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Propagation and Container Production of the Amelanchier spicata Complex

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PROPAGATION AND CONTAINER PRODUCTION OF THE AMELANCHIER SPICATA COMPLEX

By

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THESIS ACCEPTANCE STATEMENT

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By Gregory J.R. Melcher

Thesis Advisor: Dr. Bryan J. Peterson

An Abstract of the Thesis Presented
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Amelanchier is a genus in the Rosaceae containing shrubs and trees indigenous to North America that possess traits valued by the horticulture industry. *Amelanchier spicata* (dwarf serviceberry), a heterogeneous complex of hybrids indigenous to eastern North America, has agricultural and ornamental merit with notable characteristics. White blossoms emerge in early spring, edible pomes ripen in the summer, and vibrant, orange foliage occurs in the fall. I investigated phenological and physiological factors influencing adventitious rooting of stem cuttings, the effects of nitrogen source on the development of seedlings grown in container culture, and phenotypic variation among seedlings of *A. spicata* from several provenances.

Over a two-year period, I evaluated the effects of hormone carrier, auxin concentration, wounding, and collection date on adventitious rooting of softwood and semi-hardwood stem cuttings. In 2014, softwood cuttings collected from two provenances in Maine were wounded by one-sided scraping and treated with K-IBA in water or IBA dissolved in 50% ethanol at concentrations of 0, 3000, 5000, or 8000 $mg \cdot L^{-1}$. There were no significant differences in rooting across hormone concentrations or

carriers but cuttings treated with auxin had greater rooting percentage (75% vs. 50%) and root quality (2.3 vs. 1.1 out of five) compared with water and ethanol controls. In 2015, softwood or semi-hardwood cuttings were collected from one provenance in Maine on 17 June, 30 June, 14 July, and 29 July. Half of the cuttings received additional wounding by one-sided scraping and all were treated with K-IBA in concentrations of 0, 1000, 3000, and 5000 $mg \cdot L^{-1}$. Wounding always promoted greater rooting percentages and root ratings for all hormone concentrations. Wounded cuttings in a softwood condition had 93% rooting compared with 47% rooting for wounded semi-hardwood cuttings. The greatest average root quality resulted from wounded cuttings collected 14 July and treated with 5000 $mg \cdot L^{-1}$ K-IBA.

In 2015, we assessed the effects of nitrogen source (NH_4-N, NO_3-N, or NH_4NO_3-N) on the growth of *A. spicata* seedlings from four provenances in Maine and New Hampshire. Plants in container culture were grown for 61 days in a medium of 1:1 perlite:vermiculite, receiving nutrients in a modified, balanced Hoagland's solutions that varied by nitrogen source. In addition to nitrogen form, we adjusted fertilizer solutions to pH 4.5 and 6.5 to establish a factorial with six treatment combinations. Substrate pH measured during the study varied by nitrogen form and was not influence by solution pH. Although there were significant differences in stem caliper, stem dry weight, leaf number, and SPAD across nitrogen forms, plant growth was uniform, indicating that either NH_4-N or NO_3-N is suitable for commercial production of *A. spicata*. In contrast, seedlings from different provenances varied greatly by stem dry weight, leaf dry weight, root dry weight, foliar nutrient concentration, stem caliper, stem length, leaf number, and SPAD. Moreover, phenotypic traits such as leaf and stem morphology were distinctly

different among provenances. Our results indicate that there is much variation in the growth traits of *A. spicata* derived from different provenances, even on a localized spatial scale, and further evaluation of germplasm is merited.

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CHAPTER 1

LITERATURE REVIEW

1.1. *Amelanchier* Medic.

Amelanchier Medic. (serviceberry or shadbush) is a genus of shrubs and trees in the Rosaceae comprising approximately twenty species. *Amelanchier* is native to North America, Europe, and Asia (Kabuce and Priede, 2010). Common habitats include roadsides, riversides, forest and meadow edges, open fields, shallow rocky substrates, and acidic soils (Flessner, 2001; Underwood, 2012). Taxonomic delineation within the genus is difficult due to frequent hybridization between species (Campbell et al., 1987). Investigation of genetic complexity has led to the recent identification of several new species and microspecies (Kevin Cushman, personal communication). Historically, *Amelanchier* was used by Native Americans and settlers for culinary purposes, often consumed alone, in desserts, or incorporated in meals with vegetables or meat. Moreover, the wood was utilized in constructing tools and weapons (Flessner, 2001).

Amelanchier spicata (Lam.) K. Koch. (dwarf serviceberry) is a shrub to small tree indigenous to North America. In natural habitats, plants typically are between a half and one and a half meters tall, but vary in habit from rhizomatous shrubs of less than half a meter to trees reaching five meters (Underwood, 2012; Kabuce and Priede, 2010). Shoot morphology and size are not representative of plant age. Traits such as stem caliper are controlled by environmental conditions, intraspecific competition, and interspecific interactions (Dobravolskaitė, 2010). Racemes of attractive white blossoms are produced in the spring, with flowers numbering from four to fifteen, each having five oblong petals and sepals (Underwood, 2012). The serrated leaves of three to six cm in length are

simple, alternate, and elliptic. Mature fruits are purple to blue-black pomes, ripening between July and August in Northeastern United States. Like many of the species in the genus, plants of *A. spicata* are apomictic and produce fruits and clonal seeds consistently without cross-fertilization (Campbell et al., 1987).

Amelanchier is tolerant to a variety of soil conditions, including substrates of pH 5.5-8, and is cold hardy to temperatures of -50°C, making this an appealing crop for commercial production in the United States and Canada (Barney et al., 2009). The flavor of the fruit has been described and compared to blueberries and other pomes such as apples and crabapples (Barney et al., 2009). There are currently about 26 cultivars that are used ornamentally and agriculturally (Barney et al., 2009; St-Pierre, 2005). Examples of popular cultivars used primarily for fruit production include 'Honeywood', 'Martin', 'Northline', 'Pembina', 'Smokey', and 'Thiessen'. Other cultivars such as 'Altaglow', 'Paleface', and 'Regent' are favored for ornamental purposes (Barney et al., 2009; Flessner, 2001; St-Pierre, 2005). Most commercial cultivars of saskatoon are *A. alnifolia* or a hybrid of *A. alnifolia* (Barney et al., 2009; Flessner, 2001; St-Pierre, 2005). These cultivars grow up to four to five meters tall, potentially increasing the difficulty for collecting fruit. *A. spicata* would be beneficial to the industry due to its shorter stature, which would increase accessibility to fruit. Moreover, the diversity of local germplasm provides an opportunity to introduce new cultivars that are adapted to the more acidic edaphic conditions of the northeastern United States.

A. spicata has multi-season appeal, with several noteworthy characteristics that are valued in the horticulture industry. In the spring, a profusion of white blossoms provides an attractive color, especially as a thick groundcover. A delicious and healthy

crop of blue-black fruits, similar to blueberry in size and flavor, ripens during the summer season. A rich, red-orange autumnal coloration of the leaves provides a decorative focal point in any landscape and has the potential to replace invasive ornamental species, such as *Euonymus alatus* (Thunb.) Siebold. Birds voraciously consume fruits and disperse seeds, making *A. spicata* a noteworthy selection for the planting of wildlife habitat.

1.2. Propagation

1.2.1. Rooting Media

Root development of stem cuttings is influenced by the type and composition of growing media. Media that retain moisture while providing enough aeration for gas exchange and water drainage promote better rooting (Agbo and Omaliko, 2006; Akwatulira et al., 2011). Soilless media, composed of one or more of perlite, sand, vermiculite, sphagnum peatmoss, bark, and sawdust, are commonly used to root stem cuttings (Adugna et al., 2015; Agbo and Omaliko, 2006; Akwatulira et al., 2011; Al-Salem and Karam, 2001; Anyasi, 2011; Fowler, 2010; Ofori et al., 1996; Tchoundjeu et al., 2002). Mineral soils are not recommended for propagation by cuttings due to high bulk density, high water retention, and lack of porosity. These properties lead to anaerobic conditions, which decrease respiration and potentially lead to stem or root rot (Handreck and Black, 2002). In addition, the propagation medium needs to be pasteurized to ensure that pathogens do not infect and harm cuttings (Fowler, 2010).

Medium composition influences root development and structure (Adugna et al., 2015; Akwatulira et al., 2011). Adugna et al. (2015) determined that rooting media composed of a 1:1:1 mixture of forest soil, decomposed animal manure, and sand resulted

in greater leaf number, leaf fresh and dry weight, shoot length, shoot fresh weight, and root and shoot ratio in *Vanilla planifolia*. These cuttings produced robustly branched rooting suitable for transplantation. Although pure sand resulted in the longest roots and greatest rooting percentage, roots were greatly elongated and frail. Agbo and Omaliko (2006) determined that pure sawdust media significantly increased rooting percentage of semi-hardwood cuttings of *Gongronema latifolia* compared with soil and rice hulls. This response is ascribed to greater porosity in sawdust, providing improved aeration and water drainage. Similarly, *Milicia excelsa* had superior rooting in sawdust compared with coarse sand, find sand, and a mixture of coarse sand and sawdust. This is attributed to water and air content; sawdust had the greatest water content (40%) and air content (40.9%), nearly double that of other tested media (Ofori et al., 1996). These data indicated there was a positive correlation between water content and mean root number. Moreover, *Warburgia ugandensis* cuttings developed the greatest percentage of callus and roots when propagated in milled pine bark compared with forest soil and sand, for the same reasoning aforementioned (Akwatulira et al., 2011). Increasing the organic component of soilless media can decrease rooting success. In *Arbutus andrachne*, increased peatmoss in ratio to perlite decreased rooting percentage, root length, and fresh and dry root weight, with pure perlite outperforming any other medium (Al-Salem and Karam, 2001).

The optimal medium composition varies by species and the environmental conditions to which each is adapted. Different studies indicate preference to specific compositions favored by the experimental plant. The morphological characteristics of the species, hydromorphic versus xeromorphic, determine the preferred medium (Akwatulira

et al., 2011; Ofori et al., 1996; Tchoundjeu et al., 2002). For example, media with higher organic content, and concurrently higher water retention, would most likely be favored by hydromorphic species.

1.2.2. Stages of Adventitious Root Formation

Plants can develop roots from existing initials or atypical anatomical locations, the latter describing adventitious formation. Commonly, basal portions of stem cuttings are induced by exogenous applications of auxin, or other rooting hormones, and wounding to promote rooting. The process of adventitious root development has been described and divided into four stages: dedifferentiation, induction, outgrowth in stem, and root elongation (Davies and Hartmann, 1988; De Klerk et al., 1999). During the first stage in apple microcuttings, parenchyma cells of the vascular cambium dedifferentiate and build sensitivity to auxin. These cells start accumulating starch and are the site from which the root primordia will emerge. This process is also referred to as remeristemation. In the next phase, activated cells, now extremely sensitive to auxin, divide and start organizing into root initials that will give rise to root primordia. Next, auxin becomes inhibitory and the recognizable root primordium is visible; the root primordia differentiate. Finally, the primordium elongates outward as a new, functional root (Davies and Hartmann, 1988; De Klerk et al., 1999). Adventitious root formation has been documented to arise from numerous plant tissues including vascular rays, secondary phloem, cambium, and pith (Davies and Hartmann, 1988; Girouard, 1967).

The ability of plants to develop roots adventitiously diminishes with increasing stock plant age (Davies and Hartmann, 1988; Girouard, 1967). Numerous methods have

been developed to induce juvenility of stock plants, including pruning, blanching, grafting, etiolation, and chemical and hormonal applications (Hartmann et al., 2011).

1.2.3. The Role of Auxin

The most widely utilized group of plant growth regulators for promoting adventitious roots is auxin. Indole-3-acetic acid (IAA) is the most abundant and studied, naturally occurring auxin found in plants; however, this form is extremely sensitive to light degradation and is unstable in solutions (Dunlap and Robacker, 1988; Nissen and Sutter, 1990; Nordström et al., 1991; Tanimoto, 2005). Therefore, exogenous applications require carriers that are more stable and resistant to photodegredation There are multiple auxin carriers that are commonly used in commercial productions to enhance rooting: naturally occurring indole-3-butyric acid (IBA), the potassium salt of indole-3-butryric acid (K-IBA), and α-napthaleneacetic acid (NAA; Hartmann et al., 2011).

IBA is more stable in solution than IAA and is able to sustain adequate concentration levels that promote rooting, longer within tissues, which promoted better rooting in *Pisum sativum* L. (Nordström et al., 1991). Niseen and Sutter (1990) indicate that IBA is more stable than IAA in Murashige and Skoog (MS) medium, commonly used for tissue culture, when exposed to light or dark conditions. IBA concentrations in liquid media decreased 60% and 30% after 20 days in light and dark environments, respectively, whereas IAA declined by 97% and 70% (Nissen and Sutter, 1990). Moreover, nutrient salts in MS media combined with light synergistically accelerate the degradation of IAA. Dunlap and Robacker (1988) determined that IAA concentrations declined by 80% in seven days in the presence of salts and light, with nitrates and iron being the key components of the nutrient solution that contributed to IAA destruction.

Auxin is produced in actively growing shoots of plants, including apical meristems, emerging leaves, and developing fruits, flowers, and seeds, before polar transport to the roots (MacAdam, 2009). Long-distance transport of auxin can occur passively via the phloem or through polar, cell-to-cell carriers (Morris et al., 2004). Translocation of auxin in the phloem is rapid and simultaneous with other metabolites while cell-to-cell transport is slower (Morris et al., 2004). Once reaching root cells, IAA can migrate acropetally or basipetally through the inner or surface tissues, respectively, with the help of carrier proteins AUX1, PIN, and MDR1 (Tanimoto, 2005).

Haissig (1970) determined that IAA plays a critical role on the development of root primordia and the removal of leaves and meristems, the sites of auxin synthesis, inhibited root formation of *Salix fragilis* L. These effects could be reversed by exogenous auxin and were more apparent during primordia formation than during root elongation (Haissig, 1970). The relative amount of auxin present during root formation varies by developmental stage. In mung bean, IAA levels peaked only during the induction phase of adventitious rooting (Nag et al., 2001). During this stage, there are also high levels of phenolic compounds that serve as rooting co-factors. This response was similarly mapped in apple microcuttings; the greatest sensitivity to auxin was during the induction phase (De Klerk et al., 1999). Likewise, Haissig (1970) noted that IAA was more influential during initiation of primordia than during root elongation in *Salix fragilis*.

In contrast, there is an inverse trend in IAA-oxidase concentration, which climaxed during initiation and expression phases (Nag et al., 2001). IAA is readily oxidized into inactive forms, a feature not shared by NAA and only marginally by IBA (De Klerk et al., 1999). Oxidation of certain auxins is prevented through conjugation with

other compounds. The majority of auxins found in plant tissues are in conjugated forms rather than the free form, with up to 90% of IAA being in a conjugated state (Epstein and Ludwig-Müller, 1993). IAA is likewise inactive in conjugated forms but the process is reversible (De Klerk et al., 1999).

Exogenous applications of auxin can be used to overcome low, naturally occurring levels to improve adventitious rooting if IAA is the only limiting factor; however, other endogenous compounds may still inhibit root formation (Hartmann et al., 2011). For some species, greater concentrations of exogenous auxin can inhibit root formation. In *Paeonia* L., stem cuttings receiving 3000 $mg \cdot L^{-1}$ IBA rooted worse than those treated with 2000 $mg \cdot L^{-1}$ (Guo et al., 2009).

1.2.4. The Role of Wounding

The ability of plants to produce roots adventitiously can occur naturally in some species or through induction. Wounding has been shown to increase cellular division near the vascular cambium and phloem, promoting callus development and subsequent adventitious root formation (Pijut et al., 2011; Wilson and Grange, 1984). Physically, this increases the total surface area of cambium-exposed tissue, a common site of root emergence, which is exposed to exogenous applications of plant growth regulators (Davies and Hartmann, 1988). More importantly, wounding induces systemic physiological responses such as the production and/or translocation of phenolics, invertases, and jasmonic acid, or by influencing source-sink relationships (Ahkami et al., 2008; Hartmann et al., 2011; Wilson and Staden, 1990).

Following wounding, plants experience up-regulation of biosynthetic genes that code for enzymes associated with the production of jasmonates and invertases, which

ultimately affect source-sink relationships (Roitsch and González, 2004; Wasternack, 2007). Commonly, these signal transductions are defense responses to herbivory, but have been noted to play a role in adventitious rooting. In *Petunia hybrida,* Ahkami et al. (2008) described a three-phase metabolic response to wound-induced root development that included 'sink establishment,' 'recovery,' and 'maintenance'. Initial wounding leads to an increased accumulation of jasmonic acid and gene-induced activation of enzymes that breakdown sucrose, creating a resource sink. This elicits the translocation of carbohydrates from photosynthetically active sources to the site of wounding. Following source-sink transport, the sugars are either used to fuel cellular dedifferentiation or are converted to starch, to be utilized later for adventitious root formation and growth (Ahkami et al., 2008).

Jasmonates and invertases are not the only compounds up-regulated from gene expression following wounding. Phenolic compounds are known co-factors that are intrinsically beneficial to adventitious rooting in endogenous quantities or by exogenous applications (Wilson and Staden, 1990). Often, phenolics act synergistically with auxin. Stresses, like wounding, augment the activity of polyphenol oxidase that oxidizes phenolics to quinones (Kim et al., 2001). The regulation of polyphenol oxidase activity is associated with numerous genes within plants and is highly variable across species (Mayer, 2006). In apple cuttings, oxidized derivatives of phenolics are more efficient at root promotion, particularly the products from the reaction between polyphenol oxidase and phloridzin (Bassuk et al., 1981).

The effects of wounding are species-dependent and can be beneficial or detrimental to rooting percentage and root quality. (Al-Salem and Karam, 2001; Blazich

and Bonaminio, 1983; Graves, 2002; King et al., 2011). Wounding softwood cuttings of *Taxodium distichum* (baldcypress) reduced total and average lengths of adventitious roots and resulted in poorer rooting percentage and root quality than not wounding cuttings (King et al., 2011). These results partially contradict other research with baldcypress; however, discrepancies are attributed to collection time of the cuttings and relative tissue suberization (King et al., 2011; Zhou, 2005). Stem cuttings of *A. laevis* rooted well with two-sided scraping, while wounding had no effect on *A. alnifolia* (Bishop and Nelson, 1980; Still and Zanon, 1991).

The degree and location of wounding are variables that additionally impact rooting responses. MacKenzie et al. (1986) applied three levels of wounding to hardwood cuttings of *Malus* Mill., denoted as shallow incision, deep incision, and split base treatments. Compared with controls, split base and deep incision wounding increased rooting percentage to 94% and 26%, respectively, from 6%. Differences in rooting potential were attributed to changes in the physical environment and the positioning of callus derived from the cambium (MacKenzie et al., 1986). Softwood stem cuttings may be particularly sensitive to over-wounding. For example, survival of softwood cuttings of *Taxus wallichiana* Zucc. declined with heavy wounding (Saumitro and Jha, 2014).

1.2.5. Phenology

The collection time of emerging shoots is documented as a critical aspect influencing the adventitious rooting potential of many species (Agbo and Obi, 2007; Hartmann et al., 2011; Haynes et al., 2003; Jull et al., 1994; Rosier et al., 2004; Still and Zanon, 1991). Seasonal stem maturation is associated with anatomical and physiological changes. The rooting ability of stem cuttings requires close attention to phenological

phase changes and the collection date associated with optimal rooting varies by species. Often, actively growing softwood cuttings root better than dormant hardwood cuttings for many species (Hartmann et al., 2011). For *Coleonema aspalathoides* Juss., rooting percentage was greatest for cuttings collected with soft, tender tissues (Heller et al., 1994). Similar trends have been documented for other deciduous and evergreen species (Agbo and Obi, 2007; Hinesley et al., 1994; Pijut and Moore; Rosier et al., 2004). In contrast, cuttings in a hardwood condition promote better rooting than softwood states for other species (Camellia et al., 2009; Jull et al., 1994).

Anatomically, factors such a lignification can inhibit adventitious root formation. As stems transition from softwood to hardwood conditions, the amount of lignified tissue increases. In *Hedera helix* L., Reineke et al. (2002) found a negative correlation between relative lignification and rooting potential. Juvenile petioles of English ivy contained significantly less lignin than mature petioles and produced better rooting (Reineke et al., 2002). Adventitious roots form from the dedifferentiation of parenchyma cells into a reorganized meristem. Sclereids, lignified cells, are unable to dedifferentiate once committed as sclerenchyma tissue to form root primordia and therefore can act as barrier to initiation (Maynard and Bassuk, 1996). The significance of sclerenchyma tissue on inhibiting root emergence is debated, however, and is believed not to be the main cause of seasonal variation in rooting potential (Girouard, 1967). Although there is a strong correlation between the percentages of parenchymatous gaps in sclerenchyma sheaths and rooting success for two oak species, rooting potential is attributed to physiological factors that control anatomy and cell competence (Amissah et al., 2008).

Physiological factors, such as carbohydrate content, rooting co-factors,

endogenous auxin, and rooting inhibitors, fluctuate through seasonal development and greatly influence rooting ability of vegetative cuttings. In *Jatropha curcas* L., differences in rooting potential across softwood, semi-hardwood, and hardwood cuttings are attributed to relative amounts of stored carbohydrates; hardwood cuttings generally have a greater accumulation of carbohydrates that support greater rooting (Camellia et al., 2009). *Paeonia* cuttings rooted the best 70 days after florescence, when soluble carbohydrates were high and nitrogen content was low (Guo et al., 2009). Carbon resources are maintained in various forms within plants and differ in their ability to promote root development. For instance, soluble sugars are more influential to rooting than starches in *Olea europaea* L. (Denaxa et al., 2012). However, budbreak can act as a competitive sink if leaf emergence occurs before rooting and can reduce the allocation of resources to the sites of rooting (Rosier et al., 2004).

Endogenous concentrations of auxin vary by phenological growth phase and can impact adventitious rooting potential (Blakesley et al., 1991b). Smith and Wareing (1972) determined that endogenous IAA levels declined as annual growth of *Populus* x *robusta* Schneid. matured, as measured and indicated by a decline in rooting potential. They hypothesized that changes in photoperiod caused the variation in endogenous auxin levels; long days stimulate greater auxin synthesis and improved adventitious rooting compared with short days (Smith and Wareing, 1972). In hardwood cuttings of grape, better rooting was associated with increasing endogenous IAA concentration and decreasing abscisic acid (ABA; Kelen and Ozkan, 2003). The concentrations of different auxin states are also known to fluctuate during the season. Cuttings of *Cotinus coggygria* Scop. rooted better when collected early in the spring rather than in the summer

(Blakesley et al., 1991a). During the spring, free IAA levels were greater than those in conjugated forms, whereas the opposite was true in summer cuttings.

Rooting co-factors, like phenolics, exhibit seasonal variation. Bassuk and Howard (1981) supplied exogenous IBA to apple cuttings collected at different times in the season. Although rooting was improved with the hormone supplementation, rooting varied considerably by vegetative state, indicating the presence and seasonal fluctuation of co-factors that enhance rooting (Bassuk and Howard, 1981). There are numerous compounds that work synergistically with auxin to promote rooting, the most common being phenolics (Wilson and Staden, 1990).

When endogenous concentrations of auxin and rooting co-factors are not limiting, differences in rooting ability derive from rooting inhibitors. For chestnut cuttings, *Castanea sativa* Mill., rooting ability is associated with the presence or absence of rooting inhibitors that counter synergistic auxin and rooting co-factors (Vazquez and Gesto, 1982; Vieitez, 1974). Vieitez et al. (1987) identified derivatives of ellagic acid that inhibit root formation in mature chestnut cuttings; equal quantities mixed with auxin reduced rooting activity by 31.5%. These levels of rooting inhibitors in chestnut are not present in cuttings taken during a juvenile phase (Vieitez et al., 1987).

1.2.6. Propagation of *Amelanchier* by Cuttings

Harris (1961), determined that propagating *A. alnifolia* by hardwood stem cuttings was unsatisfactory, resulting in no rooting. Even though propagation by root cuttings was effective at developing shoot growth, 90.4% without overhead mist and 91.6% with mist, softwood stem cuttings are the preferred method of propagation because there is reduced risk of plant injury normally associated with obtaining root cuttings (Harris, 1961).

Robust rooting of softwood stem cuttings is required to ensure successful transplantation (Nelson, 1987).

Asexual propagation of *A. spicata* by stem cuttings may require close attention to timing of cutting collection. Dirr and Heuser (2009) report that *A. spicata* can be propagated by softwood stem cuttings rooted in sandy soil, but they provide no information on timing of cutting collection or the identities and concentrations of plant growth regulators. However, for *A. alnifolia* and *A. laevis*, the timing of softwood cuttings is important for rooting success. Optimal dates for *A. alnifolia* were mid-June in Alberta, Canada, and late June to early July in Saskatoon, Saskatchewan (Harris, 1961; Bishop and Nelson, 1980). Terminal softwood and semi-hardwood cuttings of *A. laevis* taken from the Ohio State University campus showed optimal rooting when collected between May 24 and June 15 (Still and Zanon, 1991). Concentrations of IBA in talc between 3000 and 8000 $mg \cdot L^{-1}$ promoted rooting in *A. alnifolia*, and a concentration of 5000 $mg \cdot L^{-1}$ K-IBA promoted root development in *A. laevis* (Harris, 1961; Dirr and Heuser, 2009; Still and Zanon, 1991). Softwood cuttings of *Amelanchier* require consistent misting to decrease moisture loss before the development of roots (Harris, 1961; Dirr and Heuser, 2009; Still and Zanon, 1991; Bishop and Nelson, 1980). The addition of heat during rooting may promote root production but is not required when cuttings are collected at their optimal phenology (Harris, 1961; Bishop and Nelson, 1980). In addition, etiolation of stock plants aids in promoting rooting of softwood stem cuttings, with 95% rooting without auxin application (Nelson, 1987).

1.2.7. Propagation of *Amelanchier* by Seed

Polyploidy and apomixis are features shared by many hybrid species of *Amelanchier*, the majority of which are triploid or tetraploid (Campbell et al., 1985). Apospory, the non-meiotic development of a megasporangium cell into a megagametophyte, is exhibited by all apomicts of the genus (Campbell et al., 1987). Because plants of *A. spicata* are apomicts, they produce fruits and seeds reliably in the absence of cross-fertilization (St-Pierre, 2005). As a result, F1 generations produced by seed are identical or nearly-identical clones of the parent plant (St-Pierre, 2005). Acharya et al. (1989) determined that genotype accounted for most of the variation in germination for seeds collected from different provenances, while annual environmental variation had minimal impact.

Limited information is available on seed propagation of *A. spicata*. Dirr and Heuser (2009) reported that warm and cold stratification of seeds promoted germination, but with no indication of stratification requirements. In other species of *Amelanchier*, embryo dormancy can be overcome by cold stratification lasting two to six months (Brinkman and Strong, 2008). Scarification of *A. laevis* seeds by a 15-minute soak in concentrated sulfuric acid, followed by stratification, increased germination percentages (Hilton et al., 1965). Weber et al. (1982) found that acid scarification plus treatment with a benzyladenine and thiourea mixture also increased germination.

1.3. Container Production

1.3.1. Container Production Media

To be suitable for horticulture, nursery plants that have been propagated successfully also must be amenable to culturing in containers. There are numerous classifications of soilless substrates that have been developed to maximize growth and reduce costs during production of plants in containers, including the U.C.-type, Cornell Peat-Lite, composted hardwood bark, softwood bark, and compost mixes (Davidson et al., 2000). These media are commonly composed of sand, sphagnum peat moss, vermiculite, perlite, and barks in varying proportions (Davidson et al., 2000; Hummel et al., 1990; Li et al., 2015; Peterson and Graves, 2009; Smith et al., 2004; Stanton and Mickelbart, 2014). Alternative materials such as rice or cocoa bean hulls, sawdust, coconut fiber, ground corncobs, and used hops are substituted when regionally available (Davidson et al., 2000; Rodríguez and Flórez, 2012). Generally, soilless media contain combinations of organic and mineral components.

Cation exchange capacity (CEC), the ability of substrate particles to attract and retain positively charged ions, is an important factor to consider when selecting soilless media. Plant roots that release similarly charged ions take up cations held by the substrate and the ease of the exchange is dependent on ion size, hydration, valence electron number, and salt concentration (Davidson et al., 2000). Substrate pH also influences CEC by altering the ratio of hydrogen anions in the medium (Argo, 1998). Moderate to high CEC (50-100 meq/L) is recommended for container productions to ensure that cations are uniformly supplied and reduce nutrient loss from leachate, but this is contingent on fertilization frequency; substrates with low CEC can be utilized for plants receiving

supplementary, regular fertilization (Davidson et al., 2000; Handreck and Black, 2002). This can be achieved by utilizing peat moss, bark, sawdust, and vermiculite as soilless components (Handreck and Black, 2002). In contrast, perlite, polystyrene, and rockwool have low CEC and are often mixed with organic materials to improve aeration or water retention (Argo, 1998).

Water-holding capacity and aeration in container media are essential in promoting healthy plant development. Substrates with high water-holding capacity can easily become oversaturated, leading to anaerobic conditions and, subsequently, inefficient metabolic processes (Handreck and Black, 2002). Coarse sand has low available water-holding capacity (AWHC) whereas peat moss, sawdust, poppy straw, and brown coal have high AWHC (Beardsell et al., 1979). Particle size distribution influences the physical properties of soilless media, like water availability, aeration, and hydraulic conductivity (Abad et al., 2005; Richards et al., 1986). Richards et al. (1986) determined that removing pine bark particles greater than 2mm, in a pine bark, sand, and brown coal composition, increased water availability without compromising aeration. Some fine textured media lack adequate porosity and aeration, but aeration can be improved through the addition of coarse material that can occupy up to 80% of the total bulk volume (Davidson et al., 2000). The physical properties recommended by the Southern Nurserymen's Association (1997) for container media in percent volume include: 50-85% total porosity; 10-30% air space; 25-35% available water content; 25-35% unavailable water content; and 0.19-0.70 $g \cdot cm^{-3}$ bulk density.

1.3.2. Substrate pH and Nutrient Availability

Two factors that readily influence the performance of plants growing in a soilless medium include response to substrate pH and mineral nutrition, including the form of nitrogen applied via fertilizer. Information on optimal root-zone pH and mineral nutrition for *Amelanchier* is scarce; however, a general relationship between substrate pH and soluble nutrient availability in substrates is well known. Davidson et al. (2000) found that substrate nutrients are more readily available at pH less than 5.8 in organic soil, whereas Bailey and Nelson (1998) suggest that pH between 5.6 and 6.2 is optimal in soilless medium. All micronutrients, except Molybdenum, increase in availability with decreasing substrate pH. This can lead to antagonistic relationships between micronutrients when one is in excess in relation to the rest; deficiency can occur when excessive uptake of one micronutrient blocks the uptake of another (Bailey and Nelson, 1998).

Smith et al. (2004) found that increasing root-zone pH decreased foliar macronutrient nitrogen and increased calcium, magnesium, and sulfur in *Impatiens wallerana* Hook.F. and *Petunia* x *hybrida* Hort. Vilm.-Andr. Acidic substrate of containerized *Dirca palustris* L. increased stem length and shoot/root dry mass of plants from three different provenances, ranging from indigenous pH of 7.4 (North Dakota) to 5.2 (Florida; Peterson and Graves, 2009). Optimal growth occurred at pH less than six, with leaf greenness, iron, nitrogen, and zinc of plants from all provenances decreasing with increasing pH. Low-nutrient stress of plants increased with increasing pH (Peterson and Graves, 2009).

1.3.3. Nitrogen Form

Nitrogen is essential in the construction of amino acids. Ammonium can be incorporated directly into amino acids whereas nitrate is reduced to nitrite and then ammonium in shoot systems before usage (Xu et al., 2012). In order for plants to assimilate nitrogen anions or cations, a similarly charged ion must be released to maintain the same pH within the root cells. Therefore, hydrogen and hydroxide ions are released by roots during assimilation of ammonium and nitrate, respectively (Mattson et al., 2009). Hydrogen cations accumulate in the substrate and decrease pH, whereas hydroxide anions interact with free hydrogen cations to form water; the consumption of hydrogen cations increases substrate pH (Mattson et al., 2009). The conversion between nitrogen forms facilitated by microbes also alters substrate pH. Nitrification of ammonium to nitrate releases associated hydrogen ions decreasing substrate pH. In contrast, bacterial conversion of nitrate to ammonium requires the addition of hydrogen ions from surrounding media, which increases substrate pH (Mattson et al., 2009).

Often, the form of nitrogen in fertilizer solutions controls substrate pH. Witcher et al. (2011) found that fertilizers containing nitrogen in the form of either nitrate or ammonium resulted in the highest and lowest pH, respectively, for whole pine tree and peat-lite substrates. For *Kalmia latifolia* L. grown in containers at two locations, substrate pH declined when plants were supplied with ammonium (Hummel et al., 1990). This response was more drastic at one location where the initial substrate pH was greater. These trends are additionally supported by experimentation evaluating ryegrass, white clover, and maize grown in silica sand culture; plants released similarly charged ions to assimilate nitrogen variants, resulting in either acidic or alkaline media (Smith et al.,

1983). It is important to recognize the effect different forms of nitrogen have on substrate pH when selecting fertilizers because root-zone pH ultimately determines nutrient availability.

Ecologically, the dominant form of available nitrogen in substrates is dependent on substrate pH. Generally, basic, aerobic soils are dominated by nitrate, whereas ammonium dominates acidic soils and wetlands (Xu et al., 2012). The quantity and availability of different nitrogen forms vary by successional stage in natural ecosystems. At the start of primary succession, nitrogen availability is essentially non-existent and increases following community colonization (Vitousek et al., 1989). During secondary succession and following an ecological disturbance, nitrogen availability and nitrification rates are high. An opening in the canopy increases soil temperature and subsequently, decomposition rates. Nitrification rates decline as the community progressively develops and nitrogen availability is dependent on climatic conditions (Vitousek et al., 1989). In a Costa Rican tropical rainforest, nitrification rates increased for eight years following a disturbance but declined and stabilized as the ecosystem progressed (Robertson, 1984).

The form of nitrogen favored by plants is species-specific and does not necessarily coincide with the form predominantly utilized in natural systems. Preferential uptake of nitrogen in competitive, ecological settings indicates only the realized niched space of a species (McKane, 2002). In contrast, container cultures eliminate interspecific interactions and more accurately reflect uptake preferences of a species or the source that promotes optimal growth. McKane et al. (2002) determined that preferential nitrogen uptake in field settings for *Ledum* L., *Betula* L., and *Carex* L., differed from studies conducted in culture and differences were attributed to competitive ability. Other species,

such as *Abies fraseri* (Pursh) Poir., indigenous to acidic soils where ammonium dominates, are able to utilize nitrate and potentially outperform individuals supplied with ammonium (Rothstein and Cregg, 2005).

Nitrogen form has the potential to influence anatomy, morphology, and plant health. Lowbush blueberry, *Vaccinium angustifolium* Aiton, indicated preference toward ammonium; rooted cuttings supplied with nitrate resulted in necrotic leaves and overall poor health, whereas ammonium promoted vigorous growth (Townsend, 1966). *V. angustifolium* indicated no preference to nitrogen form in another study, but *Vaccinium macrocarpon* Aiton indicated preference toward fertilizers containing ammonium and grew poorly when supplied with nitrate alone (Rosen et al., 1990). Shoot and root growth increased for *Cotoneaster dammeri* C.K. Schneid, a woody shrub, and *Rudbeckia fulgida* Aiton, an herbaceous perennial, when treated with ammonium based fertilizers compared with nitrate alone. Moreover, when supplied with ammonium, the steles of both species produced more secondary xylem with larger tracheary elements (Kraus et al., 2002). Some dioecious species may exhibit differential preference for nitrogen form depending on the sex of the individual. For example, female individuals of *Populus deltoides* W. Bartram ex Marshall preferred nitrogen in the form of nitrate, whereas males displayed no preference (Li et al., 2015).

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CHAPTER 2

EFFECTS OF COLLECTION DATE, WOUNDING, AND AUXIN APPLICATION ON ADVENTITIOUS ROOT DEVELOPMENT OF *AMELANCHIER SPICATA* PROPAGATED BY STEM CUTTINGS

2.1. Abstract

Amelanchier spicata (Lam.) K.Koch (dwarf serviceberry or shadbush) is a shrub in the Rosaceae indigenous to North America with agricultural and ornamental merit. We evaluated the effects of collection date, wounding, auxin concentration, and auxin carrier on adventitious rooting of softwood and semi-hardwood cuttings over a two-year period. In 2014, softwood cuttings were collected from two provenances in Maine, wounded by one-sided scraping, and treated with K-IBA in water or IBA dissolved in 50% ethanol at concentrations of 0, 3000, 5000, or 8000 $mg \cdot L^{-1}$. There were no significant differences in rooting percentages or root ratings among tested hormone concentrations. Applications of auxin resulted in 75% rooting and an average rooting rating of 2.3 of five, compared with 50% rooting and a rating of 1.1 for controls. In 2015, cuttings were collected from one provenance on 17 June, 30 June, 14 July, and 29 July. Softwood and semi-hardwood cuttings were either wounded or not wounded and treated with K-IBA at concentrations of 0, 1000, 3000, and 5000 $mg \cdot L^{-1}$. Wounding promoted greater rooting percentages and root ratings for all hormone concentrations, with the effect more pronounced for the third and fourth collection dates. Rooting percentages for wounded cuttings treated with auxin decreased from 93% for softwood cuttings to 47% for semi-hardwood cuttings. Cuttings collected 14 July, wounded, and treated with 5000 $mg \cdot L^{-1}$ K-IBA had the greatest

average root rating (2.4). We conclude that softwood cuttings that are wounded and treated with at least 5000 mg·L^{-1} K-IBA should be used for consistent propagation of *A. spicata* by stem cuttings.

2.2. Introduction

Shrubs and trees in the genus *Amelanchier* Medik. (serviceberry; Rosaceae) make attractive plants valued by horticulturists for their flowers, fruits, and autumnal coloration of foliage. The fruits, in addition to providing food for wildlife, are emerging as a commercial crop in North America, with most production in Canada (Barney et al., 2009). The 2011 estimated farm gate value for *Amelanchier* fruit produced in Canada was $1.2 million for 308 metric tons (Minister of Industry, 2012). There are currently about 26 cultivars that are used ornamentally and agriculturally, most of which are *Amelanchier alnifolia* Nutt. or an *A. alnifolia* hybrid (Barney et al., 2009; Flessner, 2001; St-Pierre, 2005). Most of the commercial cultivars in production derive from indigenous populations in western Canada and northern United States, and are tolerant of substrates with pH 5.5 to 8 (Barney at al., 2009; Flessner, 2001). The northeastern United States and Canada is diverse in *Amelanchier*, which provides an opportunity to select new cultivars that may be adapted to the regional climate and acidic soils of the Northeast.

Amelanchier spicata (Lam.) K. Koch. (dwarf serviceberry) grows as a shrub to small tree indigenous to eastern and central North America. It has multi-season appeal, with several noteworthy characteristics that are valued in the horticulture industry. A profusion of white blossoms provides an attractive color in the spring; edible blue-black fruits, similar to blueberry in size and flavor, ripen during the summer season; and red-

orange autumnal coloration of the leaves provides a decorative focal point in any landscape. Moreover, since plants of *A. spicata* are apomicts, they produce fruits and clonal seeds reliably in the absence of cross-fertilization (Campbell et al., 1987).

Valued genotypes of woody plants are commonly propagated asexually by softwood or semi-hardwood stem cuttings. Dirr and Heuser (2009) indicated that *A. spicata* can be propagated by root cuttings in sandy soil, but no information on the use of stem cuttings was provided. However, other species of *Amelanchier* may be propagated by softwood and semi-hardwood stem cuttings treated with indole-3-butyric acid (IBA) and the potassium salt of IBA (K-IBA) at concentrations from 1000 to 25,000 $mg \cdot L^{-1}$ (Dirr and Heuser, 2009; Harris, 1961; Still and Zanon, 1991). Success of asexual propagation of *A. spicata* by stem cuttings may also be influenced by cutting phenology (e.g., softwood vs. semi-hardwood). For instance, cuttings of *Amelanchier laevis* Wiegand collected in late May to mid-June in Ohio had the best root quality among dates tested (Still and Zanon, 1991). Optimal timing for cuttings of *A. alnifolia* was mid-June in Alberta, Canada, and late June to early July in Saskatoon, Saskatchewan, with rooting diminishing as new stems lignified (Bishop and Nelson, 1980; Harris, 1961).

Wounding the basal portions of cuttings may induce cellular division near the vascular cambium and phloem, promoting callus development and adventitious root formation (Pijut et al., 2011). This is achieved by increasing the amount of cambium-exposed area that is subjected to exogenous applications of plant growth regulators, increasing endogenous production and/or translocation of growth regulators such as ethylene, phenolics, auxin, and jasmonic acid, and by influencing source-sink relationships (Ahkami et al., 2008; Hartmann et al., 2011; Liu et al., 1990; Wilson and

Staden, 1990). However, the effects of wounding are species-dependent and can be either beneficial or detrimental to rooting percentages and root quality (Al-Salem and Karam, 2001; Blazich and Bonaminio, 1983; Graves, 2002; King et al., 2011). For example, softwood and semi-hardwood stem cuttings of *A. laevis* rooted well following scraping on two sides of the stem, but wounding had no effect on *A. alnifolia* (Bishop and Nelson, 1980; Still and Zanon, 1991).

We conducted two experiments to assess propagation of *A. spicata* from softwood and semi-hardwood stem cuttings. In 2014 (Experiment 1), we evaluated the effects of provenance, stem wounding, and both IBA carrier (IBA in 50% ethanol vs. K-IBA in water) and concentration (0 to 8,000 $mg \cdot L^{-1}$) on adventitious rooting. In 2015 (Experiment 2), we evaluated the role of collection date, wounding, and K-IBA concentration on adventitious rooting of cuttings from one provenance.

2.3. Materials and Methods

2.3.1. Experiment 1

In 2014, 224 terminal stem cuttings per site were collected from a site in Cumberland County (GPS: N 43° 46.266’ W 70° 30.355’) on 3 July, and a site in Washington County (GPS: N 44° 35.972’ W 67° 49.312’) on 17 July. Although collection dates were selected to match phenology of annual shoot growth, the number of nodes and length of the cuttings differed by provenance. Cuttings from Cumberland County were 11.5 to 19 cm in length with five to eight nodes, and cuttings from Washington County were 9 to 14 cm in length with three to four nodes. Cuttings were wounded on one side of the stem by scraping with a razor blade and quick-dipped for five

seconds in water, 50% ethanol, or 3000, 5000, or 8000 $mg \cdot L^{-1}$ K-IBA in water or IBA in 50% ethanol. There were 28 cuttings per treatment for each provenance. Once treated, cuttings were stuck in a pre-moistened medium of 3 perlite:1 peat moss. Cuttings were stuck within 50-cell vacuum propagation sheets (Dillen-ITML, Middlefield, Ohio), inserted into 1020 flats, and placed under intermittent mist with 50% shading in a glass-glazed greenhouse receiving natural irradiance without supplemental lighting (Roger Clapp Greenhouses, University of Maine, Orono, Maine). Greenhouse set points were 18 and 27° C. Cuttings received eight seconds of mist every six minutes for 52 days.

The experiment consisted of a randomized complete block design, with provenance as the blocking factor. Data were collected on 25 August for cuttings from Washington County and 8 September for cuttings from Cumberland County. We recorded the presence or absence of roots, root rating, and the length of the longest root for each cutting. Root ratings were scored from 0 to 5 on a subjective scale of rooting quantity and quality, with 0 representing no roots and 5 representing many roots that were well developed (Fig. 2.1). All statistical analyses were conducted in R (version 3.2.1; The R Foundation for Statistical Computing, Vienna, Austria). We used an analysis of variance (ANOVA) to test for interactions between provenances and collection dates. Because the data did not fit parametric assumptions, Fisher's exact test was used to separate rooting percentages and Dunn's test was used to separate means of root rating and longest root for each treatment, with $\alpha = 0.05$. Dunn's tests were preceded by Kruskal-Wallis tests. We pooled data from both provenances because responses were similar and there was no interaction between collection sites for any of the measured responses.

Figure 2.1. Representative root ratings of *Amelanchier spicata* propagated by stem cuttings for Cumberland County, Maine. Root ratings are on a scale of 0-5, based on root quality and quantity. Left: 0 represents plants with no roots. Right: five represents plants with superior rooting.

2.3.2. Experiment 2

In 2015, 200 softwood or semi-hardwood terminal stem cuttings were collected on each of 17 June, 30 June, 14 July, and 29 July from the same population of *A. spicata* in Washington County, ME as Experiment 1. Progressing from the first to last collection date, random samples of 20 cuttings averaged 4.8, 5.0, 5.6, and 5.3 nodes; 10.2, 11.4, 11.3, and 11.4 cm in length; and 1.3, 1.5, 1.8, and 1.9 mm in basal stem caliper, respectively. Softwood cuttings collected 17 and 30 June had green stems that were still actively growing. On 14 July, the cuttings were transitioning to a semi-hardwood

condition and all of the terminal buds had set. The stems were mostly red with a mixture of green and brown. The cuttings collected on 29 July were semi-hardwood with predominately red, brown, and dark green stems. The coloration of the terminal buds had transitioned from green to dark red. Cuttings were dipped, either wounded or not wounded, in solutions of K-IBA in water at concentrations of 0, 1000, 3000, and 5000 $mg \cdot L^{-1}$. Wounding was applied by scraping the basal portion of the stem prior to applying hormones. We stuck cuttings individually in 50-cell vacuum propagation sheets containing a medium of 3 perlite: 1 peat moss and placed them into 1020 flats. Flats were placed under intermittent mist with 25% shading in a glass-glazed greenhouse receiving natural irradiance without supplemental lighting (Roger Clapp Greenhouses, University of Maine, Orono). Cuttings received eight seconds of mist every ten minutes for 61 days. The environmental temperature in the greenhouse was measured using an EL-USB-2-LCD data logger and averaged 21.6° C (Lascar Electronics, Erie, Pennsylvania).

Within collection times, we used a completely randomized design. Data were collected on 18 August, 31 August, 14 September, and 29 September, 2015. We determined if each cutting was alive or dead, scored the presence or absence of roots, subjectively rated rooting on a scale from 0-5 based on root quantity and quality, and measured the length of the longest root. We conducted statistical analyses in R (version 3.2.1; The R Foundation for Statistical Computing, Vienna, Austria) and checked for equality of variance and normality. Because the data were non-parametric, Fisher's exact test and Dunn's test were used to separate rooting percentages and root ratings, respectively, with $\alpha = 0.05$. Likewise, longest root means were grouped using Dunn's tests. We used ANOVA to test for any interactions.

2.4. Results

2.4.1. Experiment 1

Cuttings treated with IBA in 50% ethanol or K-IBA in water produced more consistent and robust rooting than those treated with ethanol and water controls. When hormone was applied, the rooting percentages, root ratings, and longest root lengths were insensitive to auxin concentrations and carriers. Although there was no interaction between collection sites for any of the measured responses, mean root ratings, longest root lengths, and rooting percentages differed by provenance. Cuttings collected from Cumberland Country had greater rooting percentages, root ratings, and longest root lengths for most hormone treatments. For each provenance, the treatments with the greatest recorded root ratings included 5000 $mg{\cdot}L^{-1}$ IBA (3.1) for Cumberland County and 8000 $mg{\cdot}L^{-1}$ IBA (2.1) for Washington County. 75% of the cuttings produced callus near the site of wounding and hormone treatment did not evidently affect its frequency. Roots commonly developed on the side of the stem opposite the site of wounding, prompting our investigation of wounding in experiment 2. Across both provenances, exogenous applications of auxin enhanced rooting percentage compared with controls (75% rooting with auxin and 50% without) and enhanced mean root rating (2.3 with auxin vs. 1.1 without; Table 2.1). Among only those cuttings that rooted, root ratings averaged 3.1 and 2.1 for auxin and control treatments, respectively. Furthermore, among cuttings treated with auxin, neither concentration nor carrier (IBA in 50% ethanol vs. K-IBA in water) affected rooting percentages, root ratings, or lengths of the longest root (Table 2.1). The longest roots on cuttings not treated with auxin averaged 3.5 cm, whereas cuttings treated with auxin had longest roots averaging 6.6 cm (Table 2.1).

Treatment (mg·L^{-1})	% Rooting[1] (W)	% Rooting[1] (C)	% Rooting[1] (B)	Root rating[2] (W)	Root rating[2] (C)	Root rating[2] (B)	Longest root[2] (cm-W)	Longest root[2] (cm-C)	Longest root[2] (cm-B)
Water	39.3 c	57.1 bc	48.2 c	0.6 b	1.2 c	0.9 b	1.2 c	5.0 b	3.1 b
Alcohol	50.0 bc	53.6 c	51.8 bc	1.1 ab	1.3 bc	1.2 b	3.2 bc	4.6 b	3.9 b
3000 IBA	71.4 ab	71.4 abc	71.4 ab	1.8 ab	2.7 ab	2.2 a	4.8 ab	6.6 ab	5.7 a
3000 K-IBA	78.6 a	82.1 ab	80.4 a	2.0 a	2.5 abc	2.2 a	6.8 a	7.7 a	7.2 a
5000 IBA	71.4 ab	85.7 a	78.6 a	1.9 a	3.1 a	2.5 a	4.3 ab	8.1 a	6.2 a
5000 K-IBA	57.1 abc	75.0 abc	66.1 abc	1.8 ab	2.7 ab	2.2 a	4.5 ab	8.2 a	6.4 a
8000 IBA	71.4 ab	78.6 abc	75.0 a	2.1 a	2.8 a	2.5 a	5.2 ab	8.9 a	7.0 a
8000 K-IBA	82.1 a	75.0 abc	78.6 a	1.8 ab	2.8 a	2.3 a	5.0 ab	9.3 a	7.1 a

[1]Fisher's exact test, $\alpha = 0.05$.

[2]Kruskal-Wallis and Dunn's tests, $\alpha = 0.05$.

Table 2.1. Experiment 1: Root ratings, rooting percentages, and longest root lengths of *Amelanchier spicata* collected from two provenances in Maine. Means followed by the same letter within columns are not significantly different ($P \leq 0.05$). W = Washington County, C = Cumberland County, B = Both Counties.

2.4.2. Experiment 2

Root rating and rooting percentage varied by collection date, with both declining as cuttings transitioned to a semi-hardwood condition. Because there were no interactions among collection dates, hormone concentrations, and wounding treatments, we pooled the data to determine which treatment combination produced the greatest quality of roots. Across collection dates, 5000 mg·L^{-1} K-IBA with wounding consistently promoted significantly greater root ratings (Table 2.2). Wounding significantly increased rooting percentage and root rating for all auxin concentrations (Table 2.3). Rooting percentage

and root rating significantly decreased as annual growth lignified during later collection times (Table 2.3).

Treatment ($mg{\cdot}L^{-1}$)	Root rating[1]
Water (W)	1.0 c
Water (NW)	0.5 e
1000 K-IBA (W)	1.3 b
1000 K-IBA (NW)	0.6 e
3000 K-IBA (W)	1.5 b
3000 K-IBA (NW)	0.7 de
5000 K-IBA (W)	1.8 a
5000 K-IBA (NW)	0.9 cd

[1] Kruskal-Wallis and Dunn's tests, $\alpha = 0.05$.

Table 2.2. Experiment 2: Average root quality for all growth stages of *Amelanchier spicata* cuttings collected from one provenance in Maine. Means followed by the same letter are not significantly different ($P \leq 0.05$) based on Dunn's test. W= Wounded treatments; NW= Non-wounded treatments.

Cuttings collected on the first two collection dates had the greatest rooting percentages, with 100% rooting among cuttings in some treatment groups. When cuttings were transitioning to semi-hardwood (14 July), 5,000 $mg{\cdot}L^{-1}$ K-IBA promoted the greatest quality roots across cuttings with a relatively high rooting percentage (88%).

	17 June		30 June		14 July		29 July	
K-IBA (mg·L^{-1})	% Rooting[1]	Root rating[2]	% Rooting[1]	Root rating[2]	% Rooting[1]	Root rating[2]	% Rooting[1]	Root rating[2]
Water (W)	88 ab	1.4 cd	84 ab	1.5 ab	40 b	0.6 cd	24 abc	0.4 bc
Water (NW)	48 c	0.8 e	52 c	0.9 c	8 c	0.1 e	12 bcd	0.1 cd
1000 K-IBA (W)	84 ab	1.4 bc	96 a	1.6 ab	76 a	1.4 b	40 ab	0.6 ab
1000 K-IBA (NW)	68 bc	0.9 de	60 bc	1.0 c	24 bc	0.3 cde	4 cd	0 cd
3000 K-IBA (W)	100 a	1.9 ab	88 ab	1.5 ab	76 a	1.8 ab	48 a	0.6 a
3000 K-IBA (NW)	88 ab	1.2 cde	76 abc	1.2 bc	20 bc	0.3 de	0 d	0 d
5000 K-IBA (W)	96 a	2.0 a	96 a	1.9 a	88 a	2.4 a	52 a	1.0 a
5000 K-IBA (NW)	64 bc	1.1 cde	84 ab	1.6 ab	40 b	0.8 c	12 bcd	0.1 cd

[1]Fisher's exact test, α= 0.05.

[2]Kruskal-Wallis and Dunn's tests, α= 0.05.

Table 2.3. Experiment 2: Root ratings and rooting percentages of *Amelanchier spicata* collected from one provenance in Maine on four dates. Means followed by the same letter within columns are not significantly different ($P \leq 0.05$) based on Fisher's exact or Dunn's tests. W= Wounded treatments, NW= Non-wounded treatments.

Across cuttings that were wounded and treated with auxin, average rooting percentages were the greatest for cuttings collected 17 June and 30 June (93% rooting), and decreased to 80%and 47% for cuttings collected 14 July and 29 July, respectively. Root ratings were consistently greatest for wounded cuttings treated with 5000 $mg \cdot L^{-1}$ K-IBA, averaging 2.0, 1.9, 2.4, and 1.0 for stems collected on 17 June, 30 June, 14 July, and 29 July, respectively. Among cuttings that rooted, the greatest mean root rating was 2.8, recorded for cuttings treated with 5,000 $mg \cdot L^{-1}$ K-IBA collected on 14 July (data not shown).

We observed a mixture of locations on the stem from which roots emerged, including directly from callus, from the edges of the scrape wound, or from the side of the stem opposite the wound. We observed roots emerging from the wound or wound-edge for 155 cuttings; at least 45 cuttings had rooting away from the wound; and 32 cuttings had a mixture of rooting

2.5. Discussion

Successful rooting of *A. spicata* propagated by stem cuttings was greatly influenced by collection date and wounding, and marginally influenced by hormone concentration. Hormone carrier did not affect any of the measured responses. Softwood cuttings were easier to root than semi-hardwood cuttings, and on average developed better root systems. Roots for all treatment combinations decreased in vigor and consistency as annual growth matured. Additionally, wounding was essential in promoting robust rooting. Regardless of the hormone concentration they received, cuttings at all stages of development benefitted from wounding applications, with

wounding becoming increasingly important for root formation as cuttings developed into a semi-hardwood condition.

2.5.1. Effects of Hormone Carrier and Concentration

The first experiment produced no evident differences among cuttings in root ratings, longest roots, or rooting percentages among hormone concentrations or carriers, as long as auxin was applied (Table 2.1). For each of the measured responses, auxin outperformed both water and ethanol controls. This indicates that exogenous applications of auxin are beneficial in promoting adventitious rooting of *A. spicata*, but that relatively low auxin concentrations are sufficient. A mean root rating of 3.1, excluding non-rooted individuals and averaged across all auxin treatments, is comparable to a root score of 3 suggested by Bishop and Nelson (1980). They determined that a score of 3 (medium root production) is the minimum requirement for successful transplantation.

Because there were no significant differences in root ratings between auxin carriers in the first experiment, cuttings were only treated with K-IBA in the second experiment. In experiment 2, the greatest mean root rating excluding non-rooted individuals for was 2.8, for wounded cuttings treated with 5,000 $mg \cdot L^{-1}$ K-IBA and collected mid-season (14 July; data not shown). It was unclear from the root ratings and lengths of the longest root within collection dates what auxin concentration consistently promoted the best rooting. Cuttings treated with 3000 or 5000 $mg \cdot L^{-1}$ K-IBA resulted in the comparable root ratings, rooting percentage, and longest root lengths for most collections, except for the last date for which none of the hormone treatments were significantly different. After pooling the data across collection dates and wounding treatments, 5000 $mg \cdot L^{-1}$ K-IBA promoted significantly greater root quality and longer

root lengths than the other auxin concentrations and water control (Table 2.2). This indicates that 5000 mg·L^{-1} K-IBA is the preferred concentration for commercial production among these tested concentrations.

Rooting percentage of the pooled data in experiment 2 indicated that 1,000 to 5,000 mg·L^{-1} K-IBA were similarly effective and promoted rooting more reliably than water alone. The greatest rooting percentages based on auxin treatment alone occurred for early collected cuttings. 3000 mg·L^{-1} K-IBA promoted 94% rooting for 17 June and 5000 mg·L^{-1} K-IBA resulted in 90% rooting for 30 June. Still and Zanon (1991) observed that K-IBA concentrations of 10,000 and 15,000 mg·L^{-1} did not improve rooting percent of *A. laevis* compared with 5,000 mg·L^{-1}. Stem cuttings of *A. laevis* and *A. alnifolia* reportedly rooted well with auxin concentrations between 3,000 and 8,000 mg·L^{-1} (Dirr and Heuser, 2009; Harris, 1961). Our results agree with all of these findings. In combination with wounding treatments, cuttings collected 17 and 30 June and supplied with exogenous auxin averaged 93% rooting for both dates. The treatment combination of 3000 mg·L^{-1} K-IBA with wounding promoted 100% rooting on 17 June. Although increasing hormone concentration may not benefit rooting percent of *A. spicata*, especially for softwood cuttings, it could improve root quality. None of the hormone concentrations applied to cuttings collected 30 July resulted in consistently suitable rooting.

2.5.2. Effects of Wounding

Wounding had a profound impact on rooting percentage and root quality. In experiment 2, additional wounding promoted better root quality and rooting percentages for each hormone concentration on each collection date. Cuttings collected on 17 and 30

June resulted in 93% rooting when wounded and treated with K-IBA, a 20 percentage point increase compared with non-wounded counterparts. This is comparable to Harris (1961), who reported 91.6% rooting of *A. alnifolia* under mist and superior to Bishop and Nelson (1980) with rooting averages between 42% and 77% for cuttings wounded and treated with hormones. The same response occurred for water controls for the first two collection dates, with 86% and 50% rooting for wounded and non-wounded treatments, respectively. These effects were more pronounced when cuttings transitioned to semi-hardwood conditions. Cuttings collected 14 July and 29 July experienced a 52 and 42 percentage point increase in rooting from applied wounding, respectively. Rooting percentages for non-wounded semi-hardwood cuttings of *A. spicata* are not viable for commercial propagation.

In the both experiments, roots commonly developed on the side of the stem opposite the site of wounding or from the wound edge. Roots emerged away from the site of wounding in experiment 2 for 25%, 21%, 7%, and 15% of the observations for 17 June, 30 June, 14 July, and 29 July, respectively; this observation occurred more frequently for softwood cuttings. We applied scrape-wounding to one side of the stem, whereas other studies with *Amelanchier* implemented two-sided wounding (Still and Zanon, 1991). In many taxa, adventitious rooting typically occurs to the greatest degree around the perimeter of a wound (Hartmann et al., 2011). Conversely, tissue damage from wounding might cause roots to develop away from the wound among cuttings of *Amelanchier*. For example, the survival of stem cuttings of *Taxus wallichiana* decreased with severe wounding on the basal portion of the stem compared with light wounding, especially for cuttings in a softwood condition (Saumitro and Jha, 2014). Although root

ratings and rooting percentages were greater for wounded treatments, roots were not consistently robust. Often, wounding promoted production of callus that failed to differentiate into root primordia. Callus production and adventitious rooting are regulated by nutritional status and growth regulator interactions (Nanda and Jain, 1971; Nanda et al., 1974). The failure of callus to differentiate into formalized primordia could therefore be attributed to an unbalanced ratio of sucrose and auxin (Nanda et al., 1974). This problem could be overcome by increasing the concentration of exogenous auxin that is applied to cuttings.

2.5.3. Effects of Collection Date

In the first experiment, cuttings from Cumberland County were collected fourteen days earlier than those from Washington County. Overall, Cumberland County had greater root ratings for each hormone concentration and carrier than those from Washington. Collection date is a factor that could contribute to these differences; however, cuttings from both provenances were collected at similar phenological stages. Moreover, there are numerous differences in stock plant physiology and environmental conditions that contribute to differences in rooting potential, such as age, water status, temperature, and light (Hartmann et al., 2011).

The timing of cutting collection has been documented to greatly influence root quality and rooting percentage of *Amelanchier* species (Bishop and Nelson, 1980; Harris, 1961; Still and Zanon, 1991). Root rating and rooting percentages decreased as cuttings transitioned from softwood to semi-hardwood, as exhibited by all treatment combinations. For related species *A. alnifolia* and *A. laevis,* average rooting percentages and root ratings were greater for softwood cuttings than for semi-hardwood cuttings

(Bishop and Nelson, 1980; Still and Zanon, 1991). Among softwood cuttings of these species, rooting percentage and root ratings of early season cuttings were not as great as midseason cuttings collected prior to bud set (Bishop and Nelson, 1980; Still and Zanon, 1991). Similarly, softwood cuttings in our experiment that were wounded and treated with auxin rooted at 93% compared with 47% for those in a semi-hardwood state. In contrast to data on *A. laevis* and *A. alnifolia*, our data indicate that the earliest collection times resulted in the greatest rooting percentages. Moreover, even though rooting percentage averaged only 80% for cuttings collected 14 July, the average root rating was the greatest at 1.9 for wounded cuttings treated with auxin. This collection time also included the treatment combination with the greatest root rating, 2.4, for wounded cuttings treated with 5000 $mg \cdot L^{-1}$ K-IBA. Although cuttings collected 14 July were not actively growing and the terminal buds had set, this was a relatively recent transition as indicated by the bud coloration and stem lignification. It is likely that this collection occurred around the threshold indicated by Bishop and Nelson (1980), for which they recommended collecting cuttings at the latest point in the season while plants are actively growing.

It is widely accepted that rooting potential is greater in many species when plants are maintained in a juvenile phase (Hartmann et al., 2011). Cuttings from our experiments were collected from sexually mature plants in the wild that did not receive any formal maintenance or manipulation. We predict that maintaining stock plants for juvenility or vigor would increase rooting success. Studies conducted with related species utilized stock plants of known cultivars, including ‘Pembina’ and ‘Smoky,’ or campus-based individuals with known ages (Bishop and Nelson, 1980; Nelson, 1987; Still and

Zanon, 1991). Moreover, Nelson (1987) determined that cuttings taken from mature stock plants of 'Pembina' and 'Smoky' benefit from rejuvenation by etiolation. Cuttings taken from plants that were grown in the absence of light prior to treatment showed improved rooting percentages and root mass compared with non-etiolated counterparts. Future studies could include the collection of cuttings from stock plants established in a common garden.

2.6. Conclusions

Rooting quality still remains to be the most significant concern for propagation of *A. spicata* by stem cuttings. For example, the average root rating in the second experiment was consistently below the recommended quality rating of 3 (Bishop and Nelson, 1980). Only one treatment combination, 5000 $mg \cdot L^{-1}$ K-IBA with wounding, promoted rooting close to the recommended quality, after removing non-rooted cuttings. It is possible that the greatest concentration we evaluated in experiment 1 (8,000 $mg \cdot L^{-1}$ K-IBA or IBA) was an inadequate maximum threshold, and that root quality would increase following treatment of cuttings with concentrations as high as the 25,000 $mg \cdot L^{1}$ suggested by Still and Zanon (1991).

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CHAPTER 3

AMELANCHIER SPICATA FROM FOUR PROVENANCES IN NEW ENGLAND DIFFER IN EARLY SEEDLING GROWTH AND ARE INSENSITIVE TO FERTILIZER NITROGEN SOURCE

3.1. Abstract

Amelanchier species are cultivated for ornamental and agricultural purposes in the United States and Canada. *Amelanchier spicata* (dwarf serviceberry), a heterogeneous complex of hybrids indigenous to eastern North America, has multi-season appeal but is not cultivated in North America. We evaluated growth and development of seedlings from four provenances in Maine and New Hampshire. Because serviceberries in New England often occur on acidic soils, which may be rich in ammonium, we also evaluated the responses of plants from these provenances to nitrogen source (NH_4-N, NO_3-N, or NH_4NO_3-N). Plants in container culture, grown in a substrate of 1:1 perlite:vermiculite, were supplied with modified and balanced Hoagland's solution that varied by nitrogen source for 61 days. Nutrient solution pH was adjusted to 4.5 or 6.5, creating a total of six combinations of nitrogen source and solution pH. Nitrogen source, rather than solution pH, influenced pH of the substrate. Seedlings supplied with NH_4-N or NH_4NO_3-N had marginally greater stem dry weight, stem caliper, SPAD, and foliar Fe and Al concentration, but produced fewer leaves and assimilated less Mg, Zn, and Ca than those receiving NO_3-N alone. Though statistically significant, these differences were not dramatic, and both nitrogen sources promoted broadly acceptable growth and development of seedlings for commercial production. In contrast, there was substantial

variation in growth and development among provenances, measured by differences in stem dry weight, leaf dry weight, root dry weight, foliar nutrient concentration, stem caliper, stem length, leaf number, and SPAD. Plant origin was easily distinguishable by leaf and stem morphology. Most notably, one provenance consistently exhibited branching at the cotyledonary node, while the others maintained single, upright stems. These results show that there is significant regional diversity in germplasm that might merit further evaluation, and that these diverse plants readily utilize nitrogen in the forms of NH_4-N or NO_3-N during container production.

3.2. Introduction

Amelanchier spicata (Lam.) K. Koch (dwarf serviceberry) is a shrub in the Rosaceae indigenous to North America with noteworthy characteristics valued by the horticulture industry. The white flowers, edible pomes, and vibrant autumn foliage make this species attractive as a woody ornamental or alternative fruit crop. Although this species is not used commercially, there are approximately 26 cultivars of *Amelanchier alnifolia* Nutt. (Saskatoon) that are used ornamentally or agriculturally, with predominant production occurring in Canada (Barney et al., 2009; Flessner, 2001; St-Pierre, 2005).

Nitrogen form has been widely documented to influence morphology, anatomy, and physiology of many plant species and often affects overall health and biomass (Finn et al., 1990; Kraus et al., 2002; Lavoie et al., 1992; Merhaut and Darnell, 1996; Rothstein and Cregg, 2005; Townsend, 1966). This concept is essential when selecting fertilizers for commercial applications or choosing sites for plant establishment. The form of nitrogen that is dominantly available to plants is correlated with substrate pH.

Ammonium-based fertilizers decrease root-zone pH as plants release hydrogen ions for nitrogen assimilation, whereas nitrate uptake increases pH through the release of hydroxide ions (Mattson et al., 2009). Commercial cultivars of *A. alnifolia* grown for fruit production are derived from germplasm indigenous to northwestern North America and are tolerant of pH 5.5 to 8 (Barney at al., 2009; Flessner, 2001). However, northeastern North America is diverse in species of *Amelanchier* that inhabit more acidic edaphic conditions. Because *A. spicata* occupies habitats edaphically different from Saskatoon, it is unclear whether cultural practices suitable for the latter are similarly suitable for commercial production *A. spicata*.

Ecologically, plant species exhibit differential uptake for varying forms of nitrogen through time and space (McKane et al., 2002). In competitive environments, nitrogen selection reflects only the realized niche of a species (McKane et al., 2002). As a result, the source of nitrogen that promotes optimal growth of a species (fundamental niche) is not always the same form selected for in competitive environments. Members of the genus *Vaccinium* L. are indigenous to acidic soils where ammonium (NH_4-N) dominates. Numerous studies have indicated preferential uptake and greater accumulation of NH_4-N in shoots, and plants often exhibit detrimental responses when fertilized with nitrate (NO_3-N; Merhaut and Darnell, 1995; Rosen et al., 1990; Townsend, 1966). In contrast, *Abies fraseri* (Pursh) Poir., which also inhabits environments dominated by NH_4-N, is able to utilize nitrate and may outperform individuals fertilized with ammonium (Rothstein and Cregg, 2005).

Currently there is no information on the effects of nitrogen source on the development of *Amelanchier* species or whether there is preferential uptake and

assimilation. In spring 2015, we evaluated the growth of *A. spicata* germplasm established in containers and supplied with NO_3-N, NH_4-N, or NO_3NH4-N at two levels of fertilizer solution pH. This is the first study, of which we are aware, evaluating germplasm of *A. spicata* from different provenances for horticultural merit under controlled conditions in container culture.

3.3. Materials and Methods

3.3.1. Plant Material

We collected fruits of plants in the *A. spicata* complex from four provenances in Maine and New Hampshire (GPS coordinates: N 43° 01.130’ W 70° 44.025’-Rockingham County; N 43° 24.143’ W 70° 37.440’-York County; N 43° 46.266’ W 70° 30.355’-Cumberland County; N 44° 35.972’ W 67° 49.312’-Washington County) that represent morphologically distinct members of the *A. spicata* complex. Each provenance served as treatments in order to eliminate biases from environmental and physiological variation, and were categorized by county: Rockingham (ROCK), Washington (WASH), York (YORK), or Cumberland (CUMB). All seeds were stratified for approximately five months at 1.6° C in moist sphagnum moss. Several weeks after germination, we potted seedlings in 510 mL vacuum pots (8.9 cm x 8.9 cm; Dillen-ITML, Middlefield, Ohio) containing a 1:1 perlite:vermiculite medium. Plants were grown in containers for 70 days. Prior to transplanting, we measured fifteen seedlings from each provenance and measuring stem and root length and counting the number of true leaves. WASH ranged from six to eleven true leaves, with root and stem averages of 8.1 and 6.2 cm, respectively. Unlike the other provenances, WASH seedlings exhibited distinct and

consistent branching at the cotyledonary node. ROCK plants had five to twelve true leaves, an average stem length of 7.0 cm, and an average root length of 9.1 cm. CUMB seedlings possessed five to eight leaves, 6.7 cm average stem length, and 9.5 cm average root length. YORK ranged from four to seven true leaves and averaged 6.3 and 10.2 cm for stem and root lengths, respectively. Seedlings were grown in a glass-glazed greenhouse receiving only natural irradiance, with an average photosynthetic photon flux of 1108 $\mu mol \cdot m^{-2} \cdot s^{-1}$ measured at plant height at solar noon and a mean daily temperature of 25.1° C (Roger Clapp Greenhouses, University of Maine, Orono, Maine). Fertigation started on 4 March, 2015 and ended 3 May, 2015 (61 days).

3.3.2. Nutrient Solutions and Fertigation

We supplied seedlings with micro- and macronutrients in a modified, balanced Hoagland's solution (Hoagland and Arnon, 1950) derived from Li et al. (2015) that varied by nitrogen source (Table 3.1). Nitrogen forms included nitrate (NO_3-N), ammonium (NH_4-N), and balanced NO_3NH4-N. The experiment was a three-way factorial design consisting of four provenances, three nitrogen sources, and two solution pH values (4.5 or 6.5). There were fifteen seedlings per treatment combination for each provenance, (N= 360), which were divided across three blocks in a randomized complete block design. Nitrapyrin (0.92 $mg \cdot L^{-1}$), dissolved in dimethyl sulfoxide (DMSO; 0.04%), was added to each fertilizer solution to inhibit microbial nitrification in NH_4-N treatments. Solution pH was adjusted to 4.5 and 6.5 using 1M sodium hydroxide (NaOH) and 1M hydrochloric acid (HCl). Initially, 100ml of fertilizer was applied to containers every two days; but application was reduced to every four days after day 18, when we observed mild salt injury (browning of leaf margins). Distilled water, adjusted to pH 4.5

and 6.5, was used to irrigate plants between fertigations such that plants were either irrigated or fertigated every two days. Plants were fertigated for 61 days prior to harvest.

Compound	(NO_3-N)	(NH_4-N)	(NH_4NO_3-N)
KCl	0	1.5	1.5
$CaCl_2·2H_2O$	0	1.25	0.3125
$MgSO_4·7H_2O$	0.5	0.5	0.5
$KH_2(PO_4)$	0.25	0	0
KNO3	1.25	0	0
$Ca(NO_3)_2·4H_2O$	1.25	0	0.9375
$NH_4H_2PO_4$	0	0.25	0.25
NH_4Cl	0	3.5	1.625
H_3BO_3	0.0116	0.0116	0.0116
$MnCl_2·4H_2O$	0.0046	0.0046	0.0046
$ZnSO_4·7H_2O$	0.00019	0.00019	0.00019
$Na_2MoO_4·2H_2O$	0.00012	0.00012	0.00012
$CuSO_4·5H_2O$	0.00008	0.00008	0.00008
$FeSO_4·7H_2O$	0.0125	0.0125	0.0125
$Na_2EDTA·2H_2O$	0.0125	0.0125	0.0125
Pyridine	0.004	0.004	0.004
Total Nitrogen	3.75	3.75	3.75

Table 3.1. Balanced Hoagland's solution varying by nitrogen source, and used to fertigated seedlings of *Amelanchier spicata*. Concentrations are in millimolar (mᴍ). Modified from Li et al., 2015.

3.3.3. Data Collection and Statistical Analysis

We harvested the shoots of individual blocks on 12, 13, and 14 May, 2015. Roots of each block were placed in cold storage until they could be processed on 15 May, 2015. For each seedling, we measured the number of leaves, stem length, stem caliper, foliar nutrient concentration, and leaf, root, and stem dry weights for each seedling. Substrate

pH, and substrate electrical conductivity (EC) of each seedling was measured for one block, utilizing the PourThru extraction method and a portable pH/EC/TDS/Temperature meter (Cavins et al., 2000; Hanna Instruments, Woonsocket, Rhode Island). We measured the SPAD of a recently matured leaf from each seedling by averaging three readings taken from the center, left, and right of the central vein (SPAD 502; Minolta, Ramsey, NJ). Harvested shoots were placed in a heated drying room for two weeks prior to weighing. To characterize root-zone conditions, substrate EC and pH measurements were obtained from one of the three blocks on two dates (31 March), using the PourThru method as described above.

We made composite leachate samples for nutrient analysis for each combination of pH and nitrogen form at the end of the experiment. Nitrate and ammonium were determined colorimetrically by O.I. Analytical's Alpkem Ion Analyzer (College Station, Texas). All other leachate nutrients were measured using a Thermo Elemental (TJA) ICP-OES Spectrometer (Analytical West, Inc., Corona, California). Foliar nutrients were quantified on 24 November, 2015 by taking a composite sample of leaf tissue for each treatment and provenance across blocks. Concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), aluminum (Al), and boron (B) were determined. Composite leachate and foliar nutrient analyses were conducted by the Analytical Laboratory at the University of Maine (Orono, Maine).

Statistical analyses were conducted in R (version 3.2.1; The R Foundation for Statistical Computing, Vienna, Austria). All data were subjected to the Shapiro-Wilk test and Levene's test to confirm normality and homogeneity of variance, respectively. For

data that met both assumptions, we used analysis of variance (ANOVA) followed by Tukey's test for separation of means, with α= 0.05. Logarithmic and multiplicative inverse transformations were implemented in several cases to obtain normality. We conducted non-parametric Kruskal-Wallis tests on data that were non-normal, even after transformations, and separated means using Dunn's test. Interactions between provenance and fertilizer treatment for measured responses were analyzed using ANOVA.

3.4. Results

Phenotypic variation among seedlings was strongly affected by provenance and less consistently influenced by nitrogen form. We found no statistical interactions between provenance and fertilizer treatment for any of the measured traits. Nutrient solution pH had no impact on substrate pH or EC; we therefore pooled data across solution pH to evaluate the cumulative effects of nitrogen form. There were significant, but minor, differences in stem caliper, stem dry weight, leaf number, and SPAD across nitrogen forms, but plants grew relatively uniformly across fertilizer treatments (Figure 3.1). In comparison, seedlings derived from different regions varied in all measured traits and were clearly distinctive in morphology (Figure 3.2).

Substrate pH and EC varied by nitrogen and neither was influenced by solution pH (Table 3.2). Generally, substrates supplied with NO_3-N had the highest pH, followed by NH_4NO_3-N and NH_4-N respectively. Inversely, plants fertigated with NH_4-N had higher average EC readings, followed by NH_4NO_3-N and NO_3-N respectively. There were no differences in pH or EC between solution pH within nitrogen forms, with the exception of NH_4-N during the first sampling (Table 3.2).

Figure 3.1. Representative seedlings of *Amelanchier spicata* collected from York County, Maine. Seedlings were supplied with NO_3-N, NH_4-N, or NH_4NO_3-N in fertilizer solutions adjusted to pH 4.5 or 6.5. Fertilizer treatments from left to right: NH_4NO_3-N (6.5), NH_4-N (6.5), NH_4-N (4.5), NO_3-N (6.5), NH_4NO_3-N (4.5), and NO_3-N (4.5).

The nutrient concentration of leaf tissue samples varied both by nitrogen source and by provenance. Seedlings supplied with NH_3NO_3-N or NH_4-N alone had greater foliar Fe and Al concentration than those supplied with NO_3-N alone (Table 3.3). In contrast, those receiving NO_3-N had marginally greater Mg, Zn, and Ca than those provided with NH_4-N. Moreover, provenances differed by foliar nutrients, with WASH having greater N, Mg, P, B, and Mn concentration (Table 3.3).

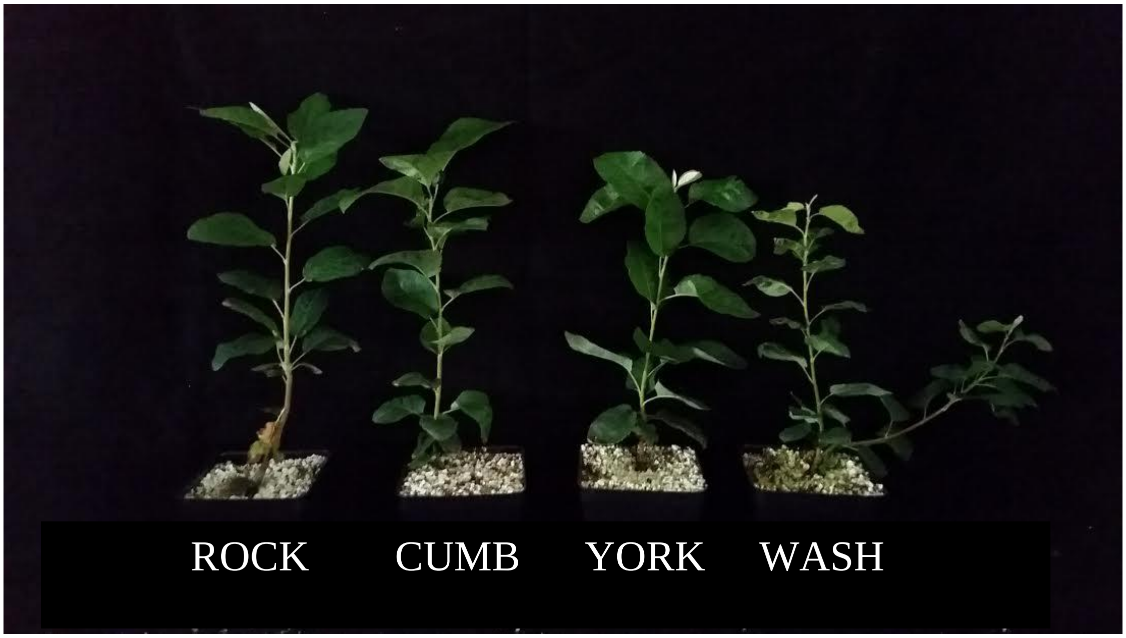

Figure 3.2. Representative seedlings of *Amelanchier spicata* from provenances in Maine and New Hampshire treated with NO_3-N at pH 6.5. Provenances from left to right: Rockingham, Cumberland, York, and Washington. Notice the branching of WASH at the cotyledonary node.

In this study, seedling provenance accounted for greater variation among measured traits compared to minimal differences from nitrogen form. Nitrogen form did not affect root dry weight, leaf dry weight, or stem length. Seedlings supplied with NO_3NH_4-N or NH_4-N alone had slightly greater stem caliper, stem dry weight, and SPAD, but fewer leaves, than those treated with NO_3-N. In contrast, seedling provenance had a large impact on plant growth and development.

31 March, 2015			
Nitrogen Form	**Solution pH**	**pH[1]**	**EC[2]**
NH_4	4.5	4.5 c	1.1 b
NH_4	6.5	4.7 bc	1.2 a
NH_4NO_3	4.5	4.9 b	0.9 c
NH_4NO_3	6.5	4.8 bc	0.9 c
NO_3	4.5	5.5 a	0.6 d
NO_3	6.5	5.5 a	0.7 d

[1]ANOVA and Tukey's tests, $\alpha = 0.05$.

[2]Kruskal-Wallis and Dunn's tests, $\alpha = 0.05$.

Table 3.2. Substrate pH and EC collected using the PourThru methodology as affected by nitrogen source and solution pH treatments. Means followed by the same letter within columns are not significantly different. EC are in units of $mS \cdot cm^{-1}$.

	N (%)	Ca (%)	K (%)	Mg (%)	P (%)	Al (mg·L^{-1})	B (mg·L^{-1})	Cu (mg·L^{-1})	Fe (mg·L^{-1})	Mn (mg·L^{-1})	Zn (mg·L^{-1})
Nitrogen Form											
NH_4	2.1 a[1]	0.73 b[1]	1.3 a[1,3]	0.50 b[1]	0.15 a[2]	62.8 b[1,4]	92.4 a[1,4]	7.8 a[1]	134 a[1]	1079 a[1]	14.1 b[1,3]
NH_4NO_3	2.0 a	0.79 ab	1.3 a	0.56 ab	0.14 a	55.5 b	92.4 a	8.2 a	119 ab	1198 a	17.7 a
NO_3	2.2 a	0.83 a	1.4 a	0.58 a	0.15 a	40.4 a	92.5 a	8.1 a	104 b	1072 a	17.6 a
Provenance											
WASH	2.4 a[1]	0.70 b[1]	1.3 ab[1]	0.57 a[2]	0.19 a[1]	67.3 a[1,4]	120.2 a[1]	9.6 a[1]	127 a[1,3]	1373 a[1]	18.7 a[1]
ROCK	2.0 b	0.83 a	1.5 a	0.50 b	0.14 b	44.3 a	75.6 b	7.8 a	121 a	1008 b	14.7 a
YORK	2.1 b	0.77 ab	1.3 b	0.54 ab	0.14 b	50.5 a	89.6 b	7.7 a	125 a	1035 b	16.7 a
CUMB	2.0 b	0.84 a	1.2 b	0.57 ab	0.13 b	49.6 a	84.3 b	7.0 a	103 a	1049 b	15.9 a

[1]ANOVA and Tukey's tests, α= 0.05.

[2]Kruskal-Wallis and Dunn's tests, α= 0.05.

[3]Logrithimic transformation.

[4]Multiplicative inverse transformation.

Table 3.3. Foliar nutrient concentration of composite leaf samples of *Amelanchier spicata* from Maine and New Hampshire by nitrogen source and provenance. Means followed by letters within columns are not significantly different. Values for N, Ca, K, Mg, and P are percentages (%); values for Al, B, Cu, Fe, Mn, and Zn are in parts per million (mg·L^{-1}).

Nitrogen Form	SPAD	Leaf Number	Stem Dry Weight (g)	Leaf Dry Weight (g)	Root Dry Weight (g)	Stem Length (cm)	Stem Caliper (mm)
NH_4	41.2 a[1]	24.6 b[2]	0.77 a[2]	1.77 a[2]	1.10 a[2]	19.4 a[2]	4.7 a[1]
NH_4NO_3	39.8 ab	25.0 ab	0.83 a	1.80 a	1.22 a	20.1 a	4.9 a
NO_3	38.3 b	25.8 a	0.71 b	1.67 a	1.13 a	20.2 a	4.5 b
Provenance							
WASH	41.7 a[2]	36.6 a[2]	0.66 b[2]	1.23 d[2]	0.80 d[2]	20.0 b[2]	4.5 c[1]
ROCK	41.0 a	24.2 b	0.89 a	1.51 c	1.07 c	23.3 a	4.7 b
YORK	37.7 b	20.0 c	0.69 b	1.97 b	1.18 b	18.9 b	4.7 bc
CUMB	38.6 b	19.4 c	0.83 a	2.24 a	1.55 a	17.5 c	5.1 a

[1]ANOVA and Tukey's tests, $\alpha = 0.05$.

[2]Kruskal-Wallis and Dunn's tests, $\alpha = 0.05$.

Table 3.4. Phenotypic measurements of seedlings of *Amelanchier spicata* from four provenances in Maine and New Hampshire supplied with fertilizer solutions differing in nitrogen source (NH_4 vs NO_3). Means followed by the same letter within columns are not significantly different according to Tukey's or Dunn's tests.

3.5. Discussion

Our results indicate that substrate pH is predominantly influenced by nitrogen form, rather than nutrient solution pH. Although the substrate pH in this experiment displayed normal trends in response to nitrogen source, these values were outside the threshold pH values that promotes optimal nutrient availability. Plants supplied with NO_3-N had EC levels below what is recommended to support seedling growth but grew normally nonetheless. Visually plants displayed no concerning signs of nutrient toxicities or deficiencies, but foliar nutrient analysis indicated discrepancies compared to *Amelanchier* standards. This could be attributed to species-specific responses. There were

significant differences in some of the measured traits resulting from nitrogen source; however, these variations were minor and either NO_3-N or NH_4-N is suitable for commercial production of *A. spicata*. In contrast, provenance accounted for much of the variation among measured characteristics; seedlings from different provenances were morphologically distinct. This indicates that germplasm selection for horticultural implementation requires close attention to regional variation and further investigation is required.

Substrate pH was influenced by nitrogen form and not by pH of the fertilizer solution. It is well established that plants release similarly charged ions in order to assimilate varying nitrogen forms (Mattson et al., 2009). For example, plants incorporate positively charged ammonium by releasing hydrogen cations, which accumulate in the substrate and decrease substrate pH. Likewise, hydroxide anions are released into the substrate to allow for the uptake of negatively charged nitrate. Hydroxide anions interact with free protons in the medium to create water and the consumption of hydrogen increases pH. The oxidation of ammonium to nitrate by microbial communities releases hydrogen cations that likewise lower substrate pH (Mattson et al., 2009). Our nutrient solutions contained a nitrification inhibitor (nitrapyrin) to prevent microbes from converting between nitrogen forms. As a result, the changes in substrate pH should be due to plant-substrate interactions alone. Similar trends in substrate pH have been observed with other genera grown in containers (Hummel et al., 1990; Smith et al., 1983; Witcher et al., 2011)

Even though the trend of substrate pH in response to nitrogen form was not unusual, Bailey and Nelson (1998) suggest a pH between 5.6 and 6.2 for optimal nutrient

availability in soilless media. Plants treated with NO_3-N reached substrate pH levels comparable to this recommendation based on leachate samples collected 31 March. In organic potting media, Mg and Ca are less available as substrate pH decreases below 4.5 (Handreck and Black, 2002). Therefore, the higher substrate pH resulting from nitrate fertilizer would allow for increased availability and subsequent assimilation in leaves. In contrast, Fe availability increases as media acidifies, consistent with the greater incorporation of Fe in plants receiving NH_4-N (Handreck and Black, 2002). Furthermore, Zn availability follows a trend similar to Fe; however, both micronutrients respond antagonistically to increasing levels of the other when excessively available in the substrate; high concentrations of Fe in substrate result in lower assimilation of Zn and vice versa (Bailey and Nelson, 1998). This supports our observation of lower Zn concentration in the leaves of seedlings treated with NH_4-N, which had higher Fe concentrations, even though the substrate pH was optimal.

Moreover, EC values for NO_3-N treatments were consistently below what is considered adequate (1.0-2.6) to support seedling growth (Cavins et al., 2000). Despite low EC readings for plants receiving NO_3-N, growth was robust with no signs of nutrient deficiencies. These measurements could be explained by increased nutrient uptake by the seedlings, reflected by the greater accumulation and storage in leaves.

Although seedlings indicated no observable signs of nutrient deficiencies or toxicities, the foliar nutrient concentration differed for all treatments in Ca, B, and Mn from previous studies evaluating other species of *Amelanchier*, with manganese being the most obvious discrepancy (Zatylny and St-Pierre, 2006). Zatylny and St-Pierre (2006) indicate a mean concentration of $mg \cdot L^{-1}$ Mn in leaves of *A. alnifolia*, whereas leaves of *A.*

spicata in this study averaged more than 1000 mg·L^{-1} regardless of treatment. Zatylny and St-Pierre (2006) noted several leaf samples that likewise contained Mn levels above 1000 mg·L^{-1}, although the cause was unknown. In apples, concentrations of Mn greater than 300 mg·L^{-1} are considered toxic (Bennet, 1993). Boron concentrations were slightly greater than the standard, while calcium was half the average amount found in *A. alnifolia* (Zatylny and St-Pierre, 2006). Neither nutrient was considered toxic or deficient by apple standards (Bennet, 1993). Overall, plants did not have visual abnormalities that indicated improper fertilization and deviation from *A. alnifolia* standards may be attributable to species-specific responses (Figure 1, Figure 2).

Seedlings supplied with NH_4-N had higher average SPAD values than those receiving NO_3-N alone. Mattson et al. (2009) suggested that ammonium-based fertilizers promote plants with darker green coloration and larger leaves. Although we did not find statistical differences in total leaf dry weight among nitrogen forms, plants receiving NH_4-N produced fewer leaves. Therefore, the leaves produced by *A. spicata* in response to NH_4-N were indeed larger than those of plants fertilized with NO_3-N. Furthermore, seedlings exhibited more resource partitioning to the stems when supplied with NH_4-N, as suggested by the stem dry weight and caliper. At this stage in growth and development, differences in stem size were negligible and did not contribute to or hinder the overall aesthetic or commercial value of the seedlings; thinner stems appeared normal and provided sufficient structural support.

Provenance accounted for most of the phenotypic variation in this study, with all measured traits differing by provenance. We could distinguish provenance based on leaf morphology alone, which ranged from small, orbicular leaves characteristic of WASH, to

the narrow, elliptical shape of ROCK. Moreover, all mature plants of *A. spicata* we sampled in native populations were exclusively shrub-like in habit. Interestingly, plants of only one provenance (WASH) exhibited branching consistent with a shrubby nature. In contrast, ROCK stems grew the longest and were consistently straight and upright. The wide range of traits further promotes the horticultural merit of this species and allows for germplasm selection that suits specific purposes. Additional studies need to be conducted to determine if branching habits are stable in different environments. In addition, the duration of active growth and amount of seasonally accumulated biomass should be investigated. Phenotypic differences of seedlings due to provenance have been commonly documented in other genera of woody plants and are associated with of the longitude of seed origin, maternal carryover, and genetic variability (Ginwal et al., 2005; Loha et al., 2006; Renison et al., 2005; Sniezko and Stewart, 1989).

3.6. Conclusions

A. spicata seedlings appear to be relatively insensitive to nitrogen source and are able to utilize either NO_3-N or NH_4-N for healthy development. We found growth to be uniform and robust across nitrogen treatments, with measurable differences in growth probably inconsequential for commercial production. The variation in phenotypes across provenances emphasizes the diversity of germplasm within the species, highlighting their horticultural value. Additional studies should be conducted to evaluate germplasm for ornamental or agricultural value in landscape environments.

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BIOGRAPHY OF THE AUTHOR

Gregory J.R. Melcher was born in Farmington, Maine on April 5, 1992. His passion for plant life was predominantly influenced by his mother and grandmother, Mary and Eva, who often traveled across the state to visit the variety of greenhouses and nurseries Maine has to offer. It was not until his father, Larry, suggested studying biology that he decided to pursue a degree in plant sciences. He graduated summa cum laude from the University of Maine in 2014, earning a B.S. in Botany with a double major in French. His studies were diverse and consisted of subjects including plant physiology, ecology, and taxonomy. Gregory was granted the opportunity to conduct and assist with novel research in a fungal pathology laboratory and herbarium for three years, under the guidance of Drs. Seanna Annis and Christopher Campbell. The experience he gained was invaluable and inspired his pursuit of an advanced degree.

While working toward his M.S. degree, he was awarded numerous scholarships and represented the Plant, Soil, and Environmental Sciences department on the Graduate Student Government. He hopes to incorporate his love for both science and language into future career prospects. Gregory is a candidate for the Master of Science degree in Horticulture from the University of Maine in August 2016.