced in 1977. 'Smoky' originated from Beaverlodge, Alberta. 'Smoky' was discovered by W. D. Albright, selection was done with Dr. W. T. Macoun, reselected and introduced by J. A Wallace in 1956. 'Success' is a hybrid from Pennsylvania. H. E. Van

Deman of Kansas acquired the selected seedling in 1873 and introduced it in 1878. Originating from Hepburn, Saskatchewan, 'Thiessen', as a wild plant, was discovered by Maria Loewen Thiessen in 1906. Seedlings were obtained from her farm by G. Krahn and introduced in 1976 (Zatylny and St-Pierre, 2003).

Juneberry cultivar trials have been conducted in Canada, for the purpose of identifying higher yielding juneberry cultivars, as well as identifying various desirable characteristics that exist among selected cultivars. In their study, Zatylyn et al. (2002) identified 'Pearson II', 'Smoky' and 'Honeywood' as their top yielding cultivars. Characteristics such as stem growth, suckering, fruit size and fruit yield were evaluated in their experiment. They noted that stem growth for 'Thiessen', 'Smoky' and 'Martin' was about 40 cm compared to about 20 cm for 'Northline', 'Honeywood', 'Success' and 'Pembina'. 'Thiessen', 'Pembina' and 'Martin' produced significantly fewer suckers than 'Northline' and 'Parkhill'. 'Thiessen' and 'Martin' were the cultivars that produced the largest fruit, while 'Parkhill' and 'Success' produced the smallest fruit. 'Smoky', 'Honewood', 'Thiessen', 'Martin', 'Parkhill' and 'Northline' were the highest yielding cultivars, while 'Pembina' and 'Success' were the lowest yielding cultivars. Davidson and Mazza (1991) concluded that since existing juneberry cultivars have been selected and cloned from the wild species, desirable characteristics such as shorter plants, higher yielding shrubs, bigger berry size and higher soluble solid content can be attained through controlled crossing.

Economic Importance of Juneberry

The juneberry industry has been steadily growing in Canada. There were 240 juneberry growers in 2002 with approximately 3.66 km^2 of land under cultivation (St-Pierre,

2003). In 2001 and 2002 about 1.5 million pound of juneberry were produced. A 5 million dollar was generated from the processing of about 226796 kg of juneberry in 2001. The juneberry was marketed either fresh, frozen or as processed products. An amount of 6.5 million dollars was received as revenue for juneberry when fruits were sold as fresh or processed in 2001. Jobs are provided for about 300 people full time or part time by the Saskatchewan fruit industry (St-Pierre, 2003).

Environmental Requirements in Juneberry Establishment

Factors such as planting site, soil pH, and weed control may also affect the proper growth and establishment of juneberry. Weeds are a problem because they compete with plants for water and nutrients. Losses of newly planted woody plants in a field setting are most commonly as a result of weed competition (Geyer and Long, 1998). Juneberry plants are easily adaptable with regards to soil and climate requirements and depending on the variety, are cold hardy to near -60°F (Barney et al. 2009). Even though juneberry plants are adaptable to a range of soil types, they grow best on well-drained soils such as sandy loams and loams. Drip irrigation is generally beneficial and is recommended over sprinkler irrigation since it reduces foliar diseases (St-Pierre, 2005).

Zatylyn et al. (2002) reported that juneberry cultivar survival rates varied from a of 60 to 97%. Although many factors influence survival rate, the authors concluded that poor quality of parental material for planting and genetic differences among cultivars influenced the survival outcomes in their study.

Poorly-drained soils and lands with permanent or seasonal high water tables are not recommended for commercial juneberry production (Barney et al. 2009). Juneberry appears to grow best at slightly acidic soil pH values (6.0 to 7.0), where all nutrients are easily

absorbed by plant roots. High concentrations of organic matter are not usually required for juneberry production unless a nutrient deficiency is diagnosed in the soil (St-Pierre, 2005).

Pests and Diseases of Juneberry

In as much as yield and fruit quality of juneberry has been the focus of the cultivar trials, insects and diseases attack juneberry roots, leaves, buds, flowers and fruits, and often affect plant survival and fruit yield and quality. When environmental conditions are favorable for the development of *Entomosporium mespili* (Entomosporium Leaf and Berry Spot), yields of juneberry can be reduced up to 100% (Holtslaf et al., 2004). Woolly elm aphid (*Eriosoma americanum*) is an insect that feeds on the roots of juneberry and can adversely influence the survival of juneberry less than five years of age (Barney et al., 2009). Other pests that attack juneberry are *Holocampa montanicola* (saskatoon sawfly), *Epinotia bicordana* (saskatoon bud moth), *Anthonomus quadrigibbus* (apple curculio). *Gymnosporangium nidusavis* (Juniper Rust), *Erysiphe polygoni* (Powdery Mildew), *Erwinia amylovora* (Fireblight) are some diseases that affect juneberries (Barney et al., 2009).

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CHAPTER I. REDUCING POST-ROOTING DORMANCY IN JUNEBERRY (*AMELANCHIER ALNIFOLIA*) USING PLANT GROWTH REGULATOR AND TEMPERATURE TREATMENTS

Abstract

A growth chamber study was conducted for ten weeks to evaluate post-rooting dormancy juneberry through tissue culture. Temperature of 6 and 25°C were main plots, while 'Thiessen' and 'Northline' cultivars (pre-rooted or no pre-rooted), and PGR treatments were sub-plots. Pre-rooted plantlets with 750 mg/L GA, 100 mg/L BA, and 250 mg/L GA + 100 mg/L BA treatments recorded the greatest leaf number. 'Thiessen' pre-rooted plantlets grown at 25°C also had the greatest leaf number. Application of 250, 500 and 750 mg/L GA for pre-rooted plantlets had the highest plant height.

Pre-rooted juneberry plantlets given 100, 200, 300 mg/L BA and 250 mg/L GA + 100 mg/L BA had the greatest branching. The highest branching was also recorded for 'Thiessen' plantlets grown at 25°C. Fresh weight and dry weight accumulated the most biomass with pre-rooted 'Thiessen' plantlets as well as producing the greatest root volume.

Introduction

Juneberry plants can be propagated using softwood cuttings, etiolated shoot cuttings, suckers, seeds, and root divisions (Mazza and Davidson, 1993). However, micropropagation through tissue culture technique has the advantage of faster and more prolific reproduction of juneberry. This process is efficient since the genetic composition of the cultivar, when desirable, is not altered in anyway as encountered during propagation with seeds (Mazza and Davidson, 1993). However, rooting juneberry shoots after proliferation in the laboratory has been challenging and inconsistent. A diverse response to rooting makes it difficult to

determine the source of variation even within specific cultivars (Pruski et al., 1990). Plants that eventually root continue to encounter survival and growth issues especially during their early growth stages. Knowing the optimal level of soil moisture and humidity at the rooting and initial establishment of the juneberry plantlets has been very challenging with no clear remedy.

Juneberry transplant dormancy (which is the cessation or drastic reduction in growth of plantlets) has been identified as a shortfall that inhibits successful mass production via micropropagation techniques (Mazza and Davidson, 1993). Pruski et al. (1990) reported that plantlets given gibberellic acid (GA) and cytokinin (specifically BA) had an increase in the length of shoots and number of stems compared to the control group that were given no plant growth regulators. Unfortunately, the study did not measure root growth in response to the increased shoot growth. Teng and Timmer (1993) reported that GA stimulated hybrid poplar (*populous* x *euramericana*) shoot growth, but at the expense of root growth. They concluded that the stimulated shoot growth from GA was only a temporary benefit that actually hindered establishment. The current study was conducted to determine if plant growth regulators and temperature could be used to stimulate shoot growth without reducing root growth. Overcoming post-rooting dormancy will improve commercial production variability.

Objectives

The overall objective of this research was to increase the survival rate of juneberry (*Amelanchier alnifolia* NUTT.) regeneration through the micropropagation procedure by reducing post-rooting dormancy as commonly observed with this species. The specific objective was to determine the effect of two temperatures (6 and 25ºC), two rooting

conditions (pre-rooted and not pre-rooted) and four rates of each plant growth regulator (gibberellic acid at 0, 250, 500 and 750 mg/L and benzyladenine at 0, 100, 200 and 300 mg/L) and two combination treatments of 250 mg/L GA + 100 mg/L BA and 250 mg/L GA + 200 mg/L BA on post-rooting dormancy of two juneberry cultivars ('Thiessen' and 'Northline').

The research question:

> Can post-rooting dormancy in juneberry be reduced using plant growth regulators and temperature treatments?

Hypothesis:

> The temperature of 25°C and plant growth regulators will have an effect on reducing post-rooting dormancy on the pre-rooted 'Thiessen' cultivar.

Materials and Methods

Establishment of tissue culture

Prior to the onset of cultivar rooting in the laboratory experiment in 2010 and 2011, shoot proliferation of the two cultivars, 'Northline' and 'Thiessen' was undertaken. Plantlets of these cultivars were obtained from already existing juneberry plantlets stock maintained through tissue culture in the North Dakota State University Plant Science department. This existing stock was produced from dormant buds obtained from juneberry plants currently grown at the North Dakota State University Research and Arboretum site near Absaraka, North Dakota. Micropropagation techniques were used to produce plant materials needed for the post-rooting dormancy study for each of the cultivars. Multiplication of juneberry plants was done under aseptic conditions in the laboratory. Explants used were buds from the stated cultivars. In order to eliminate surface contamination, buds were treated with 10 to

15% commercial bleach for 10 to 15 minutes. Buds were then rinsed in sterilized water four times; the fourth rinse was for 5 minutes in duration while the first three rinses were only seconds in duration. After the final rinse, buds were placed on sterilized paper towel to absorb the surface water present before insertion in the shoot-proliferation medium. These sterilized buds were placed on sterile medium for the purpose of multiplication of shoots that were then rooted and subjected to treatments for the experiment.

Shoot proliferation

Juneberry shoot proliferation medium was prepared in 1 liter of water using 30 g granulated sugar[1] (sucrose), 0.43 mM of adenine hemisulfate (ADS)[2] solution, 0.03 mM of thiamine hydrochloride[3] solution, 0.56 mM of *myo*-inositol[4], 1.23 mM of sodium phosphate[5] ($NaH_2PO_4.H_2O$), 4.3 g of Murashige and Skoog[6] (MS) basal salt mixture, and 0.01 mM of N6-benzyladenine[7] (BA), all solidified in 7 g of agar[8]. The BA granules were dissolved in sodium hydroxide[9] before the concentration was formulated. The pH of the medium was adjusted to 5.7 before autoclaving. Magenta boxes (6.5 cm by 6.5 cm by 8 cm and 6.5 cm by 6.5 cm by 10 cm) were filled with 35 ml of the formulated medium. The medium was autoclaved at a temperature of 121ºC and a pressure of 137900 Pa for 20 minutes. The medium was then allowed to cool and harden before placement of the sterilized buds. Magenta boxes with buds were kept in growth chambers under light intensity of between

[1] Granulated sugar, Supervalu Inc. Eden Praire, MN 55344.
[2] Adenine hemisulfate, *Phyto*Technologies Laboratories™, P. O. Box 13481, Shawnee Mission, KS; 66282-3481.
[3] Thiamine hydrochloride, *Phyto*Technologies Laboratories™, P. O. Box 13481, Shawnee Mission, KS; 66282-3481.
[4] *Myo*-inositol, *Phyto*Technology Laboratories™, P.O.Box 13481, Shawnee Mission, KS; 66282-3481.
[5] Sodium phosphate, Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103.
[6] Murashige and Skoog, *Phyto*Technology Laboratories™, P.O.Box 13481, Shawnee Mission, KS; 66282-3481.
[7] N6-benzyladenine, *Phyto*Technology Laboratories™, P.O.Box 13481, Shawnee Mission, KS; 66282-3481.
[8] Agar, *Phyto*Technology Laboratories™, P.O.Box 13481, Shawnee Mission, KS; 66282-3481.
[9] Sodium hydroxide, Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103.

21.9 to 48.2 µmol.m^{-2}.s^{-1} with a 16/8 h photoperiod and a temperature of approximately 20°C for four to six weeks. Subculture was done every four to six weeks until an appropriate number of shoots were obtained to conduct the experiment.

In vitro rooting

One half of the shoots for each of the two cultivars were sub-cultured using the shoot proliferating medium. The remaining half of shoots for each cultivar was used to induce root production. Using sterilized forceps and scissors, shoots between 2 - 2.5 cm were removed and placed into a beaker with deionized water to prevent them from drying out in the lamina flow hood. Using a forceps, the base of each shoot was dipped into a 1:1 mix of commercial rooting powders: Rootone[10] containing 0.2 % naphthalene acetic acid (NAA), and Rhizopon[11] containing 0.1 % indole-3- butyric acid (IBA). The dipped shoots were then planted at a depth of approximately 1 cm into the potting mixture held in sealable rectangular containers (12 cm by 18 cm by 6.5 cm). The potting mixture consisted of a 1:2 mix of autoclaved sand[12] and sunshine mix No. 1[13]. Sand was added since previous research showed that the sunshine mix No. 1 retained moisture for a longer period of time, which increased rot problems at the shoot base or initiated roots. The mixture of sand and sunshine mix No. 1 was autoclaved at a temperature of 121ºC and a pressure of 262010 Pa for 2 to 2.5 hours. Autoclaved potting mix was kept in the laminar flow hood to cool. The quantity of the potting mixture used was 591 cm^3 (2 cm depth) per container. The potting mixture was moistened with deionized (DI) water before filling the rectangular containers. The

[10] Rootone, GardenTech., P.O. Box 95437. Palatine, IL 60095-0437.
[11] Rhizopon® AA #1 (0.1), Phytotronics, 13688 Rider Trail N, Earth City MO 63045.
[12] Sand,TCC Materials Spec blended Construction Products, St. Paul, MN 55117.
[13] Sunshine mix No. 1, Sun Gro Horticulture Distribution Inc. F1153, 15831 N.E. 8th Street, Suite 100 Bellevue. Washington 98008.

rectangular containers had transparent lids for light penetration and a dark base to enhance root formation. Nine plantlets (experimental unit) were placed into each container.

Growth chamber experiment

There were 108 rectangular containers (54 per cultivar), each holding nine juneberry shoots treated with the rooting powder that were kept in growth chambers with temperatures of 25°C during the day and 18°C during the night for a four week period in order for roots to form. The remaining half of the shoots were maintained in shoot proliferating medium for an additional four weeks, and then *in vitro* rooting was carried out as previously described. Therefore, plants in the first set of 108 containers (pre-rooted) had an opportunity to produce roots for four weeks, while plants in the later set of 108 containers (not pre-rooted) did not have root formation prior to the plant growth regulator application. All 216 containers were then treated with the plant growth regulator treatments.

Different concentrations of plant growth regulators, specifically GA_{4+7} and BA, were applied to the pre-rooted and non-rooted juneberry shoots. Gibberellic acids - GA_{4+7}[14] with a molecular weight of 331g. (GA_4 - $C_{19}H_{24}O_5$ with a molecular weight of 332g and GA_7 - $C_{19}H_{22}O_5$ with a molecular weight of 330g) and a 1:1 ratio of GA_4 and GA_7 was used. The N6-Benzyladenine[15] ($C_{12}H_{11}N_5$) has a molecular weight of 225g. Three different rates of 0 (control), 250 mg/L, 500 mg/L and 750 mg/L were used for gibberellic acid (specifically GA_{4+7}) while 0 (control), 100 mg/L, 200 mg/L and 300 mg/L were used for N6-Benzyladenine (BA). Two combinations of 250 mg/L GA + 100 mg/L BA and 250 mg/L GA + 200 mg/L BA were used, providing nine plant growth regulator treatments.

[14] ProVide plant growth regulator GA_{4+7}, Valent BioSciences Corporation 870 Technology Way, Suite 100 Libertyville, IL 60048.
[15] N6-Benzyladenine, *Phyto*Technology Laboratories™, P.O.Box 13481, Shawnee Mission, KS; 66282-3481.

Plant growth regulator treatments were applied as a foliar spray (one spray per plant) after four weeks for the pre-rooted juneberry and two days after planting for the non-rooted (not pre-rooted) treatments. Containers of both rooted and non-rooted juneberry plantlets were placed into six growth chambers for six weeks. Containers were placed into a split plot arrangement using three growth chambers set at 25°C and three growth chambers set at 6°C, respectively. Growth chambers having 25°C were set to 18°C for their night temperatures, while growth chambers at 6°C were maintained at the same temperature for both day and night. The temperature treatments were used to study how temperature can interact with plant growth regulators to influence post-rooting dormancy in the selected cultivars.

Each replication included a growth chamber at a 6°C and a growth chamber 25°C. Growth chambers were similar except for temperatures and were randomly assigned to the temperature treatments. All fluorescent light tubes in the growth chambers were replaced with new ones at the beginning of the experiment. Each growth chamber had four shelves and each shelf had two fluorescent light bulbs. The intensity of light among the six growth chambers were similar but ranged within shelves between 21.9 to 48.2 $\mu mol.m^{-2}.s^{-1}$ depending on location in the chamber.

The experimental design was a randomized complete block design (RCBD) with split plot arrangement and three replicates. Temperatures were main plots, while subplots were cultivar, rooting conditions and plant growth regulators. Thus, within any growth chamber (main plot) there were ‘Thiessen’ pre-rooted, ‘Thiessen’ not pre-rooted, ‘Northline’ pre-rooted and ‘Northline’ not pre-rooted assigned to one of the four shelves. Each shelf contained nine containers (nine growth regulator combinations). Experimental units (containers) were re-randomized daily within each shelf to reduce the influence of light or

temperature variations that may exist across the shelf. In addition, shelves were periodically (one week interval) re-randomized within the growth chamber so that each container was at a particular height in the growth chamber for an equal amount of time and exposed to similar variation in light intensity and temperature as well.

Data collection

After six weeks in the growth chambers, plant heights were measured from the surface of the potting mixture to the top of the stem. Number of leaves and branching was counted on plantlets. The number of leaves was determined by counting all open leaves whether fully expanded or not. A branch constituted a distinct growth of an axillary bud with nodes, internodes, and leaves. Root volume was measured among the different treatment combinations using WinRHIZO (software that utilizes a scanner to determine root volume) and data were analyzed. Only plantlets that had roots had their root volumes taken. Plants were gently pulled apart to distinguish the rooted from the un-rooted. Plantlets with roots were firm and lifted the potting mix around the base when lifted. Plants having roots among the treatments were randomly uprooted, the roots washed carefully by shaking in water to remove most of the potting mixture without breaking the roots. The root volume was determined with WinRHIZO by placing the plants horizontally in a thin film of water to aid in root separation in a specialized container and scanner. The software computed the root volume. Fresh weight and dry weight of the rooted plants of the various treatments were measured. After the fresh weight was taken, plants were put in paper envelopes, dried at 65-68°C for 24 hours and then reweighed for dry weight measurements.

Greenhouse experiment

All remaining juneberry plants (rooted or unrooted) from the growth chambers were transplanted into 15cm long cones with sunshine mix no. 1. The cones had more depth to encourage better root formation and an open top for unrestricted elongation of plants. Each cone was labeled with the initial temperature, cultivar and growth regulator treatments. The temperature in the greenhouse was between 15-20ºC. Cones were arranged on trays and covered with Agrofabric Pro 17[16] to help conserve moisture and prevent drying out of the plantlets. Trays were kept in the greenhouse for four weeks to observe the effect of treatments on post-rooting dormancy. Continued growth of plants was measured by recording the number of leaves, length of upright stem, and branching of plants.

The experiment was repeated with specific changes based on information learned. An additional hour and a half was added to the time the potting mixture was autoclaved, because mold was frequently found on plants in the growth chambers of the previous experiment. The additional time in the autoclave was to better sterilize the potting mixture with the hope of eliminating or reducing mold incidence in the repeated experiment.

The six-week period that juneberry plants were maintained in the growth chamber was reduced to a four-week period because the treatments with GA_{4+7} resulted in plants that elongated to lid surfaces of the rectangular containers. The lids, in addition to restricting further elongation of the plantlets, also accumulated moisture that caused shoot tip necrosis. Apart from shortening the growth period to four weeks, the moistening of the initial potting mixture was reduced in the repeated experiment. This action was taken to possibly help minimize mold growth.

[16] Agrofabric Pro 17, Hummert International 4500 Earth City Expressway, Earth City, MO 63045

In the first experiment, the cones were covered with agrofabric pro 17 which was to help increase the humidity. In the second experiment, instead of covering the trays with agrofabric pro 17, plants were sprayed with water in the mist room for six seconds every 20 minutes for the first week, four seconds every thirty minutes for the second week, and two seconds every thirty minutes for the third week. The trays were removed from the mist room after the third week.

Statistical analysis

Data were analyzed using Proc GLM of the statistical software, SAS 9.3. Post-rooting dormancy of juneberry was evaluated as a randomized complete block design with a split-plot arrangement. The juneberry variable measured included number of leaves, stem length, branching, fresh weight, dry weight and root volume. The null hypothesis was that, temperatures and plant growth regulators have no effect on juneberry post-rooting dormancy. Dead plants were given a value of zero rather than considered missing data to enable SAS to estimate the actual values present in some replications and not categorize the values as non-estimable.

Residual variance for years were combined when found to be homogeneous. Fisher's protected LSD at 0.05 level of significance was used where appropriate to separate treatment means. The ANOVA with computed values for the significant and non-significant factors are listed in the appendix.

Results and Discussion

The experiment varied temperature and plant growth regulator concentrations for two juneberry cultivars to better understand ways to overcome post-rooting dormancy through micropropagation. The variables measured to estimate the extent of post-rooting

dormancy included number of leaves, stem length, branching, fresh weight, dry weight and root volume. All three-way interactions were discussed using the significant two-way interactions that covered all the factors involved in the three-way interactions.

Number of leaves

The interaction between plant growth regulator and rooting status was significant at $P \leq 0.05$. Juneberry plantlets that were subjected to four weeks of pre-rooted conditions with growth regulator treatments of 750 mg/L GA, 100 mg/L BA and 250 mg/L GA + 100 mg/L BA, produced the greatest number of leaves, which was greater than all remaining rooting conditions and plant growth regulator treatments (Table 1).

Increasing the concentration of BA above 100 mg/L for pre-rooted plants reduced the number of leaves suggesting that the optimum BA concentration for leaf number with pre-rooted plants was 100 mg/L BA. On the other hand, the greatest leaf production of the pre-rooted plants occurred when the highest straight GA concentration of 750 mg/L was used. This result suggests that the optimum concentration may have not been reached and that concentrations greater than 750 mg/L GA may have resulted in more number of leaves. None of the plant growth regulator treatments increased leaf production compared to the control when plants were not pre-rooted. Plantlets receiving the combined treatments of 250 mg/L GA + 100 mg/L BA and 250 mg/L GA + 200 mg/L BA responded similarly to the straight BA treatments. It appears that the 250 mg/L GA did not have any effect on the leaf number for the pre-rooted treatments because the same effect of the straight BA treatments was recorded in the combined treatments as well.

Table 1. Juneberry leaf number averaged over cultivar and temperature as influenced by plant growth regulator and rooted treatments.

Plant growth regulator	Pre-rooted	Not pre-rooted
--------- mg/L---------	----------no.----------	
Control	1.7 b[z]	1.4 bc[z]
250 GA	1.9 b	1.4 bc
500 GA	1.9 b	1.0 bc
750 GA	3.7 a	1.6 bc
100 BA	4.1 a	1.9 b
200 BA	2.8 b	1.2 bc
300 BA	2.9 b	2.3 b
250 GA + 100 BA	4.0 a	1.3 bc
250 GA + 200 BA	2.3 b	0.5 c

[z]Means with the same letter(s) are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

A significant two-way interaction ($P < 0.0001$) occurred between temperature and rooting status for leaf production. Pre-rooted plantlets maintained at 25°C with the presence of roots may have been the cause of more leaf formation (Table 2), as improved plant growth may be more certain with better root formation. At 6°C, pre-rooted plantlets did not perform any better from the not pre-rooted plantlets at both temperatures.

Table 2. Juneberry leaf number averaged over cultivar and plant growth regulator as influenced by temperature and rooted treatments.

Temperature	Rooting status	Number of leaves
--°C--		------no.-----
6	Pre-rooted	1.9 b[z]
6	Not pre-rooted	1.8 b
25	Pre-rooted	3.7 a
25	Not pre-rooted	1.0 c

[z]Means with the same letter(s) are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

A significant two-way interaction ($P < 0.0001$) occurred between temperature and cultivar for leaf production. 'Thiessen' plantlets maintained at 25°C produced the most leaves followed by 'Thiessen' plantlets maintained at 6°C (Table 3). 'Northline' responded

poorly and similarly at both temperatures. Post-rooting dormancy has been reported to be more of a problem with 'Northline' than 'Thiessen' (Pruski et al., 1990) and our data supports their findings.

Table 3. Juneberry leaf number averaged over rooting status and plant growth regulator as influenced by temperature and cultivar treatments.

Temperature	Cultivar	Number of leaves
---°C---		------no.-----
6	'Thiessen'	2.3 b[z]
6	'Northline'	1.4 c
25	'Thiessen'	3.6 a
25	'Northline'	1.1 c

[z]Means with the same letter(s) are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

There was also a two-way interaction ($P < 0.01$) between rooting status and cultivar for leaf production. Pre-rooted 'Thiessen' plantlets produced the most leaf (Table 4). Whereas 'Thiessen' not pre-rooted plantlets were not different from pre-rooted 'Northline' in leaf number. This goes to confirm again that 'Thiessen' may have a better response to treatment effects even under unfavorable growth conditions. Although not significantly different from pre-rooted 'Northline', not pre-rooted 'Thiessen' was numerically higher in leaf number.

Table 4. Juneberry leaf number averaged over temperature and plant growth regulator as influenced by rooted and cultivar treatments.

Rooting status	Cultivar	Number of leaves
		------no.-----
Pre-rooted	'Thiessen'	4.0 a[z]
Pre-rooted	'Northline'	1.6 b
Not pre-rooted	'Thiessen'	2.0 b
Not pre-rooted	'Northline'	0.8 c

[z]Means with the same letter(s) are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Stem length

There was a significant interaction ($P < 0.001$) for stem length as influenced by plant growth regulator and rooting status. Plants grew taller when pre-rooted plantlets received straight GA treatments (Table 5). Stem length was generally similar to the control plantlets for all growth regulator treatments when plantlets were not pre-rooted. Results suggest that the straight GA concentrations could only cause internode elongation when plantlets were pre-rooted. Also the GA concentrations did not indicate optimum or minimum growth trends. However, the BA and GA combinations for the pre-rooted treatments suggest that the effectiveness of GA was reduced by the presence of the BA treatment in stem elongation.

Table 5. Juneberry plant height averaged over temperature and cultivar as influenced by rooted and plant growth regulator treatments.

Plant growth regulator	Pre-rooted	Not pre-rooted
------ mg/L------	----------cm----------	
Control	1.5 bc[z]	1.2 bc[z]
250 GA	3.1 a	1.5 bc
500 GA	3.0 a	1.5 bc
750 GA	3.3 a	1.1 bc
100 BA	2.0 b	1.1 bc
200 BA	1.6 b	1.0 c
300 BA	1.7 b	1.0 c
250 GA + 100 BA	2.0 b	1.2 bc
250 GA + 200 BA	2.0 b	1.3 bc

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

The interaction of temperature and pre-rooted plantlets was also significant for stem length ($P < 0.0001$). Plantlets that had been subjected to pre-rooting treatments produced the longest stems at both temperatures (Table 6). This result suggests that pre-rooted plants have the potential of growth irrespective of the varied temperatures used in this study.

Table 6. Juneberry plant height averaged over plant growth regulator and cultivar as influenced by temperature and rooted treatments.

Temperature	Rooting status	Stem length
--°C--		----cm----
6	Pre-rooted	2.2 a[z]
6	Not pre-rooted	1.6 b
25	Pre-rooted	2.3 a
25	Not pre-rooted	0.8 c

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Temperature and plant growth regulator interaction was also significant at $P < 0.0001$ for stem length. Plantlets treated with the straight GAs and placed under the 25°C temperature were not different in stem length from all other plantlets receiving all the growth regulator treatment combinations under the 6°C temperature except for control under 6°C (Table 7). Results suggest that 250 mg/L GA may be the optimum concentration for stem length for plants when grown at the 25°C temperature, since numerically stem length was reduced with higher concentration even though they were not statistically different.

Table 7. Juneberry plant height averaged over rooting conditions and cultivar as influenced by temperature and plant growth regulator treatments.

Plant growth regulator	6°C	25°C
------ mg/L------	----------cm----------	
Control	1.5 b[z]	1.2 bc[z]
250 GA	2.1 a	2.5 a
500 GA	2.1 a	2.4 a
750 GA	2.0 ab	2.4 a
100 BA	1.8 ab	1.3 bc
200 BA	1.8 ab	0.8 c
300 BA	1.7 ab	1.3 bc
250 GA + 100 BA	2.0 ab	0.9 c
250 GA + 200 BA	2.0 ab	1.2 bc

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Stem length for plants placed under the 6°C temperature were similar to the control except for 250 and 500 mg/L GA plant growth regulator treatments.

There was an interaction of temperature and cultivar which was also significant at $P < 0.01$ for stem length. Although at 25°C 'Thiessen' plantlets exhibited greater stem length than 'Northline', this was not noted at 6°C (Table 8).

Table 8. Juneberry plant height averaged over plant growth regulator and rooting status as influenced by temperature and cultivar treatments.

Temperature	Cultivar	Stem length
--°C--		----cm----
6	'Thiessen'	1.9 a[z]
6	'Northline'	1.9 a
25	'Thiessen'	1.9 a
25	'Northline'	1.3 b

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Branching

There was an interaction between temperature and rooting status ($P < 0.0001$) for plantlet branching. Pre-rooted plantlets grown under the 25°C temperature condition produced the highest number of branches compared to the other treatment combinations (Table 9). The 6°C temperature did not differ in branching with regards to rooting status but had higher number of branching when compared to 25°C not pre-rooted plantlets. This

Table 9. Juneberry branching averaged over cultivar and plant growth regulator as influenced by temperature and rooted treatments.

Temperature	Rooting status	Branching
--°C--		-----no.----
6	Pre-rooted	0.6 b[z]
6	Not pre-rooted	0.6 b
25	Pre-rooted	0.9 a
25	Not pre-rooted	0.3 c

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

suggests that the 6°C temperature may not have been conducive for branching even when plantlets had roots or not.

The interaction between plant growth regulator and rooting treatments was significant at $P < 0.01$. Only pre-rooted juneberry plantlets treated with 100 mg/L BA had more branches than the control plants (Table 10). Pre-rooted plants treated with either 200, 300 mg/L BA or 250 mg/L GA + 100 mg/L BA had similar branching as pre-rooted plants treated with 100 mg/L BA suggesting that the optimum BA concentration for increased branch was 100 mg/L or less, but only when plants had roots. This result also suggests that BA treatments are better initiators of branching than the GA treatments.

Previous research by Grossman et al. (2012) recorded increased branching on herbaceous perennial liners with BA treatments. Similarly, various combinations of GA and BA, increased number of lateral shoots in maiden apple cultivars (Kapłan, 2010). BA treatments also increased bud break in *Rhododendron indicum* (Formosa azalea) and *Photinia fraseri* (Fraser pholinia) ornamentals (Keever and Foster, 1990).

Table 10. Juneberry branching averaged over cultivar and temperature as influenced by plant growth regulator and rooted treatments.

Plant growth regulator	Pre-rooted		Not pre-rooted	
------ mg/L------	----------no.----------			
Control	0.7	b[z]	0.7	b[z]
250 GA	0.6	bc	0.7	b
500 GA	0.6	bc	0.5	bc
750 GA	0.5	bc	0.3	c
100 BA	1.2	a	0.4	bc
200 BA	0.9	ab	0.4	bc
300 BA	0.9	ab	0.4	bc
250 GA + 100 BA	0.8	ab	0.4	bc
250 GA + 200 BA	0.7	b	0.6	bc

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

There was an interaction between temperature and cultivar treatments ($P < 0.0001$) for branching. All treatment combinations were significantly different from each other (Table 11). 'Thiessen' plantlets placed under both temperatures had more branching than 'Northline'. This result suggests that the 'Thiessen' cultivar has a better adaptation to the two temperatures for branching than 'Northline'. Interestingly, 'Northline' at 6°C exhibited greater branching than 'Northline' at 25°C.

Table 11. Juneberry branching averaged over rooting status and plant growth regulator as influenced by temperature and cultivar treatments.

Temperature	Cultivar	Branching
--°C--		----no.----
6	'Thiessen'	0.7 b[z]
6	'Northline'	0.5 c
25	'Thiessen'	1.0 a
25	'Northline'	0.3 d

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

A two-way interaction of rooting status and cultivar was significant at $P \leq 0.05$ for branching. Similar to the leaf number results, 'Thiessen' plantlets that were pre-rooted had more branches compared to any other cultivar and rooting status combination (Table 12). Results suggest that post-rooting dormancy is not as great a problem with pre-rooted 'Thiessen' compared to pre-rooted 'Northline'.

Table 12. Juneberry branching averaged over plant growth regulator and temperature as influenced by cultivar and rooted treatments.

Rooting status	Cultivar	Branching
		----no.----
Pre-rooted	'Thiessen'	1.1 a[z]
Pre-rooted	'Northline'	0.5 b
Not pre-rooted	'Thiessen'	0.6 b
Not pre-rooted	'Northline'	0.3 c

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Fresh weight

Fresh weight was measured to determine if biomass of the juneberry plantlets was influenced by treatments. A two-way interaction involving rooting status and cultivar occurred at $P < 0.0001$. 'Thiessen' plantlets that were pre-rooted had greater fresh weight compared to the other treatments (Table 13). The fresh weight for pre-rooted 'Northline' was similar to not pre-rooted 'Thiessen' but greater than the not pre-rooted 'Northline'.

Table 13. Juneberry fresh weight averaged over temperature and plant growth regulator as influenced by rooted and cultivar treatments.

Rooting status	Cultivar	Fresh weight
		------mg------
Pre-rooted	'Thiessen'	131.0 a[z]
Pre-rooted	'Northline'	34.9 b
Not pre-rooted	'Thiessen'	17.8 bc
Not pre-rooted	'Northline'	8.5 c

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Dry weight

The two-way interaction of rooting status and cultivar was significant at $P < 0.0001$ for dry weight. Similar to fresh weight results, pre-rooted 'Thiessen' had greater dry weight compared to the other treatments (Table 14). Pre-rooting, regardless of the cultivar resulted in greater dry weight when compared to those not pre-rooted. Results suggest that pre-

Table 14. Juneberry dry weight averaged over temperature and plant growth regulator as influenced by rooted and cultivar treatments.

Rooting status	Cultivar	Dry weight
		------mg------
Pre-rooted	'Thiessen'	35.4 a[z]
Pre-rooted	'Northline'	9.4 b
Not pre-rooted	'Thiessen'	3.5 c
Not pre-rooted	'Northline'	1.9 c

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

rooting is more important for biomass or dry matter accumulation.

Root volume

The interaction of rooting status and cultivar was significant at $P < 0.001$ for root volume. Similar to fresh and dry weight results, pre-rooted 'Thiessen' had the largest root volume and significantly greater root volume compared to the other treatments (Table 15). This reinforces the suggestion that pre-rooting may be more important than cultivar sensitivity to dormancy.

In their study with herbaceous perennial liners, Grossman et al. (2012) reported increased branching with BA application but with reduced rooting. Plant growth regulators used in the present study did not influence root production. Reduced rooting may not have been observed in the current study due to root growth with pre-rooted plantlets especially with the 'Theissen' cultivar. The length of time that the not pre-rooted plantlets were allowed to grow before measurements were taken and when the experiment was terminated may also have contributed to the lack of root volume differences with plant growth regulators.

Table 15. Juneberry root volume averaged over temperature and plant growth regulator as influenced by rooted and cultivar treatments.

Rooting status	Cultivar	Root volume
		----------cm^3----------
Pre-rooted	'Thiessen'	0.534 a[z]
Pre-rooted	'Northline'	0.051 b
Not pre-rooted	'Thiessen'	0.003 b
Not pre-rooted	'Northline'	0.003 b

[z]Means followed by the same letter are not significantly different based on Fisher's protected LSD at $P \leq 0.05$.

Additional observations

Four weeks after planting the juneberry cultivars for the pre-rooted treatments, the survival rate for 'Thiessen' was 92% while only 46% of the 'Northline' survived. 'Northline' was observed to have a low tendency of rooting and establishment compared to 'Thiessen'. Genetic differences among cultivars were considered responsible for diverse treatment responses. More than ninety apple cultivars and related species of *Malus* were found to have varying chilling and heat requirements based on categorized genotypes. The variation in temperature requirements among the apple cultivars and *Malus* species caused a variation in their break of dormancy. The rate of the release of dormancy was found to be different among the genotypes (Hauagge and Cummins, 1991).

Statistical analysis could not be computed for plantlets transplanted to individual containers in the greenhouse because of the increased number of deaths that occurred. However, visual observations were recorded throughout the experiment. Shoots that did not produce roots still showed visible changes in stem length and branching after plant growth regulator applications. Treatments responsible for increased stem length (250, 500 and 750 mg/L GA along with the 25°C temperature) also caused the juneberry plantlets to grow spindly and weak. The apex of the stems for all plantlets from these treatments withered and died after exposure to greenhouse conditions.

Observed growth was better with juneberry plantlets kept at 6°C upon transfer to the greenhouse where the temperature was warmer and more conducive for plant growth. The observed growth may also be because the chilling requirement was satisfied as reported by Schwartz and Hanes (2010) for several temperate woody plants. Plantlets receiving the treatment combination of 250 mg/L GA + 100 mg/L BA had vigorous and balanced growth.

However, the one-time foliar application of GA and BA was not long lasting. A repeated spray may have sustained the treatment effects.

Conclusions

Significant two-way and three-way interactions indicate the complexity of post-rooting dormancy and the difficulty when trying to overcome this dormancy. Leaf production was greatest with pre-rooted plantlets when the lowest BA concentration (100 mg/L) was used or when the highest GA concentration (750 mg/L) was used. These results suggest that the optimum concentration for either plant growth regulator may have not been reached and that concentrations lower than 100 mg/L BA or greater than 750 mg/L GA may have resulted in more leaf number. In contrast, all of the unrooted plantlets responded similarly for leaf production regardless of the plant growth regulator treatments. Pre-rooted plantlets maintained at 25°C as well as the 'Thiessen' cultivar maintained at the same temperature produced the most leaves. At a temperature of 6°C leaf production was similar for the rooting treatments but 'Thiessen' and 'Northline' maintained at the same temperature of 6°C were different with 'Thiessen' producing more leaves. However, pre-rooted 'Thiessen' plantlets produced the highest number of leaves than the not pre-rooted 'Thiessen' or at both rooting statuses for 'Northline'.

Plantlets were taller when pre-rooted and sprayed with 250 or 500 or 750 mg/L GA. Pre-rooted plantlets placed in temperatures of 6°C or 25°C did not differ in stem length and was taller than the not pre-rooted plantlets at both temperatures. This suggests that the presence of good rooting in plantlets increases the stem growth irrespective of the temperature difference of 6 and 25°C. 'Thiessen' and 'Northline' plantlets grown at a temperature of 6°C were similar in their stem growth with 'Thiessen' plantlets grown at a

temperature of 25°C but differed from plantlets of 'Northline' grown at 25°C temperature. The 25°C temperature recorded more variation in treatment response. This may suggest that small concentration differences are better expressed under the warmer temperature of 25 vs. 6°C. The highest stem growth were treatments of 250 mg/L GA, 500 mg/L GA for both temperatures and 750 mg/L GA under 25°C temperature, and these were different from the control. 'Thiessen' and 'Northline' recorded the greatest stem length under both temperatures except for 'Northline' at 25°C.

Pre-rooted juneberry plantlets receiving 100, 200, 300 mg/L BA or 250 mg/L GA + 100 mg/L BA had more branches compared to other plant growth regulator and rooting treatments. 'Thiessen' plantlets that were pre-rooted and grown at 25°C had more branches compared to any other cultivar, pre-rooting and temperature combinations. Fresh weight, dry weight and root volume was greater with pre-rooted 'Thiessen' plantlets compared to other cultivar and rooting combinations.

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CHAPTER II. JUNEBERRY (*AMELANCHIER ALNIFOLIA*) CULTIVAR EVALUATION IN NORTH DAKOTA

Abstract

A juneberry variety trial was conducted near Absaraka, North Dakota to evaluate yield, fruit and plant characteristics for juneberry cultivars most commonly grown in Canada. An RCBD with four replicates was used. Planting of rooted and established cultivars were done in 2004. This experiment was to assist in characteristic identification of the selected ten juneberry cultivars and a native biotype for future commercialization.

Fruit diameter, soluble solid content, and yield (total and marketable) along with plant size measurements, were taken during the 2010 and 2011 season. 'Thiessen', 'Martin', 'Parkhill', 'Pembina', 'Regent' and Native produced the most total yield. However, marketable weight was greatest only for 'Thiessen', 'Martin', and 'Parkhill'. 'Thiessen' and 'Martin' fruits were larger and heavier than the rest of the cultivars. The tallest and widest plants were 'Thiessen', 'Martin', Native, 'Parkhill' and 'Regent'. Soluble solid content was similar among the cultivars.

Introduction

Juneberry is native to the Northwest Territories and southern Yukon, the northern plains of the United States and the prairies of Canada (Mazza and Davidson, 1993). Juneberry is a shrub with a sweet edible pome of the Rosacea family. Among the North American Indian tribes, juneberry played a significant role in their everyday lives and was considered their staple food (St-Pierre, 2005).

The pome has several essential nutrients and it is dark purple in color. Flavonols, anthocyanins and phenolics are essential nutrients known to be in high quantities in the

pome (Bors, 2010). The sweetness of the pomes and the high nutritional content makes juneberry a desirable, healthy fruit choice. Compared to *Vaccinium corymbosum* (blueberry), *Fragaria ananassa* (strawberry) and *Rubus spp.* (raspberry), juneberry's nutritional value is identified as higher (Bhagwa et al., 2011). Indian tribes of the Plains also used the fruit to make pemmican, a food staple, while various plant parts were also used for medicine (St-Pierre, 2005).

Juneberry, as a fruit crop, has great commercial potential (Pruski et al., 1990). However, large scale commercial production of juneberry has only occurred in Canada. In the 1970's, the first commercial juneberry orchard was planted in Canada. Subsequent orchards were established in the 80's and 90's (St. Pierre, 2005). A fruit processing sector has been successfully established in Saskatchewan where jams, jellies, sauces, frozen fruit, dried fruit, and teas are processed from juneberry fruit. In North Dakota, large scale juneberry plantings have not been explored. The provinces of Manitoba, Alberta, and Saskatchewan in Canada are the largest juneberry producers. The Canadian Census of Agriculture indicated that the juneberry acreage in Saskatchewan increased 21% from 2001 to 2006. In 2009, about 1,300 juneberry acres were established in Saskatchewan. The Saskatchewan province of Canada contributes about one-third of Canada's commercial juneberry acreage (Saskatchewan Ministry of Agriculture, 2010).

Many juneberry cultivars were selected by nurserymen growing a stand of plants in their nursery. Several cultivars such as 'Parkhill', 'Regent', and 'Success' have been found to be hybrids where *Amelanchier alnifolia* is one of the parents (Zatylny and St-Pierre, 2003). Presently there are about twenty six named juneberry cultivars. The first named cultivar was 'Success' and was released in 1887 in the United States (Zatylny et al., 2002).

Objectives

The juneberry cultivar trial was undertaken to evaluate characteristic differences in plant size, plant yield, fruit diameter, fruit weight and soluble solid content for eleven juneberry cultivars established under North Dakota environmental conditions. One of the eleven juneberry cultivar is a native species available from the USDA Natural Resource Conservation Service. As juneberry commercialization in North Dakota is envisaged, cultivar evaluations will be more important in moving the industry forward.

Research question:

> Are there differences in yield, fruit and plant characteristics among the ten selected juneberry cultivars and the native biotype?

Hypothesis:

> There are differences in yield, fruit and plant characteristics among the ten selected juneberry cultivars and the native biotype.

Materials and Methods

Experimental design

This specific variety trial experiment was undertaken in 2010 and 2011 calendar years. The experiment was conducted at the NDSU Horticulture Research Arboretum near Absaraka. Ten juneberry cultivars (‘Honeywood’, ‘Lee II’, ‘Martin’, ‘Northline’, ‘Parkhill’, ‘Pembina’, ‘Regent’, ‘Smoky’, ‘Success’, and ‘Thiessen’), and a native biotype (Native). Native was available from the USDA Natural Resource Conservation Service were rooted from cultures at the NDSU Plant Science Department. The Native biotype will be considered as a cultivar for the purpose of table titling. Rooted and established cultivars

were planted in 2004. The experimental design was a randomized complete block design (RCBD) with four plants of each cultivar within each experimental unit and four replicate.

Data collection

All plants had been established for at least five years when data were collected in 2010. These juneberry plants were rain fed only. Two plants were randomly selected within each experimental unit and entire fruits were collected from each plant and packaged into separate 13 X 13 X 8 cm disposable Styrofoam containers[17]when most of the fruit for each cultivar had turned a deep purple, yet prior to any fruit drop. The packaged fruits were kept in a cooler set at a temperature of 3ºC until all data were collected.

Total fruit weights from the sampled cultivars were taken prior to separation of dried, green, crushed, insect damaged and diseased fruits. The diameter of ten fruits were determined and averaged to get the final value. Plant heights and widths were measured for each harvested plant. The highest vertical branch was the point at which the height was taken while the width covered the longest horizontal branches apart. The weight of 50-fruit was measured. The soluble sugar was measured with the aid of a refractometer[18]. The brix value was obtained by squeezing the fluid of two or three fruits into the sample stage of the refractometer. After each sample was taken, the sample stage was thoroughly cleaned with Kimwipes[19] before the next measurement was taken. The weight of fifty fruits was also weighed among the selected cultivars.

[17] 5" X 5" X 3" disposable Styrofoam containers – Dacotah Paper Co. 3940 15th Avenue NW, Fargo ND 58103.
[18] Refractometer Pal-1 – ATAGO U.S.A. Inc., 12011 NE First Street, Bldg. C, Suite 110, Bellevue, WA 98005.
[19] Kimwipes – Kimtech Science, Kimberly-Clark Global Sales, Inc., Roswell, GA 30076-2199.

Statistical analysis

Data were subjected to analysis of variance using SAS Proc GLM (SAS institute version 9.3, Cary, NY). Test for homogeneity was performed for the years before values were pooled together. The null hypothesis for the experiment stated that there were no differences among juneberry cultivars for all measure characteristics.

Years were considered as random effect while cultivars were considered as fixed effects. Differences among the treatment means were separated using F-protected LSD at $P \leq 0.05$ where appropriate. The ANOVA's for variables analyzed are listed in the appendix.

Results and Discussion

Different juneberry cultivars have different characteristics for plant stature and fruit production and size. There are about twenty six named juneberry cultivars that exist with diverse characteristics from plant to fruit (St-Pierre et al., 2005). The objective of the research was to evaluate the productivity of juneberry cultivars that have been successfully grown in North Dakota and characterize them according to their distinct characteristics. Desirable cultivars were noted for future commercialization purposes. Homogeneity of variance was done based on the F-test and the data from the two years were combined as a result. Pomes will be referred to as fruit from here after.

Yield

The interaction between year and cultivar for total fruit weight was statistically significant at $P \leq 0.05$. The average total fruit weight for 2011 was more than three times the weight of 2010 (Table 16). There was a trend for all cultivars to exhibit higher yields in 2011. Total yield differences between years was attributed to the higher rainfall that was recorded in 2011 (44.70 cm) than 2010 (32.39 cm), as reported by the North Dakota

Agricultural Weather Network. Winter weather was also noted to have been milder prior to the 2011 season compared to 2010. A late spring frost in 2010 may have also contributed to lower total yields in 2010. Juneberry is an early blooming shrub with periodic frost damage (Bors, 2010).

In 2010, 'Parkhill', 'Thiessen', 'Martin', 'Pembina', 'Regent' and Native were the highest yielding cultivars, producing significantly more fruit than 'Honeywood', 'Lee II', 'Northline', 'Success' and 'Smoky' (Table 16). In 2011, 'Parkhill' had the highest total yield, followed by 'Thiessen' and then 'Martin'. 'Smoky' was the lowest yielding cultivar, producing significantly less fruit than all other cultivars.

Table 16. Effect of year by juneberry cultivar treatment interaction for total fruit weight per plant averaged across two experiments.

Cultivar	2010		2011	
	----------g----------			
'Parkhill'	138.2	ab[z]	919.7	a[z]
'Thiessen'	176.9	a	790.6	b
'Martin'	182.5	a	593.2	c
'Pembina'	162.4	a	422.6	d
'Regent'	155.3	a	378.7	d
Native	150.4	a	369.3	d
'Honeywood'	29.3	c	200.5	e
'Lee II'	15.3	c	182.6	e
'Northline'	5.3	c	164.1	e
'Success'	73.5	bc	162.8	e
'Smoky'	5.1	c	29.6	f

[z]Means followed by the same letter are not significantly different within a column based on Fisher's protected LSD at $P \leq 0.05$.

The marketable fruit weight, which comprised only the ripe and unblemished fruits, was statistically different for the selected cultivars ($P < 0.01$). The marketable yield for 'Thiessen' was the highest and significantly greater than 'Pembina', 'Regent', Native,

‘Success’, ‘Lee II’, ‘Honeywood’, ‘Northline’ and ‘Smoky’ (Table 17). ‘Martin’, a cultivar selected from ‘Thiessen’ plants, and ‘Parkhill’ had marketable yields similar to ‘Theissen’.

Comparison between the total and the market weight helped to identify cultivars that were high yielding with uniform ripening. Uniform ripening of fruits will reduce the number of harvests required in order to collect the ripe fruit and is conducive to mechanical harvesting. ‘Parkhill’ for instance, had the highest averaged total weight of 529 g, but only a mean marketable weight of 163 g. The amount discarded was more than twice the marketable weight for ‘Parkhill’, an undesirable commercial characteristic.

Table 17. Mean marketable weight of fruit for eleven juneberry cultivars averaged across two experiments.

Cultivar	Marketable weight	
	----------g----------	
‘Thiessen’	295.7	a[z]
‘Martin’	236.1	ab
‘Parkhill’	163.4	abc
‘Pembina’	155.4	bc
‘Regent’	144.0	bcd
Native	97.3	cd
‘Success’	64.8	cd
‘Lee II’	54.0	cd
‘Honeywood’	45.3	cd
‘Northline’	43.0	cd
‘Smoky’	8.3	d

[z]Means followed by the same letter are not significantly different within a column based on Fisher’s protected LSD at $P \leq 0.05$.

In their experiment conducted in Canada, Zatylny et al. (2002) listed ‘Smoky’, ‘Northline’ and ‘Honeywood’ among their highest yielding cultivars. St-Pierre et al. (2005) also had ‘Honeywood’, ‘Smoky’ and ‘Northline’ among their highest yielding cultivars. However, in North Dakota, ‘Smoky’, ‘Northline’ and ‘Honeywood’ were among the least productive cultivars. Diverse environmental factors most likely contributed to these

differences. Bird predation may also have contributed to yearly differences since their feeding will be more pronounced during a year when bushes have fewer fruits than a year with more abundant fruit (Hatterman-Valenti, personal communication). Adequate rainfall favors better fruit production. Flowering time also varies significantly among juneberry cultivars (Zatylny et al., 2002). All these factors could help to explain why a reported high yielding cultivar could underperform in a different environment. The ripening period has been designated as early- to mid-season for 'Northline' and 'Smoky', mid-season for 'Honeywood' and 'Parkhill' and mid- to late-season for 'Thiessen' and 'Martin' (Bors et al., 2010). The presence of frost during flowering will increase fruit abscission depending on the reproductive stage and the duration of the low temperatures.

Plant height and width

Plant height and plant width varied among the cultivars ($P < 0.01$). 'Thiessen', was the tallest cultivar, but plant height was similar to Native, 'Martin', 'Parkhill', and 'Regent' with an average range of 1.52 to 1.95 m (Table 18). 'Smoky' was the shortest cultivar, but plant height was similar to 'Pembina', 'Honeywood', 'Lee II', 'Success', and 'Northline' with an average range of 0.85 to 1.34 m. Davidson and Mazza (1991) reported that eleven year old 'Smoky' plant height ranged from 1.60 to 2.40 m. 'Smoky' plants in the current study were almost one-half as tall as those reported by Davidson and Mazza (1991), which may help to explain some of the reported yield differences.

The Native plants were the widest with more spreading branches, but plant width was similar to 'Thiessen', 'Martin', 'Parkhill', and 'Regent' (Table 18). 'Smoky' was the narrowest cultivar, similar only to 'Northline' for plant width. Generally, the taller cultivars

also had longer branches, which should support more fruit due to their larger stature. Tall plants, however, pose a problem when fruits are harvested by hand-picking.

Table 18. Mean plant height and plant width for eleven juneberry cultivars averaged across two experiments.

Cultivar	Plant height		Plant width	
	----------m----------			
'Thiessen'	1.94	a[z]	1.16	ab[z]
Native	1.86	ab	1.19	a
'Martin'	1.74	ab	1.07	abc
'Parkhill'	1.65	abc	1.01	abc
'Regent'	1.52	abcd	0.98	abcd
'Pembina'	1.34	bcde	0.73	cde
Honeywood'	1.16	cde	0.85	bcd
'Lee II'	1.13	cde	0.76	cd
'Success'	1.04	de	0.79	cd
'Northline'	1.04	de	0.64	de
'Smoky'	0.88	e	0.40	e

[z]Means followed by the same letter are not significantly different within a column based on Fisher's protected LSD at $P \leq 0.05$.

Soluble solid content

The brix value estimates the soluble solid content in an aqueous solution, in this case, the juice from the juneberry fruits. Juneberry has been reported to contain about 18% sugar and 80% water (Cornell University Cooperative Extension, 2011). The juneberry cultivars had a mean range of 15.3 to 18.8 ºBrix, which was not statistically different between the selected cultivars (Table 19). Rogiers and Knowles (1997) reported that 'Smoky' exhibited greater soluble solid content than 'Northline', 16.4 vs. 14.0 ºBrix, respectively. Although fruit soluble solid content was somewhat higher in the current study, the data did not reinforce the conclusion that 'Smoky' fruits were sweeter than 'Northline' fruits.

Table 19. Mean brix value for eleven juneberry cultivars averaged across two experiments.

Cultivar	Brix
	----------°Bx----------
'Pembina'	18.8
'Honeywood	18.2
'Smoky	18.1
Native	17.9
'Northline'	17.7
'Regent'	17.3
'Lee II'	17.1
'Parkhill'	16.5
'Thiessen'	16.5
'Martin'	16.4
'Success'	15.3
LSD (0.05)	ns[z]

[z]ns = not significant.

Fruit diameter

The fruit diameter varied among cultivars ($P < 0.01$). 'Thiessen' had numerically the largest fruits and significantly larger fruits compared to 'Honeywood', 'Success', 'Pembina', Native, 'Smoky' and 'Lee II' (Table 20). This was somewhat consistent with the results by

Table 20. Mean fruit diameter for eleven juneberry cultivars averaged across two experiments.

Cultivar	Fruit diameter	
	----------cm----------	
'Thiessen'	1.32	a[z]
'Regent'	1.20	ab
'Martin'	1.18	abc
'Parkhill'	1.17	abc
'Northline'	1.12	abcd
'Honeywood'	1.01	bcde
'Success'	0.96	bcde
'Pembina'	0.92	cdef
Native	0.89	def
'Smoky'	0.81	ef
'Lee II'	0.66	f

[z]Means followed by the same letter are not significantly different within a column based on Fisher's protected LSD at $P \leq 0.05$.

Zatylny et al. (2002) which classified ‘Thiessen’ and ‘Martin’ as having the largest fruits. ‘Regent’, ‘Martin’, ‘Parkhill’ and ‘Northline’ were similar to ‘Thiessen’ for fruit size. ‘Lee II’ produced the smallest fruit, but this was statistically similar to the fruit size for ‘Pembina’, Native, and ‘Smoky’. St-Pierre et al. (2005) also had similar results where the fruits of ‘Thiessen’ and ‘Martin’ were the largest, followed by ‘Smoky’, ‘Honeywood’ and ‘Northline’ in the medium category and ‘Success’ in the smallest category.

Weight of 50-fruit

Fifty fruits of each cultivar were weighed to estimate the specific fruit weight for each cultivar. The weight of 50-fruit was significantly different among the cultivars ($P < 0.0001$). Cultivars, ‘Martin’ and ‘Thiessen’ produced the heaviest fruit, while Native, ‘Lee II’, ‘Smoky’ and ‘Success’ fruits were the lightest (Table 21). The ranking of fruit weight for a cultivar differed slightly from the fruit diameter suggesting that the fruits from ‘Martin’ were denser than fruits produced by ‘Honeywood’, ‘Northline’ and ‘Success’. Both fruit

Table 21. Mean weight of 50-fruit for eleven juneberry cultivars averaged across two experiments.

Cultivar	50-fruit weight	
	----------g----------	
‘Thiessen’	55.2	a[z]
‘Martin’	48.5	ab
‘Parkhill’	39.9	bc
‘Regent’	37.4	bcd
‘Honeywood’	30.0	cde
‘Northline’	29.9	cde
‘Pembina’	28.3	cde
‘Success’	27.4	de
‘Smoky’	26.8	de
‘Lee II’	26.1	de
Native	21.5	e

[z]Means followed by the same letter are not significantly different within a column based on Fisher’s protected LSD at $P \leq 0.05$.

diameter and weight are influenced by rainfall patterns (suitable environment) and specific cultivar potentials.

Conclusions

The juneberry cultivar trial indicated significant differences for fruit yield, weight of 50-fruits, fruit diameter, plant height and plant width. 'Thiessen', 'Martin', 'Parkhill', 'Pembina', 'Regent' and Native were high yielding cultivars for total yield. However, only 'Thiessen', 'Martin', and 'Parkhill' maintained a high marketable yield. 'Thiessen', 'Regent', 'Martin', 'Parkhill' and 'Northline' had the largest fruits, while 'Thiessen' and 'Martin fruits were heavier than the rest. The largest cultivars (plant height and width) were 'Thiessen', 'Martin', Native, 'Parkhill' and 'Regent'. 'Smoky', 'Success', 'Northline', 'Pembina', 'Regent', 'Lee II' and 'Honeywood' were smaller in stature. The Native plants had a lower marketable yield even though they were among the largest in plant stature. These two characteristics are not desirable in a commercial fruiting cultivar and likely why the biotype is available as a conservation plant and not a fruiting cultivar. There were no cultivars differences for soluble solid content.

There was an interaction for total weight between the cultivar and year. The year 2011 was a more productive year than 2010, with greater yields for all cultivars, except 'Smoky'. Cultivars with high marketable yields and rather large fruit size are need when initiating commercial juneberry production in North Dakota.

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OVERALL CONCLUSION

The parameters that were measured at the end of the growth chamber study not surprisingly showed that the plant growth regulator Gibberellic acid (GA) caused elongation in stems under the 25℃ temperature more than the 6℃. Similarly, Benzyladenine (BA) not surprisingly caused the promotion of branching. Root establishment prior to plant growth regulator treatments made plantlets more responsive to plant growth regulator effects at temperature of 25°C. 'Thiessen' responded to treatments better than 'Northline'. The findings did suggest that good root establishment and chilling temperature without plant growth regulator treatments could reduce post-rooting dormancy in 'Northline'.

The evaluation of the genetic material identified 'Martin' and 'Thiessen' as the top desirable cultivars because of good yield potential, bigger fruit size and greater fruit mass. 'Martin' and 'Thiessen' exhibited larger plant stature and produced the highest yields and larger fruits.

The use of chilling for the reduction of post-rooting dormancy in juneberry should be undertaken for various periods of time in future research. Also, analysis of nutritional quality among fruits from each cultivar may be undertaken to verify if higher yield in a particular cultivar is at the expense of lower nutritional content.

APPENDIX

Table A1. Partial ANOVA for number of leaves of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	32.1769	3.49
Hormone	8	17.7560	4.07**
Hormone*Temperature	8	6.3292	1.45
Rooting	1	211.8200	48.58**
Temperature*Rooting	1	181.6111	41.65**
Hormone*Rooting	8	8.9308	2.05*
Hormone*Temperature*Rooting	8	5.3310	1.22
Cultivar	1	325.6945	74.69**
Temperature*Cultivar	1	69.3602	15.91**
Hormone*Cultivar	8	3.1942	0.73
Hormone*Temperature*Cultivar	8	2.8401	0.65
Rooting*Cultivar	1	35.4206	8.12**
Temperature*Rooting*Cultivar	1	73.4250	16.84**
Hormone*Rooting*Cultivar	8	4.7699	1.09
Hormone*Temperature*Rooting*Cultivar	8	2.0088	0.46
Error	350	4.3605	-

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A2. Partial ANOVA for stem length of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	11.8008	2.93
Hormone	8	7.8904	9.75**
Hormone*Temperature	8	4.1509	5.13**
Rooting	1	113.6726	140.40**
Temperature*Rooting	1	28.2133	34.85**
Hormone*Rooting	8	4.6798	5.78**
Hormone*Temperature*Rooting	8	2.1354	2.64**
Cultivar	1	11.0848	13.69**
Temperature*Cultivar	1	8.1126	10.02**
Hormone*Cultivar	8	0.4478	0.55
Hormone*Temperature*Cultivar	8	1.7786	2.20*
Rooting*Cultivar	1	1.9468	2.40
Temperature*Rooting*Cultivar	1	0.8712	1.08
Hormone*Rooting*Cultivar	8	0.6136	0.76
Hormone*Temperature*Rooting*Cultivar	8	0.9315	1.15
Error	350	0.8097	-

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A3. Partial ANOVA for branching of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	0.0028	0
Hormone	8	0.6029	1.82
Hormone*Temperature	8	0.4577	1.38
Rooting	1	10.5469	31.91**
Temperature*Rooting	1	11.5052	34.80**
Hormone*Rooting	8	0.9486	2.87**
Hormone*Temperature*Rooting	8	0.5429	1.64
Cultivar	1	19.8061	59.92**
Temperature*Cultivar	1	6.3317	19.15**
Hormone*Cultivar	8	0.3696	1.12
Hormone*Temperature*Cultivar	8	0.2626	0.79
Rooting*Cultivar	1	1.5769	4.77*
Temperature*Rooting*Cultivar	1	4.2206	12.77**
Hormone*Rooting*Cultivar	8	0.3241	0.98
Hormone*Temperature*Rooting*Cultivar	8	0.1713	0.52
Error	350	0.3306	-

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A4. Partial ANOVA for fresh weight of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	0.001200	0.21
Hormone	8	0.004030	0.82
Hormone*Temperature	8	0.001598	0.32
Rooting	1	0.523615	105.94**
Temperature*Rooting	1	0.000059	0.01
Hormone*Rooting	8	0.003428	0.69
Hormone*Temperature*Rooting	8	0.002027	0.41
Cultivar	1	0.297675	60.22**
Temperature*Cultivar	1	0.000001	0
Hormone*Cultivar	8	0.001217	0.25
Hormone*Temperature*Cultivar	8	0.001136	0.23
Rooting*Cultivar	1	0.201934	40.85**
Temperature*Rooting*Cultivar	1	0.000490	0.10
Hormone*Rooting*Cultivar	8	0.002912	0.59
Hormone*Temperature*Rooting*Cultivar	8	0.001293	0.26
Error	350	0.004943	-

** Significant at the 0.01 probability level.

Table A5. Partial ANOVA for dry weight of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	0.00058	2.21
Hormone	8	0.00041	1.02
Hormone*Temperature	8	0.00017	0.43
Rooting	1	0.04189	103.85**
Temperature*Rooting	1	0.00040	0.99
Hormone*Rooting	8	0.00029	0.71
Hormone*Temperature*Rooting	8	0.00020	0.50
Cultivar	1	0.02077	51.48**
Temperature*Cultivar	1	0.00015	0.36
Hormone*Cultivar	8	0.00012	0.29
Hormone*Temperature*Cultivar	8	0.00014	0.34
Rooting*Cultivar	1	0.01607	39.83**
Temperature*Rooting*Cultivar	1	0.00012	0.30
Hormone*Rooting*Cultivar	8	0.00021	0.52
Hormone*Temperature*Rooting*Cultivar	8	0.00012	0.31
Error	350	0.00040	-

** Significant at the 0.01 probability level.

Table A6. Partial ANOVA for root volume of juneberry cultivars in 2011 and 2012.

Source of variation	Degree of freedom	Mean square	F-value
Temperature	1	1.6492	1.43
Hormone	8	0.5015	0.77
Hormone*Temperature	8	0.6144	0.94
Rooting	1	9.0579	13.86**
Temperature*Rooting	1	1.7895	2.74
Hormone*Rooting	8	0.5115	0.78
Hormone*Temperature*Rooting	8	0.6066	0.93
Cultivar	1	6.2974	9.64**
Temperature*Cultivar	1	1.1049	1.69
Hormone*Cultivar	8	0.4637	0.71
Hormone*Temperature*Cultivar	8	0.5448	0.83
Rooting*Cultivar	1	6.2713	9.60**
Temperature*Rooting*Cultivar	1	1.0981	1.68
Hormone*Rooting*Cultivar	8	0.4708	0.72
Hormone*Temperature*Rooting*Cultivar	8	0.5385	0.82
Error	350	0.6534	-

** Significant at the 0.01 probability level.

Table A7. Partial ANOVA for total weight of fruits of eleven juneberry cultivars averaged across two experiments.

Source of Variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	23372.39	0.48
Cultivar	10	234176.80	2.22
Cultivar*Year	10	105597.78	2.18*
Error	60	48427.37	-

** Significant at the 0.01 probability level.

Table A8. Partial ANOVA for marketable weight of fruits of eleven juneberry cultivars averaged across two experiments.

Source of Variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	8924.54	0.47
Cultivar	10	63961.97	3.03*
Cultivar*Year	10	21081.65	1.12
Error	60	18855.59	-

** Significant at the 0.01 probability level.
* Significant at the 0.05 probability level.

Table A9. Partial ANOVA for plant height of eleven juneberry cultivars averaged across two experiments.

Source of variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	2.9290772	1.00
Cultivar	10	11.9286538	43.91**
Cultivar*Year	10	0.2716328	0.09
Error	60	2.9172257	-

** Significant at the 0.01 probability level.

Table A10. Partial ANOVA for plant width of eleven juneberry cultivars averaged across two experiments.

Source of variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	0.63810256	0.49
Cultivar	10	3.90552273	45.91**
Cultivar*Year	10	4.94742068	86.99
Error	60	1.29680070	-

** Significant at the 0.01 probability level.

Table A11. Partial ANOVA for the brix value of eleven juneberry cultivars averaged across two experiments.

Source of variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	29.5987876	1.51
Cultivar	10	27.4027275	4.62*
Cultivar*Year	10	5.9326920	0.30
Error	51	19.5969110	-

* Significant at the 0.05 probability level.

Table A12. Partial ANOVA for the diameter of fruits of eleven juneberry cultivars averaged across two experiments.

Source of variation	Degrees of freedom	Mean square	F-value
Rep(Year)	6	0.12136844	1.92
Cultivar	10	0.26612276	14.16**
Cultivar*Year	10	0.01879857	0.30
Error	51	0.0630797	-

** Significant at the 0.01 probability level.

Table A13. Partial ANOVA for the weight of 50-fruits of eleven juneberry cultivars averaged across two experiments.

Source of variation	Degrees of freedom	Mean Square	F-value
Rep(Year)	6	102.541975	0.89
Cultivar	10	839.633832	5.83**
Cultivar*Year	10	143.920754	1.24
Error	51	115.73732	-

** Significant at the 0.01 probability level.