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Propagation of Two Utah Native Plants: Ceanothus velutinus and Cercocarpus montanus

Asmita Paudel
Utah State University

PROPAGATION OF TWO UTAH NATIVE PLANTS: *CEANOTHUS VELUTINUS* AND *CERCOCARPUS MONTANUS*

by

Asmita Paudel

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Approved:

_____	_____
Youping Sun, Ph.D. Major Professor	Larry A. Rupp, Ph.D. Committee Member
_____	_____
John Carman, Ph.D. Committee Member	Stephen L. Love, Ph.D. Committee Member

_____

D. Richard Cutler, Ph.D.
Interim Vice Provost for
Graduate Studies

UTAH STATE UNIVERSITY
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ABSTRACT

Propagation of Two Utah Native Plants: *Ceanothus velutinus* and *Cercocarpus montanus*

By

Asmita Paudel, Master of Science in Plant Science

Utah State University

Major Professor: Dr. Youping Sun
Department: Plants, Soils and Climate

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf mountain mahogany) are native species with potential to be used in water-efficient landscapes. However, efficient propagation methods are not well developed. Our objectives were to develop efficient seed propagation, cutting propagation, and micropropagation protocols for these two species.

Seeds of both *C. velutinus* and *C. montanus* were scarified and/or stratified and treated with gibberellic acid (GA_3) to break dormancy. The results showed that *C. velutinus* seeds stratified for 2 months after being scarified at 90 ºC and treated with 500 $mg \cdot L^{-1}$ GA_3 had the greatest germination percentage (74.2 ± 2.0%), and *C. montanus* seeds treated with 50 $mg \cdot L^{-1}$ GA_3 and stratified for 2 months had the greatest germination percentage (64.2 ± 3.6%).

Terminal cuttings of *C. velutinus* were collected from May to Sept. 2019 and June to Aug. 2020 from the Tony Grove Lake area, Utah. *Ceanothus velutinus* cuttings collected in July tended to have a better rooting percentage than those collected at other

times of the year. Terminal and stem cuttings were collected in Aug. 2019 from the same area. Terminal cuttings were good compared with stem cuttings but were not significantly different in terms of rooting percentage. Likewise, different rooting hormones were tested using cuttings collected from greenhouse-grown seedlings. Hormodin 2 [3,000 $mg \cdot L^{-1}$ indole-3-butyric acid (IBA)] tended to be the better rooting hormone.

Terminal cuttings of *C. montanus* 'Coy' were collected in mid-July and different rooting hormones were tested. Hormodin 2 tended to be the better rooting hormone. A separate experiment was also conducted using terminal and stem cuttings. Stem cuttings tended to be better for *C. montanus*. Hardwood stem cuttings were collected on 11 May, 2020, and wounding study was performed. Wounding promoted adventitious root formation of *C. montanus*.

For micropropagation, Murashige and Skoog (MS) and Gamborg's B-5 (B5) medium supplemented with 1 $mg \cdot L^{-1}$ benzylaminopurine (BA) were better than other medium for establishment of *C. velutinus*. In addition, *ex vitro* rooting study was successful for rooting microshoots of *C. velutinus*. For *C. montanus*, MS + 1 $mg \cdot L^{-1}$ BA tended to be better medium for multiplication stage.

(113 pages)

PUBLIC ABSTRACT

Propagation of Two Utah Native Plants: *Ceanothus velutinus* and *Cercocarpus montanus*

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Among various water conservative approaches, the use of native plants in landscape, such as *Ceanothus velutinus* (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf mountain mahogany), is attractive. Efficient propagation methods are required to allow these native species to use in water-efficient landscaping. Sexual (seed) and asexual/vegetative (cuttings and micropropagation) propagation methods were evaluated.

Seeds of both *C. velutinus* and *C. montanus* were scarified and/or stratified and treated with gibberellic acid (GA_3) to break dormancy. The results showed hot water scarification and 2-3 months of stratification effectively broke the dormancy of *C. velutinus* seeds, and stratification for 2-3 months was needed for *C. montanus* seeds. Furthermore, GA_3 also helped to increase germination of both species.

Terminal cuttings of *C. velutinus* were collected from May to Sept. 2019 and June to Aug. 2020 from the Tony Grove Lake area, Utah. Terminal and stem cuttings were also collected in Aug. 2019 from the same area. Likewise, different rooting hormones were tested using cuttings collected from greenhouse-grown seedlings. *Ceanothus velutinus* cuttings collected in July tended to have a better rooting percentage than those collected at other times of the year. Hormodin 2 [3,000 $mg \cdot L^{-1}$ indole-3-butyric acid

(IBA)] tended to be the better rooting hormone. Terminal cuttings were good compared with stem cuttings but were not significantly different in terms of rooting percentage.

Terminal cuttings of *C. montanus* ‘Coy’ were collected in mid-July and different rooting hormones were tested. Hormodin 2 tended to be the better rooting hormone. A separate experiment was also conducted using terminal and stem cuttings. Stem cuttings tended to be better for *C. montanus*. In addition, on 11 May, 2020, hardwood stem cuttings were collected and wounding study was performed. Wounding promoted adventitious root formation of *C. montanus*.

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CHAPTER I

LITERATURE REVIEW

Water is an important natural resource-essential to health, social and economic well-being of people. In the United States (U.S.), water demand, especially in urban areas, is destined to exceed the supply (St. Hilaire et al., 2008). Urbanization, population growth and climatic change are three major factors responsible for water scarcity in urban areas. The urbanization of the western U.S. has progressed rapidly, and most populations are concentrated in cities (Kjelgren et al., 2000). The entire output of local and regional watersheds is, therefore, required to fulfill the demand for water by industry, personal and agriculture use. Urbanization also leads to the expansion of watershed-impervious areas and affects the hydrology, groundwater recharge, stream geomorphology, climate, biogeochemistry, and stream ecology, which limits further water availability for use by people (O'Driscoll et al., 2010). In Utah, the second driest state in the U.S., water demand is increasing day by day; parallel with the population growth. The 2015 report of Utah Division of Water Resources entitled "A Performance Audit of Projections of Utah's Water Needs" predicts that the population of Utah is estimated to double (nearly 6 million people) by 2060. This fast increase in population will strain currently developed water supplies. Most of the water in Utah is used for agricultural purposes. About 5.18×10^{9} cubic meter of fresh water is used every year for agricultural purposes in Utah (Strong et al., 2010). Of total available potable water, 60% is used for urban landscape irrigation (Kjelgren et al., 2000). However, water resources are limited in Utah, and more rigorous water conservation practices will likely be imposed relative to agriculture and landscape irrigation.

Native Plants

Among various approaches for water conservation, the use of native plants in landscapes has considerable potential. Native plants, by definition, are plant species that occur naturally in a region, state, ecosystem, and habitat without direct or indirect human actions. Native plants in other words are those plants that occur naturally in a region in which they evolved so that they can thrive in that region. Drought tolerance is one of the peculiar characteristics of western U.S. native plants. They can maintain aesthetics in landscape with less applied water as compared with other exotic plants. Additionally, consumers are increasing their interest in natural landscapes and show willingness to pay a premium price for native plant products (McCoy, 2011). Native plants can be planted easily in low-water landscapes and are of great value in landscape maintenance (Rupp and Wheaton, 2014). Native plants are drought, disease and pest tolerant (Dyas, 1976). Native plants that have ornamental characteristics like short stature and glossy leaves are suitable for most water-conserving landscapes (Rupp et al., 2011). Kratsch (2011) studied water requirements of native plant species in two consecutive years by irrigating them in the first year and cutting off irrigation in the second. This author noted that *Astragalus filipes* (basalt milkvetch), *Dalea searlsiae* (searls' prairie clover), *Penstemon eatonii* (firecracker penstemon), and *Stanleya pinnata* (desert princes' plume) could be grown in well-drained soils without regular irrigation. Similarly, Rupp and Libbey (1996) identified various native species for landscape use and noted that *Cercocarpus* spp. did not require additional water once it was established and therefore could be effectively

grown in low-water landscapes. Thus, the use of native plants should be encouraged to help address water conservation issues in the landscapes of Utah.

Native plants have valuable importance but some of them do not survive well when transplanted. They are susceptible to different soil-borne diseases and may be affected by native insects or pest. In addition, they are native to specific zone or higher elevations and may not thrive well in lower elevations or valleys.

However, many native plants have been shown to perform well in urban landscapes in Utah, many more species may be candidates for utilization in this way. *Ceanothus velutinus* (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf or true mountain mahogany) are examples of underutilized Utah native plants.

Ceanothus velutinus. It is a broad-leaf evergreen shrub in the Rhamnaceae family. *Ceanothus* is a common genus in North American ecosystems and includes approximately 50 species of evergreen or deciduous shrubs (Fross and Wilken, 2006). The common names of *C. velutinus* include snowbrush ceanothus, mountain balm, red root, sticky laurel, tobacco brush, and varnish leaf ceanothus. Native elevation range is 2,135 to 2,745 meters (Fross and Wilken 2006; Rupp and Wheaton, 2014). It is native to western North America, covering a range from British Columbia south to California and east to Colorado. *Ceanothus velutinus* has multiple stems with procumbent branches and usually grows up to 0.9-2.5 meters tall (Seven Oaks Native Nursery, 2019). This species possesses characteristics that could prove valuable in the landscape, including shiny, evergreen, broadleaf foliage. Leaves are alternate and produce a pungent odor when crushed. Leaf blades of *C. velutinus* are 33-81 mm long and 21-53 mm wide and are

elliptical to ovate, leathery with dark green upper surface and pale green lower surface. Flowering occurs from March to August. The attractive white flowers of *Ceanothus velutinus* are produced in terminal clusters (Fig. 1-1A) (Fross and Wilken, 2006). Three-lobed seed capsules are 3.5 to 5 mm wide, brownish with smooth to slightly wrinkled lobes (Fig. 1-1B). Seeds are ovoid and brownish; about 1 to 3 mm wide. *Ceanothus velutinus* is shade intolerant and commonly found on south-facing slopes. Importantly, it does not require heavy irrigation to complete its normal lifecycle. *Ceanothus velutinus* is a fire-adapted species, and incidence of fire is believed to enhance seed germination (Gratkowski, 1962). This is a nitrogen-fixing species and fills a soil-building role within its native habitat. In symbiosis with *Frankia* this species fixes nitrogen at rates of 20 to more than 142 Kg N ha^{-1} yr^{-1} (10,000 m^{-2}) (Binkley et al., 1982; Youngberg and Wollum, 1976).

Leaves of *C. velutinus* are green throughout the year, even in winter, but the leaf edges often curl in the winter and may turn black without cover of snow (Karban, 2008). This species spreads by sprouts, forming dense thickets which make it difficult to identify the boundary of a single individual. *Ceanothus velutinus* is a high value plant for soil stabilization and forage for wildlife (McNabb and Cromack, 1983). *Ceanothus velutinus* has a high potential to be used for restoration, but the survival of planted seedlings is low and seed production is reduced due to over-grazing (Garrison, 1953).

Cercocarpus montanus. It is a shrub to small tree and is prevalent across the western continental U.S.; being distributed within an area from Montana and South

Dakota, south New Mexico and Arizona (Vines, 1960; Woodmansee, 1969). This species can be found at elevations from 1,050 to 2,750 meters.

Cercocarpus montanus grows to a mature height of 4 meters tall (Fig. 1-2A). Leaves are oval, greenish above, and whitish below (Fig. 1-2B). Flowering occurs in May and June. Fruits are achenes with a long, white, fuzzy, curly tail, and ripen in August and September (Gucker, 2006) (Fig. 1-2B). *Cercocarpus montanus* has an extensive root system including rhizomes and plays a role in controlling erosion on dry slopes (Deitschman et al., 1974). *Cercocarpus montanus* grows on sites that are dry, erosive, and fertile (Brotherson, 1992). It is an actinorhizal plant that forms symbiosis with *Frankia* bacteria and plays an important role in nitrogen fixation (Paschke, 1997). This species generally forms pure stands or is found in mixed stands as a dominant, climactic species (Woodmansee, 1969).

Cercocarpus montanus can tolerate soils high in lime and prefers sandy soils (Brotherson et al., 1984; Plummer 1969) with pH ranging from 6.8 to 7.7 (Plummer, 1969). It grows nicely in shallow soils with 35% or greater coarse particles. This common shrub is palatable for livestock and a most valuable winter browse plant for wild animals.

Native Plant Propagation

Though native plants have significant potential value in landscapes, in many cases, use is limited because efficient propagation methods are not available. To promote the use of Utah native plants, effective propagation methods are needed. Plant propagation is the process of manipulating a plant and its environment to form additional

new plants. Sexual (seed and spores) and asexual/vegetative (cutting, layering, grafting, division, and micropropagation) propagation are the primary methods. For successful sexual or asexual propagation, a plant propagator must understand certain principles before developing propagation protocol for specific plants.

Seed Propagation

Propagation of approximately 250,000 angiosperm and 700 gymnosperm species has been successfully demonstrated through the use of seeds (Dirr and Heuser, 1987). It is an easy and reliable method of propagation, but offspring may not be identical to parents; homogeneity being an important trait for nursery products. For successful seed propagation, the embryo must be alive and capable of germination. Environmental conditions must be favorable, and seed dormancy, if present, must be overcome. Dormant seeds will not germinate immediately after dissemination even in the presence of optimal growing conditions. There are different kinds of seed dormancy, physical (exogenous) and physiological (endogenous) dormancy are commonly referenced.

Physical dormancy. Physical dormancy is often the result of the presence of a hard, impermeable seed coat. Such seed coats protect against microbial infection (Dalling et al., 2011). Seeds with physical dormancy can persist in the soil for long time, often many years (Shen-Miller et al., 1995). In addition to protecting the viability of seed, a hard seed coat inhibits water uptake and limits oxygen diffusion to the embryo, thus preventing germination. One or more layers of tightly packed palisade macroscleroids cells with water repellant chemicals are responsible for the impermeability of hard seed

coats (Werker, 1980). The impermeable layer works as the ‘water gap’ which needs to be opened for successful germination of seeds with physical dormancy (Baskin et al., 2000). In order to break physical dormancy a seed coat needs to be cracked, softened, abraded or removed using acid, hot water, or mechanical scarification. Kildisheva et al. (2011) reported that *Sphaeralcea munroana* (munro’s globemallow) seeds are physically dormant and need scarification for germination. They mentioned that *S. munroana* seeds after mechanical stratification with sharp blade demonstrated a 93% germination rate.

Physiological dormancy. Seeds with physiological dormancy are viable but even under ideal conditions are unable to germinate due to the presence of inhibiting chemicals. A low ratio of gibberellic acid (GA_3) to abscisic acid (ABA) is thought to be important for creating a dormant state in seeds. A combination of environmental and physical factors is required to modify the chemical makeup of the seed and allow for germination (Baskin and Baskin, 2004). Cold or warm stratification helps in breaking physiological dormancy. Application of gibberellins can shorten the period of cold stratification requirement of many plants (Ching, 1972). Gibberellic acid (GA_3) is the most used form of gibberellin (Hartmann et al., 2002). Utah native plant like *Acer grandidentatum* (bigtooth maple) was successfully propagated after breaking seed dormancy with combination of warm moist treatment and stratification (Woodruff et al., 2012). Similarly, *Eriogonum corymbosum* (lacy buckwheat), and *Penstemon digitalis* (beardtongue) were propagated after breaking seed dormancy with cold stratification, and GA_3, respectively (De Mello et al., 2009; Meyer and Paulsen, 2000).

Ceanothus velutinus is difficult to grow from seeds. However, if the seeds are collected in a timely fashion and handled carefully then 70-80% of the seeds germinate (Rupp and Wheaton, 2014). Seeds remain viable in the soil for several years as they have physical dormancy created by a hard seed coat. Ripening of dry capsules occurs from August to early October, and seeds can be collected from wild prior to dehiscence. *Ceanothus velutinus* seeds are physically and physiologically dormant (double dormancy). Scarification is necessary to break physical dormancy, and stratification to break physiological dormancy. Similarly, *Sphaeralcea ambigua* (desert globemallow) and *Sphaeralcea coccinea* (scarlet globemallow) had 45% and 85% germination, respectively, when a combination of mechanical scarification and 30-day stratification was performed (Dunn, 2011). *Ceanothus velutinus* seeds germinate typically after fire and temperatures of 80-95 ℃ are necessary to break its hard seed coat (Native Plants PNW, 2019). After scarification, cold treatment is also needed as stratification.

Cercocarpus montanus is commonly propagated from seeds (Rupp and Wheaton, 2014). Fruits are ripened and dispersed from July to October (Kitchen, 2008). Seeds of *C. montanus* (alder-leaf mountain mahogany) need stratification to break physiological dormancy (Rosner et al., 2003). Cold stratification is found to be successful in breaking dormancy. On the other hand, chemical treatments like GA_3, thiourea, and hydrogen peroxide had less success in breaking dormancy in *Cercocarpus ledifolius* (curl-leaf mountain mahogany) (Kitchen, 2008).

Vegetative Propagation

Sexual reproduction produces visible variability in native plant products, a characteristic not considered acceptable for most commercial markets (Dole and Gibson, 2006). In such cases, vegetative propagation can be used to produce clones that are individually identical and true-to-type to original plant. Vegetative propagation is desired for commercial nursery production as it generates more uniform plants and a whole new plant can be created from portion of parent plant (Hartmann et al., 2002).

Cutting Propagation

Cutting propagation is the most common method of vegetative propagation of woody plants (Rupp and Wheaton, 2014). Generally, stems, leaves, and roots are used for cutting propagation. Rooting ability of cuttings is species dependent, and there is also significant variation within a species (Ercisli et al., 2001). The physiological processes associated with rooting of cuttings is influenced by various factors, including timing of cutting harvest, rooting medium, application of appropriate growth regulators, and temperature of the rooting environment, and their interactions.

Rooting medium like peatmoss mixed with perlite and have adequate moisture holding capacity combined with porous characteristics that enhance drainage and provides appropriate conditions for rooting of cuttings (Gislerod, 1983). Seasonal timing of cutting collection, plant part utilized, and addition of auxins such as indole-3-butyric acid (IBA) play important roles in adventitious root formation (Dole and Gibson, 2006).

Adventitious root formation. Some species have existing root initials on vegetative cuttings while others develop adventitious roots. Formation of adventitious roots is the important goal with many species for propagation by stem cuttings (Hartmann et al., 2002). There are several factors which influence adventitious rooting in stem cuttings including rooting hormones, rooting medium, and wounding. In some species root initials are present, and roots are formed under favorable environmental conditions. On the other hand, wound-induced adventitious roots are formed after a cut is made. Wound-induced adventitious root formation is divided into four stages: dedifferentiation, induction, outgrowth of stem, and root elongation (Davies and Hartmann, 1988). Adventitious roots arise from various plant tissues including vascular rays, secondary phloem, cambium, phloem, callus, lenticels or pith (Davies and Hartmann, 1988; Davies et al., 2018; Girouard, 1967).

Time of cutting collection. Timing is an important factor for successful rooting. The seasonal growth phase of a plant when cuttings are collected often determines the rooting success (Still and Zanon, 1991). Carbohydrate content, endogenous auxin levels, rooting co-factors, and rooting inhibitors which have important roles in rooting ability change during seasonal development of plant (Melcher, 2016). Smith and Wareing (1972) stated that photoperiod causes the variations in endogenous auxins which have important roles in adventitious rooting and higher levels of auxins are synthesized in long days when compared with short days.

Firmness of the wood and the stage of terminal bud development are indicators of cutting status. *Amelanchier spicata* (dwarf serviceberry) cuttings collected in June had

higher rooting rate (88%) than July cuttings (Melcher, 2016). Similarly, hardwood and softwood cuttings of *Juglans cinerea* (butternut) rooted best when cuttings were taken in mid-May and June, respectively (Pijut and Moore, 2002). In addition, softwood to semi-hardwood cuttings of *Amelanchier laevis* (smooth serviceberry) collected in mid-May to mid-June performed best in terms of cutting rooting percent and root quality than cuttings taken in July (Still and Zanon, 1991). Moreover, *Ceanothus americanus* (New Jersey tea) cuttings taken in June had 57% rooting success which was higher than cuttings taken later in July and August (Cartabiano, 2013).

Semi-hardwood stem cuttings of *C. velutinus* collected in summer can have higher success rate for rooting after 8 weeks of sticking (Rupp and Wheaton, 2014). Rosner et al. (2000) found less than 1% rooting of *C. montanus* when cuttings were collected during the period January to April.

Type of cuttings. Type of cuttings used for propagation affects rooting of cuttings (Hartmann et al., 2002). Softwood and semi-hardwood cuttings are the most common in nursery industry. Softwood cuttings are preferred in many species since there is higher chance of gaining post-rooting growth (Smalley et al., 1987). However, softwood cuttings are delicate, wilt easily, and need special care (Hartmann et al., 2002). Terminal cuttings with a shoot apex are seen as advantageous for increasing the rooting percentage (Malan, 1992). In some species young stem tissue roots faster than old stem tissue (Dole and Gibson, 2006). *Populus tremuloides* (aspen) do not root well from stem cuttings but root cuttings have been used successfully for propagation (Snedden et al., 2010). Everett et al. (1978) reported that semi-hardwood cuttings of *Artemisia spinescens* (budsage),

Atriplex lentiformis (big saltbush), *Ceratoides lanata* (winterfat), *Grayia spinosa* (spiny hopsage), *Lepidospartum latisquamum* (wooly scalybroom), *Prunus andersonii* (Anderson's peachbrush), *Rosa woodsii* (woods' rose), *Salvia dorrii* (Dorr's sage), and *Vitis arizonica* (canyon grape) were superior to softwood or hardwood cuttings. Conversely, softwood cuttings were good compared with semi-hardwood cuttings in *A. spicata* propagation (Melcher, 2016).

Wounding. Wounding positively affects root production in stem cuttings for some species (Hartmann et al., 2002). Stem cuttings of *A. laevis* had a positive effect of wounding on rooting, but wounding had no effect on *Amelanchier alnifolia (*saskatoon serviceberry) (Bishop and Nelson 1980; Still and Zanon, 1991). *Arctostaphylos* species which are difficult to root had a beneficial effect from lateral wounding (Wisura, 1980). Wounding speeds rooting and increase the surface area of cambium-exposed tissue (Davies and Hartmann, 1988). Some species can produce adventitious roots naturally while in others wounding increases cellular division near the vascular cambium and phloem that promotes callus formation which is often followed by development of adventitious roots (Pijut et al., 2011). Wounding is beneficial for some species but can be detrimental for rooting success in other species because it creates a site of entry for harmful microorganisms (Dirr and Heuser, 1987). Adventitious root formation and root quality of *A. spicata* were enhanced by wounding (Melcher, 2016). In some hard-to root species adventitious root quantity and uniformity increases when they are wounded and then treated with an auxin (Alsup et al., 2003, Griffin and Bassuk, 1996).

Rooting hormones (Auxins). Auxins are produced in plant apical meristems and other actively growing tissues such as developing leaves, fruits, flowers, and seeds (MacAdam, 2009). Indole-3-acetic acid (IAA) is potentially valuable auxin for use in propagation that is naturally occurring and found abundantly in plants; but it is very sensitive to light and unstable in solutions (Dunlap and Robacker, 1988; Tanimoto, 2005). Therefore, stable exogenous auxins, like IBA and 1-naphthaleneacetic acid (NAA) in liquid or powder form are most commonly used as root-inducing hormones. Mixtures of IBA and NAA are often efficacious for rooting. The right concentration of auxin is necessary for root formation. High doses of auxin can lead to foliar senescence, chloroplast damage, destruction of membranes, necrosis, and even plant death (Blythe et al., 2007). Rupp et al. (2011) reported successful cutting propagation of the native woody species *Arctostaphylos patula* (greenleaf manzanita), *Arctostaphylos pungens* (point-leaf manzanita), and *Cercocarpus intricatus* (little leaf mountain mahogany) using auxin in combination with intermittent mist with bottom heat. The auxin employed was IBA/NAA at a concentration of 4,000/2,000 $mg \cdot L^{-1}$ and proved superior for rooting cuttings when compared with concentrations 0 $mg \cdot L^{-1}$ and 2,000/1,000 $mg \cdot L^{-1}$.

Micropropagation

Micropropagation, also called *in vitro* or tissue culture propagation, is an important method for the multiplication of many foliage plants, cut flowers, and potted plants. Many identical plants can be produced from one original plant using small pieces of plant shoots, roots, or reproductive structure. Explant type, culture medium and growth

regulators have important roles in the success of micropropagation (Davies et al., 2018). Nodal stem cuttings or tissue pieces from leaves, petioles, or roots are used for micropropagation, and explant type has influence on the success of tissue culture.

Culture medium consisting of agar, inorganic nutrient elements, sucrose, and vitamins supplements are used to grow tissue, and optimal medium constituents vary with species. In general, cytokinins are believed to help promote shoot growth and auxins root growth. Efficacious protocols for new shoot growth and development vary among species, and optimization of basal salts and plant growth regulator combinations is necessary (Mackay et al., 1996; Rounsaville and Ranney, 2010).

Some Utah native plants were successfully propagated via micropropagation. Pruski et al. (1990) successfully used *in vitro* culture to propagate four cultivars of *A. alnifolia* and found that shoot-tip explants were better than dormant buds when taken from actively growing plants. Combination of benzylaminopurine (BA) and GA_3 as foliar sprays were considered important in breaking dormancy and formation of axillary shoots in cultured *A. alnifolia*. In addition, use of an IAA/NAA (2.8/1.1 μM) (≈0.5/0.2 $mg \cdot L^{-1}$) mixture induced the highest rate of rooting. Similarly, Murashige and Skoog (MS) salts (full, 1/2, 1/4, and 1/8-strength) were used for rooting study of *A. alnifolia*, wherein 38% of the shoots formed roots in 1/8-strength MS medium and shoots failed to form roots in full-strength MS medium (Alosaimi and Tripepi, 2016). Clonal propagation of *P. tremuloides* is demonstrably possible by micropropagation (Haapala et al., 2004). Moreover, nodal cuttings of *A. grandidentatum* were used for tissue culture, wherein Driver-Kuniyuki Walnut (DKW) was the best medium for shoot multiplication, and IAA

induced rooting in microshoots (Bowen-O'Connor et al., 2007). In addition, *Calochortus nuttallii* (Sego lily) bulb basal sections were used for tissue culture in which Schenk and Hildebrandt (SH) basal medium and BA were successfully used for shoot multiplication and NAA for rooting (Hou et al., 1997).

No peer-reviewed literature is available for tissue culture of *C. velutinus* and *C. montanus*. They are difficult to propagate by cuttings, and micropropgation could provide another alternative for propagation. Development of efficient micropropagation protocols could be very important in creating economically viable nursery products with these two species. Using a series of controlled experiments, we plan to develop efficacious propagation strategies for the Utah native plant species *C. velutinus* and *C. montanus*. By so doing, we will expand the potential for use of native plants in water-efficient landscapes.

Fig. 1-1. *Ceanothus velutinus* flowers and fruits. (A) Flowers and (B) fruits. Pictures by Dr. Larry A. Rupp.

Fig. 1-2. *Cercocarpus montanus* in the wild and its leaves and fruits. (A) *Cercocarpus montanus* in the area of Wind Cave, Logan, UT (41°45’48’’ N, 111°43’2’’ W, elevation 1798 m) and (B) its leaves and fruits.

CHAPTER II

SEED PROPAGATION OF *CEANOTHUS VELUTINUS* AND *CERCOCARPUS MONTANUS*[1]

Abstract

Ceanothus velutinus Douglas ex Hook. [snowbrush ceanothus (Rhamnaceae)] and *Cercocarpus montanus* Raf. [alderleaf mountain mahogany (Rosaceae)] are native species with urban landscape value and potential to create unique aesthetics and conserve water. Propagation protocols for these native species are not well established. Because of dormancy, seed propagation requires scarification and (or) stratification. We designed a study to further define protocols necessary to consistently produce high rates of germination for these two species. *Ceanothus velutinus* seeds were scarified in hot water at 50, 70, or 90 ºC and soaked with gibberellic acid (GA_3) at 0, 50, 250, or 500 $mg \cdot L^{-1}$ for 24 hours before stratification for 1, 2, or 3 months. Seeds of *C. velutinus* stratified for 2 months after being scarified at 90 ºC and treated with 500 $mg \cdot L^{-1}$ GA_3 had the greatest germination percentage (74.2 ± 2.0%). Percent germination was the lowest when seeds were scarified at 50 ºC and treated without GA_3 but with 2 months stratification. *Cercocarpus montanus* seeds were treated with GA_3 at 0, 50, 250, or 500 $mg \cdot L^{-1}$ and stratified for 1, 2, or 3 months. Seed germination of *C. montanus* increased as

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stratification time increased. Seeds dipped in 50 $mg \cdot L^{-1}$ GA_3 and stratified for 2 months had the greatest germination percentage (64.2 ± 3.6%).

Introduction

The use of native plants has gained popularity in ecological landscape design, green building construction, and urban habitat development (Calkins, 2005; Hooper et al., 2008). Rocky Mountain native plants have evolved under climate and soil conditions that require less water and fertilizer than do traditional landscape plants, and they help in reducing air pollution and promoting biodiversity (USDA, 2019). Utah ranks the top fifth among US states for native plant species diversity (Hooper et al., 2008; Stein 2002).

Ceanothus velutinus Douglas ex Hook. (Rhamnaceae), common name snowbrush ceanothus, is native to western North America from British Columbia to California and eastward to Colorado. *Ceanothus velutinus* is an evergreen shrub with oval leaves and can grow up to 0.9-2.5 meters tall (Seven Oaks Native Nursery, 2019). Aromatic white flowers are found in 5-10 cm long corymbose inflorescences. *Ceanothus velutinus* prefers full sun and coarse-textured, well-drained soils. In addition, it is stress tolerant and fixes nitrogen, which plays a soil-building role (Conard et al., 1985). The fruit is a 3-lobed, dry capsule containing dark red-brown seeds when mature.

Cercocarpus montanus Raf. (Rosaceae), common name alder leaf mountain mahogany, is another native of Utah that is drought tolerant and grows well in alkaline soils (Lady Bird Johnson Wildflower Center, 2019). As a shrub or small tree, *C. montanus* grows 2.5-6 meters tall. It possesses attractive leaves that are dark green on top

and are fuzzy silver underneath. The fruits are silvery-white and showier than the flowers. The fruit is an achene, and seeds are physiologically dormant.

Seeds that are viable but unable to germinate under favorable conditions are defined as dormant (Finch-Savage and Leubner-Metzger, 2006). In some species dormancy takes the form of hard seed coats that act as a mechanical barrier that prevents germination (physical dormancy) (Abubakar and Muhammad, 2013). In order to break physical dormancy, the seed coat should be broken, softened, abraded or removed using acid, hot water or mechanical scarification. In other species, dormancy is physiological and can be overcome by cold stratification. Gibberellic acid (GA_3), an endogenous plant growth regulator, plays an important role in seed germination by inducing enzymes that weaken the mechanical resistance of seed coverings (endosperm or seed coat), stimulating cell expansion, and mobilizing seed storage reserves for embryo growth (Dewir et al., 2011; Gupta and Chakrabarty, 2013; Lecat et al., 1992). Gibberellic acid is also used to break physiological dormancy in seeds of many species (De Mello et al., 2009; Kitchen and Meyer, 1991).

Ceanothus seeds can remain viable in the soil for years under field conditions, which illustrates that they are strongly dormant (Conard et al., 1985). *Ceanothus velutinus* is difficult to propagate from seeds because both physical and physiological dormancy are present (Rupp and Wheaton, 2014). In a 2-week preliminary study, *C. velutinus* seeds without any scarification and stratification treatment did not germinate (unpublished data). However, if *C. velutinus* seeds are collected and handled carefully, 70-80% of the seeds will usually germinate (Rupp and Wheaton, 2014). Luna (2008)

indicated that dormancy in *C. velutinus* is both physical and physiological, and that scarification using hot water at a temperature of 80 to 90 ºC to break physical dormancy and then stratification in cold, moist conditions for 1-3 months are required for adequate germination. Rosner et al. (2003) reported that seeds of *C. montanus* need stratification. In the reported experiment they used a 5-10 minutes soak in concentrated sulfuric acid and 0, 30, or 60 days stratification. Ultimately, they concluded that scarification is less effective than stratification.

We conducted the present study to establish efficient protocols for seed propagation of *C. velutinus* and *C. montanus*. The specific objectives were: 1) to determine an efficacious scarification temperature, optimal stratification time, and suitable GA_3 concentration for breaking dormancy of *C. velutinus* seeds, and 2) to determine the optimal stratification time and the effective GA_3 concentration for breaking dormancy of *C. montanus* seeds.

Materials and Methods

Seeds of *C. velutinus* were purchased from the Native Seed Foundation (Polson, MT) (Fig. 2-1A). Collection site is at an elevation of 975 meters nearby Libby, Lincoln County, Montana. Seeds were collected in August 2018 and stored in a metal drum at a temperature of 1 to 7 ºC with a few perforations for air flow (Billington, 2018).

Prior to the experiment, seeds were stored at 1.9 ± 0.4 ºC in a refrigerator. An electronic data logger (Marathon, EDL, San Leandro, CA) was used to measure the temperature inside the refrigerator. Seeds were wrapped in cheesecloth and dipped in a

hot water bath (Isotemp 102, Fisher Scientific, Canada) at 50, 70, or 90 ºC for 10 seconds. They were then immediately plunged in a cold water bath with ice at 6 ºC for 1 hour. Afterward, seeds in each temperature treatment were divided into four groups and soaked in 0, 50, 250, or 500 $mg \cdot L^{-1}$ gibberellic acid solution (GA_3, Caisson Laboratories, Smithfield, UT) for 24 hours. Distilled water was then used to rinse the seeds, and floating seeds were discarded. Seeds were wrapped in moist paper towels and kept in a resealable plastic bag with some unfilled space left for aeration. They were refrigerated at 1.9 ± 0.4 ºC for 1, 2, or 3 months of stratification. We inspected seeds regularly, and water was added as needed.

We ordered seeds of *C. montanus* from Sheffield's Seed Company (Locke, NY) (Fig. 2-1B). Seeds were collected in Colorado and stored in sealed containers in a freezer (Shefield's Seed Company, 2018). Upon receipt, seeds were refrigerated at 1.9 ± 0.4 ºC until used. Seeds were soaked in 0, 50, 250, or 500 $mg \cdot L^{-1}$ GA_3 solution for 24 hours. They were then rinsed with distilled water, and floating seeds were discarded. We kept seeds at 1.9 ± 0.4 ºC for 1, 2 or 3 months of stratification as previously described. Routine inspection was performed, and water was added as needed.

Stratified seeds of *C. velutinus* and *C. montanus* were placed in Petri dishes (Genesee Scientific, Morrisville, NC) on moist blotter paper (Hoffman Manufacturing, Corvallis, OR). Each treatment included 12 Petri dishes as replicates and each Petri dish contained 30 *C. velutinus* seeds. We used similar procedures for *C. montanus* except only 20 seeds were placed in each Petri dish. Petri dishes were then sealed with Parafilm (American National Can™; Menasha, WI) and the seeds were allowed to germinate at 25

ºC under cool-white fluorescent lamps (184.5 ± 9.3 µmol·m^{-2}·s^{-1}) for 2 weeks. We used a quantum light meter (MQ-100, Apogee Instrument, Logan, UT) to measure fluorescent light intensity. Germinated seeds were counted as radicles emerged.

The experiment was conducted using a complete randomized design (CRD). Three-way and two-way analyses of variance were conducted for *C. velutinus* and *C. montanus*, respectively. Trend analysis was performed to locate the rate of seed germination. Means separation among treatments was adjusted using Tukey's method for multiplicity at $\alpha = 0.05$. All statistical analyses were conducted using SAS software (SAS university edition, Cary, NC).

Results

As the temperature for scarification increased from 50 to 90 ºC, the percent germination increased linearly ($p < 0.0001$) (Table 2-1 and Fig. 2-2). On average, 17.4, 34.4, and 63.1% of seeds germinated when they were dipped in the hot water bath at 50, 70, and 90 ºC, respectively.

Stratification time significantly affected seed germination of *C. velutinus* ($p < 0.0001$) (Table 2-1). Seeds stratified for 2 or 3 months showed greater germination than those stratified for only 1 month (Fig. 2-2). The percent germination of *C. velutinus* seeds was 31.2, 38.8, and 44.9% when stratified for 1, 2, or 3 months, respectively. The percent germination increased linearly with increase in time for stratification ($p < 0.0001$).

Gibberellic acid enhanced the germination of *C. velutinus* seeds ($p < 0.0001$) (Table 2-1). When seeds were dipped in GA_3 solutions at 0, 50, 250, or 500 mg·L^{-1},

percent germination were 34.7, 39.4, 39.7, and 39.4%, respectively. The 0 GA_3 treatment produced statistically less germination than all treated seeds, which were not different from each other.

Scarification temperature, stratification time, and GA_3 treatment also had interactive effects on seed germination of *C. velutinus* ($p < 0.0001$). Seeds scarified in hot water at 50, 70, or 90 ºC and stratified for 2 and 3 months had better germination rates than 1 month stratified seeds (Table 2-1 and Fig. 2-2). In addition, seeds scarified at 90 ºC and stratified for 2 or 3 months had higher germination rates compared with seeds stratified for 1 month and scarified at 70 and 90 ºC. Similarly, the combination of longer stratification time, 90 ºC scarification and addition of GA_3 increased the germination rate of *C. velutinus* seeds.

Cercocarpus montanus seeds stratified for 1, 2 and 3 months had 34.8, 55.6, and 57.8% germination, respectively (Fig. 2-3). Seed germination increased linearly with duration of stratification ($p < 0.0001$) (Table 2-2). Gibberellic acid also enhanced the germination of *C. montanus* seeds ($p = 0.031$) (Table 2-2). When seeds were dipped in GA_3 solution at 0, 50, 250, or 500 $mg \cdot L^{-1}$, the germination rate was 45.7, 52.3, 50.7, and 49%, respectively (Fig. 2-3). For *C. montanus* seeds, stratification times and gibberellic acid levels interacted significantly ($p < 0.0002$) (Table 2-2). Pretreatment of seeds with GA_3 before stratification increased the germination rate. Seeds stratified for 1 month had higher germination at 500 $mg \cdot L^{-1}$ GA_3 compared to lower concentrations of GA_3 (Fig. 2-3). For 2 and 3 months stratified seeds, the germination rate increased slightly or remained the same with the increasing concentration of GA_3.

In our study, a hot water treatment of *C. velutinus* seeds at 90 ºC followed by a 2 months stratification and 500 $mg \cdot L^{-1}$ GA_3 treatment produced the highest percent germination at 74.2 ± 2 %. For *C. montanus*, seeds dipped in 50 $mg \cdot L^{-1}$ GA_3 and stratified for 2 months had the greatest germination percentage at 64.2 ± 3.6%.

Discussion

Presence of a hard seed coat inhibits seed germination, an effect that may be due to prevention of water or oxygen uptake or the presence of some chemical inhibitors within the coat (Taiz and Zeiger, 2002). The hard seed coat must be weakened to break physical dormancy. In this study, hot water scarification was used to weaken the seed coat of *C. velutinus*. Our results indicate that *C. velutinus* seeds should be scarified at temperatures as high as 90 ºC to break their physical dormancy and enhance the rate of germination. Similarly, Radwan and Crouch (1977) reported that seeds of *Ceanothus sanguineus* Pursh (Rhamnaceae), common name redstem ceanothus, effectively germinated when treated with hot water at 90 ºC or boiling water at 100 ºC. Moreover, seeds of *Ceanothus fendleri* A. Gray (Rhamnaceae), common name Fendler's ceanothus, exposed to 70 or 90 ºC for 10 minutes had greater germination (25 and 35%) than those exposed to no heat (12%) (Huffman, 2006).

Once physical dormancy is broken for double-dormant seeds, stratification can further help to increase seed germination. Schramm and Johnson (1981) found 70% germination when seeds of *Ceanothus americanus* L. (Rhamnaceae), common name New Jersey tea, were dipped in boiling water for 1.5 minutes and then stratified for 10 weeks,

but boiling water treatment alone or stratification treatment alone had low germination rates (28 to 30%). Stratification time to break physiological dormancy of seeds varies with plant species. In this experiment, long stratification periods of 2 or 3 months were particularly beneficial for both *C. velutinus* and *C. montanus*. Radwan and Crouch (1977) indicated that germination increased with an increase in stratification times for *C. sanguineus*. The highest germination percentage (84%) occurred after 4 months of stratification. Similarly, Rosner et al. (2003) stratified *C. montanus* seeds for 0, 30, and 60 days and observed that 60 days stratification was the most effective for seed germination.

Gibberellic acid is widely used to enhance the germination of seeds with physiological dormancy (Baskin and Baskin, 2014; Bonner, 2008). Endogenous levels of abscisic acid (ABA) (growth inhibitor) and GA_3 (growth promoter) are thought to be responsible for physiological dormancy of seeds (Hilharst and Karseen, 1992). Hence, the growth potential of embryos is enhanced when seeds are pretreated with GA_3 (Rascio et al., 1998). A good example is that germination of *C. velutinus* seeds scarified at 90 ºC, stratified for 1 month, and treated with 50 $mg \cdot L^{-1}$ of GA_3 was 25% greater than seeds with the same scarification and stratification, but without GA_3 treatment (Fig. 2-2). Seed germination rates for *Ziziphus joazeiro* (Rhamnaceae), common name Juazerio, were enhanced by GA_3 treatment at rates of 346, 692 or 1038 $mg \cdot L^{-1}$, and no significant difference occurred among GA_3 treatments (Araujo et al., 2009), which is similar to our results. All of these results indicate that GA_3 promotes seed germination (De Mello et

al., 2009; Fang et al., 2006). Gibberellic acid may be a cost-effective and time saving alternative to cold stratification to effectively enhance germination.

In addition, Kitchen and Meyer (1991) stated that cold stratification helps to increase the effects of GA_3 in breaking seed dormancy. Stratification along with application of hormones and (or) scarification have been used to break seed dormancy in many species (Duan et al., 2004; Macchia et al., 2001). For instance, the combined effect of cold treatment and GA_3 increased the germination percentage in Intermountain region penstemon species and *Morus nigra* L. (Moraceae), common name black mulberry (Kitchen and Meyer, 1991; Koyuncu, 2005). In our study, the combination of cold stratification and GA_3 treatment enhances the seed germination rate of both *C. velutinus* and *C. montanus*.

Conclusions

Both *C. velutinus* and *C. montanus* seeds exhibited dormancy. Scarification in hot water at 90 ºC together with stratification for 2 or 3 months effectively broke the dormancy of *C. velutinus* seeds. In addition, GA_3 also helped to increase *C. velutinus* seed germination. For *C. montanus* seeds, a combination of stratification for 2 months and GA_3 treatment at 50 $mg \cdot L^{-1}$ effectively broke seed dormancy.

Table 2-1. A summary of analysis of variance for effects of scarification, gibberellic acid, stratification, and their interactions on the seed germination of *Ceanothus velutinus*.

Source	Degree of freedom	F value	Pr > F
Temperature	2	1168.49	<0.0001
Gibberellic acid	3	9.62	<0.0001
Month	2	101.95	<0.0001
Temperature × Gibberellic acid	6	3.4	0.0028
Temperature × Month	4	30.75	<0.0001
Gibberellic acid × Month	6	7.55	<0.0001
Temperature × Gibberellic acid × Month	12	4.12	<0.0001

Table 2-2. A summary of analysis of variance for the effects of stratification and gibberellic acid, and their interactions on the seed germination of *Cercocarpus montanus*.

Source	Degree of freedom	F value	Pr > F
Gibberellic acid	3	3.04	0.0314
Month	2	83.47	<0.0001
Gibberellic acid × Month	6	4.89	0.0002

Fig. 2-1. Seeds of *Ceanothus velutinus* and *Cercocarpus montanus*. (A) *Ceanothus velutinus* and (B) *Cercocarpus montanus*.

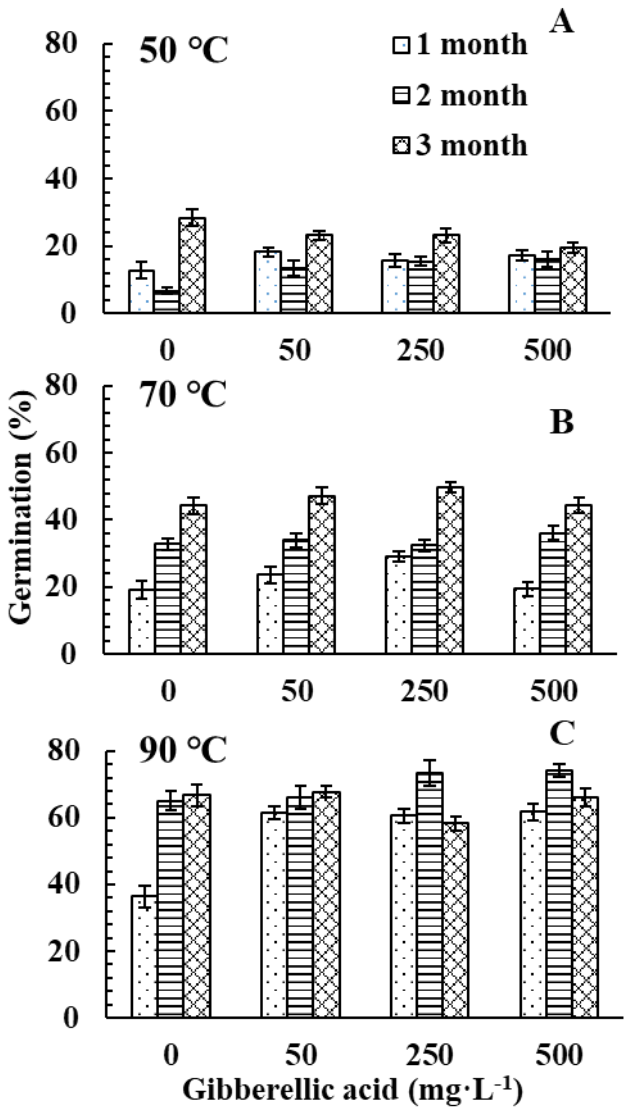

Fig. 2-2. The effects of scarification temperatures, stratification periods, and gibberellic acid concentrations on germination of *Ceanothus velutinus* seeds. Seeds were scarified at (A) 50, (B) 70, or (C) 90 ºC, treated with gibberellic acid at concentrations of 0, 50, 250, or 500 mg·L^{-1}, and subsequently stratified for 1, 2 or 3 months.

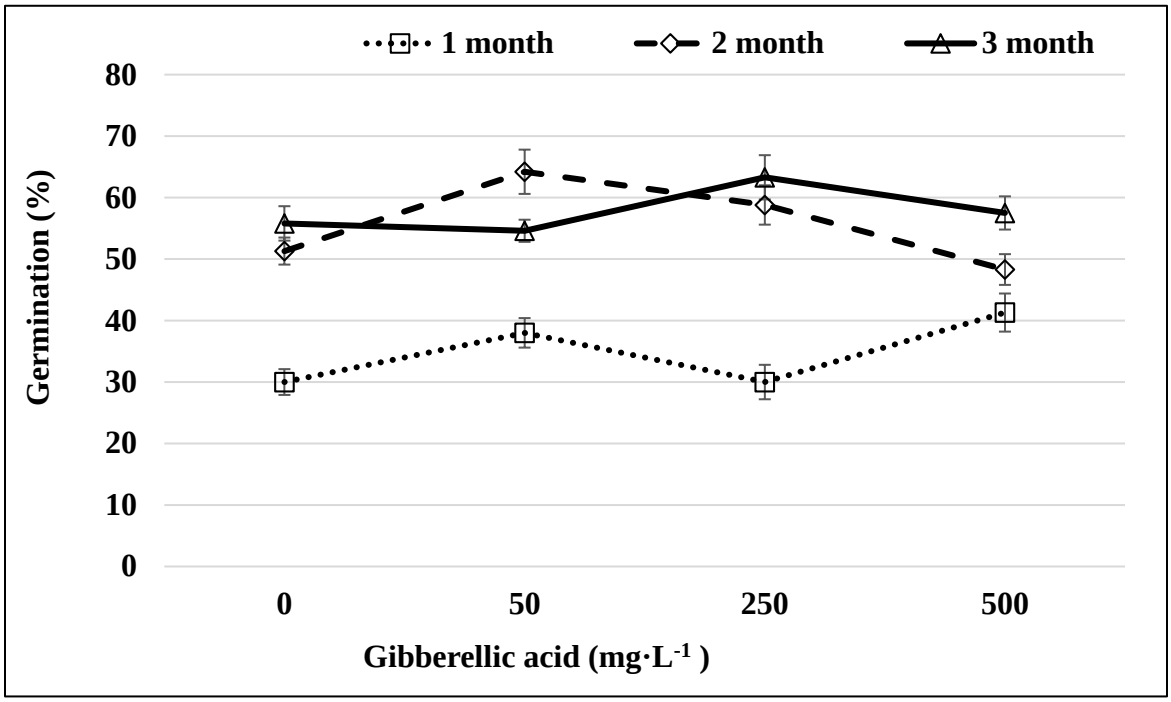

Fig. 2-3. Germination of *Cercocarpus montanus* seeds treated with different concentrations of gibberellic acid and different time of stratification. Seeds were treated with 0, 50, 250, or 500 mg·L^{-1} of gibberellic acid and stratified in a refrigerator for 1, 2 or 3 months.

CHAPTER III

CUTTING PROPAGATION OF *CEANOTHUS VELUTINUS* AND *CERCOCARPUS MONTANUS*

Abstract

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf mountain mahogany) are potential native species for use in water-efficient landscaping. Experiments were designed to develop effective cutting propagation protocols to allow both species to express their potential in landscapes. Terminal cuttings of *C. velutinus* were collected from May to Sept. 2019 from Tony Grove Lake area, Utah. Cuttings were dipped in 1,000/500 or 3,000/1,500 $mg \cdot L^{-1}$ indole-3-butyric acid (IBA)/1-naphthaleneacetic acid (NAA) as Dip'N Grow or talc-based rooting hormone Hormodin 1 (1,000 $mg \cdot L^{-1}$ IBA) or Hormodin 2 (3,000 $mg \cdot L^{-1}$ IBA) and stuck in a rooting medium consisting of perlite and peatmoss (4:1). Cuttings collected in July when treated with Hormodin 2 had 22% rooting which tended to be better than cuttings collected in other months and subsequently treated with other rooting hormones. Likewise, cuttings were collected from June to Aug. 2020 from the same area and similar results were observed. For cuttings taken in Aug. 2019 from the same area, terminal cuttings were good compared with stem cuttings but were not significantly different in terms of rooting percentage. In another experiment, rooting hormones were tested using cuttings collected from greenhouse-grown seedlings. Hormodin 2 tended to be the better rooting hormone.

Terminal cuttings of *C. montanus* 'Coy' collected in mid-July 2019 were treated with 2,000/1,000, 3,000/1,500, or 4,000/2,000 $mg \cdot L^{-1}$ IBA/ NAA as Dip'N Grow or

Hormodin 1, Hormodin 2, or a combination of both (Hormodin 1 + 1,000/500 mg·L^{-1} IBA/NAA as Dip‘N Grow or Hormodin 1 + 3,000/1,500 mg·L^{-1} IBA/NAA as Dip‘N Grow). Although there was no significance among hormone treatments, cuttings treated with Hormodin 2 had the highest rooting percentage (37%). A separate experiment was conducted for terminal and stem cuttings using 3,000/1,500 mg·L^{-1} IBA/NAA as Dip‘N Grow. Stem cuttings tended to be better for rooting. On 11 May, 2020, hardwood stem cuttings were collected for a wounding study. Cuttings rooted at 38.9%, 52.8% and 86.1% when lacking a wound, following perpendicular cuts, or when one side of the stem was scraped, respectively.

Introduction

Vegetative propagation is often superior to sexual propagation for commercial nursery production because uniform plants can be generated (Dole and Gibson, 2006; Hartmann et al., 2002). Cutting propagation utilizes a portion of plant leaf, stem, or root for propagating ornamental plants identical to the parent plant. Various factors such as seasonal timing of collection, tissues used for cuttings, type and concentration of plant rooting hormones (e.g. auxins), and type of wounding, play important roles in root formation on cuttings (Dole and Gibson, 2006).

Plants exhibit different seasonal physiological conditions, so the scheduling of cutting collection impacts the success of cutting propagation. If an ideal time for cutting collection is missed, a year of production may be lost. During the growing season, plants experience seasonal growth and development patterns with carbohydrate content, endogenous auxin, rooting co-factors, and/or rooting inhibitors changing over time. Each

of these play important roles in rooting capability (Melcher, 2016). *Amelanchier spicata* (dwarf serviceberry) cuttings had a higher rooting rate (88%) when collected in June than in July (Melcher, 2016). Similarly, 57% rooting was obtained on *Ceanothus americanus* (New Jersey tea) cuttings when softwood cuttings were collected in June (Cartabiano and Lubell, 2013).

The type of cuttings used for propagation also affects rooting (Hartmann et al., 2002). Softwood and semi-hardwood cuttings are most common utilized in the nursery industry. Softwood cuttings are preferred in many species since there is high chance of achieving post-rooting growth (Smalley et al., 1987). On the other hand, softwood cuttings are delicate, wilt easily, and successful application requires special care (Hartmann et al., 2002). Terminal and basal (subterminal) cuttings are two types of stem cuttings and woody species may respond uniquely to one or the other. Terminal cuttings with shoot apices are generally seen as advantageous for increasing rooting percentages (Malan, 1992).

Basal treatment of stem cuttings with synthetic auxins such as indole-3-butyric acid (IBA) can enhance rooting rate as well as increase the quantity of adventitious roots (Hartmann et al. 2002). However, the success of rooting depends on the type and concentration of auxin and plant genotype. Graves (2002) reported that *Rhamnus caroliniana* (Carolina buckthorn) showed the highest percentage of rooting when cuttings were dipped in 3,000 $mg \cdot L^{-1}$ IBA. Results showed a reduction in rooting percentage at 8,000 $mg \cdot L^{-1}$ IBA. High doses of auxin can lead to foliar senescence, chloroplast damage, destruction of membranes, necrosis and even plant death (Blythe et al., 2007).

Wounding may enhance adventitious rooting on stem cuttings (Hartmann et al., 2002). Some species can produce adventitious roots naturally while in others wounding increases cellular division near the vascular cambium and phloem, which promotes callus formation - followed by adventitious roots (Pijut et al., 2011). The adventitious root formation and root quality of *A. spicata* were enhanced by wounding (Melcher, 2016). *Arctostaphylos* species, which are difficult to root, showed beneficial effects following lateral wounding along the bottom (1 cm) of the cutting (Wisura, 1980).

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf or true mountain mahogany) flourish in low-water landscapes. However, few studies have been conducted on propagation of these two landscape worthy species. Seed propagation has been recently investigated for *C. velutinus* and *C. montanus*. However, off-type plants are produced from seed propagation (Dole and Gibson, 2006).

Though Rupp and Wheaton (2014) experimented with IBA as a rooting hormone for cutting propagation of *C. velutinus*, currently, there exists no peer-reviewed literature regarding cutting propagation of *C. velutinus*. Gucker (2006) reported that vegetative regeneration is possible from root crowns and rhizomes of *C. montanus*. However, there are still questions as to the correct time for cutting collection, type of auxins and their concentrations, and proper plant parts for propagating *C. velutinus* and *C. montanus*. The purpose of this study was to develop an efficient protocol to successfully propagate *C. velutinus* and *C. montanus* via stem cuttings. Factors evaluated during the experiment were: timing of cutting collection, types of cuttings, plant rooting hormones, and type of wounding.

Materials and Methods

Evaluation Series 1: *Ceanothus velutinus*

Timing of cutting collection. Terminal cuttings (≈15 cm) were collected in the middle of each month from May to Sept. 2019 from the Tony Grove Lake area, Utah (lat. 41°52'34"N, long. 111°34'21"W, elevation 2010 m). Nine separate clonal clumps were labeled for cutting collection. Cuttings were collected in the morning on 15 May, 13 June, 16 July, 16 Aug., or 20 Sept. 2019. Cuttings were immediately wrapped with moist paper towels, placed on ice in a cooler, transported to the USU campus, and kept in a walk-in cooler at 4 °C overnight.

Terminal cuttings were recut to 12-13 cm in length and stripped of bottom leaves leaving 5 leaves at the top (Fig. 3-1A). Wounds (3 or 4 perpendicular cuts to the wood around the base) were created using a sharp blade on both sides of the cuttings. Cuttings were quickly dipped in distilled water and subsequently treated with a talc-based rooting hormone Hormodin 2 (ai. 0.3%, 3,000 $mg \cdot L^{-1}$ IBA, OHP, Mainland, PA), Hormodin 1 (1,000 $mg \cdot L^{-1}$ IBA) or liquid-based rooting hormone at a concentration of 1,000 or 3,000 $mg \cdot L^{-1}$ IBA plus 500 or 1,500 $mg \cdot L^{-1}$ NAA in 25% ethanol as Dip‘N Grow (1% IBA, 0.5% 1-napthaleneacetic acid (NAA), Dip‘N Grow, Clackamas, OR). Afterwards, the cuttings were laid on paper towels for about 1 minute. Cuttings were implanted vertically into inserts (180 ml inserts, 8 cm depth, Landmark Plastic Corporation, Akron, OH) containing a moist medium of perlite (Expanded Perlite; Malad City, ID) and peat moss (100% Canadian Sphagnum peat moss, SunGro Horticulture, Agawam, MA) at a

volumetric ratio of 4:1. Cuttings were placed on a white-cloth-covered intermittent mist bench and supplied with bottom heat at 23 ºC using a heating mat (Propagation Mat, Grower's Nursery Supply, Salem, OR). Cuttings were misted at a frequency set to maintain conditions above 40 vapor pressure deficit (VPD) units using a Water Plus VPD mist controller (Phytotronics, Earth City, MO) in a greenhouse covered with 60% shade cloth (Fig.3-1B). Cuttings were drenched with Aliette® fungicide (80% fosetyl aluminium, 6.18% nonylphenol ethoxylate, 4.4% lignosulfonic acid, 0.16% crystalline quartz; Bayer CropScience, Research Triangle, NC) at a rate of 2.5 gram per gallon. Cuttings were evaluated at the end of 6 weeks.

In 2020, terminal cuttings (≈15 cm) were collected from June to Aug. from the same plants. Cuttings were collected in the morning on 15 June, 1 July, 16 July, 30 July, 15 Aug. 2020. Cuttings were processed and stuck in the rooting medium as described previously and only Hormodin 2 was used as a rooting hormone.

Stem and terminal cuttings. On 16 Aug. 2019, cuttings (≈30 cm) were collected from the Tony Grove Lake area as described above. Cuttings were divided into terminal cuttings (10-12 cm long) and subtended stem cuttings (10-12 cm long). Bottom leaves were removed leaving the top 3-5 leaves. Cuttings were subsequently processed, and same rooting hormones and rooting substrate were used as describe above. Cuttings were evaluated at the end of 8 weeks.

Plant growth regulator. Terminal cuttings from greenhouse seedlings (≈12-13 cm long) were stripped of bottom leaves leaving 4 leaves at the top. Cuttings were

subsequently processed, and identical rooting hormones and rooting substrate were used as described in the previous experiments. Cuttings were evaluated at the end of 8 weeks.

Evaluation Series 2: *Cercocarpus montanus*

Stem and terminal cuttings. On 11 July 2019, propagation experiments were conducted for *C. montanus* ‘Coy’ using cuttings collected from plants located in a landscape in Hyde Park, Utah (Fig. 3-2A). *Cercocarpus montanus* ‘Coy’ is an evergreen selection with smaller leaves that has been selected for the landscape use (Paudel et al., 2020). Healthy cuttings (≈24 cm long) were collected, wrapped in moist paper towels, and placed on ice in a cooler at 4 ºC until used. An experiment was performed to determine the best part of the stem for use in cutting propagation. The cuttings were cut into halves with the top part as terminal cuttings (10-12 cm) and the bottom portion as stem cuttings (10-12 cm). Terminal and stem cuttings were wounded as described for *C. velutinus* above and quick-dipped in a solution of 3,000 $mg \cdot L^{-1}$ IBA and 1,500 $mg \cdot L^{-1}$ NAA as Dip‘N Grow in 25% ethanol and stuck in a rooting substrate containing perlite and peatmoss (4:1). Cuttings were placed on the bench with intermittent mist system set to maintain 40 VPD units and supplied with bottom heat at 23 °C for eight weeks (Fig. 3-2B).

Plant growth regulator. The second experiment was performed to determine the best rooting hormone and the optimal concentration for cutting propagation. Terminal cuttings (12-14 cm) were prepared as aforementioned and treated with plant growth regulators as Dip‘N Grow in 25% ethanol and talc-based Hormodin (Table 3-1).

Wounding. On 11 May, 2020, hardwood stem cuttings, previous season growth (10-12 cm) were collected, wrapped in moist paper towels, and stored in a cooler at 4 ºC overnight. Three treatments were designed to determine the best way of wounding. Either cuttings were left unwounded, perpendicular cuts (3-4) to the wood around the base, or scraped at the base by removing the bark on one side. Hormodin 2 was used as a rooting hormone. Cuttings were placed on the bench with an intermittent mist system set to maintain 60 VPD units. Other protocols were followed as described above for *C. montanus*.

Experimental design and statistical analyses. The experiments were conducted using a completely randomized design. At harvest, callus formation and rooting on each of stem cuttings were recorded. Number of roots was counted, and the length of the longest root (cm) was also measured. An analysis of variance was conducted on all data. Callus formation and rooting on cuttings were treated as a binary data (0, 1). The nine separate clonal clumps for cutting collection were considered as a random variable. All statistical analyses were performed with PROC GLIMMIX or PROC MIXED procedures using a Statistical Analysis Software (SAS) university edition (SAS Institute, Cary, NC). Hierarchical cluster analyses were conducted using Ward method in JMP 13.2.1 (SAS Institute, Cary, NC) for plant growth regulator experiment with mean values of the percent rooted cuttings, number of roots per cuttings, and length of the longest root.

Results and Discussion

Evaluation Series 1: *Ceanothus velutinus*

Timing of cutting collection. Percent callus formation and rooting were significantly ($p < 0.0001$) influenced by the timing of cutting collection. Callus formation was the highest in August, followed by July, when compared with other months (Fig. 3-3A). Hormodin 2 treated cuttings tended to show greater callus percentage. Similarly, cuttings collected in July had a greater rooting percentage than those for other months (Fig. 3-3B). In addition, Hormodin 2 treated cuttings had greater percent rooting. Cuttings collected in July and treated with Hormodin 2 produced 22% rooting. There was no significant effect of the timing of cutting collection or rooting hormone on the number of roots and length of roots. Numerically, number of roots and length of roots increased from May to August and again decreased in September (Fig. 3-3C and D).

Similarly, *C. velutinus* cuttings collected in the middle of July 2020 had numerically greater callus (68.1%) and rooting percentage (23.6%) (Table 3-2).

These results indicated that *C. velutinus* cuttings collected in July rooted better than cuttings collected in other months from the wild at an elevation of 2010 m. Based on the physiological status of the cuttings, those collected on 15 May and 13 June were considered hardwood cuttings; those on 16 July and 16 Aug. were softwood to semi-hardwood cuttings, while those on 20 Sept. were semi-hardwood cuttings. Given that cuttings collected in May and June had lower callus or rooting rates, softwood and semi-hardwood cuttings may be most suitable for *C. velutinus* cutting propagation. Cartabiano and Lubell (2013) showed that *C. americanus* had a higher rooting percentage when softwood cuttings were collected in June. This contrast might be due to differences in species, elevation, location of parent plants, a different method of wounding. In our

study, *C. velutinus* plants in Logan Canyon usually start new growth in May, and the early season growth produced is of insufficient length for softwood cuttings by the middle of June. In addition, phenological growth phase affects the endogenous auxins and impacts rooting potential (Blakesley et al., 1991). As stems transit from young to mature phase, lignified tissues in the plants increase and can inhibit adventitious root formation (Melcher, 2016). In addition, it has been reported that plant genetic variation results in different rooting ability (Mabizela et al., 2017). However, in our study, there was no significance in rooting among cuttings that were taken from nine *C. velutinus* clumps.

Stem and terminal cuttings. Type of cuttings affected the callus percentage (p = 0.0001). There was no significance in the type of cuttings in terms of the rooting percentage, number of roots per cutting, and length of the longest root formed. Similarly, there was no significant effect of the rooting hormone for callus and root formation. In addition, no significant interaction was observed between type of cuttings and rooting hormone. Numerically, terminal cuttings had greater callus and root formation with more roots and longer roots when compared with stem cuttings (Table 3-3 and Fig. 3-4). In our study, terminal cuttings were usually younger and softer than stem cuttings. Similarly, Dole and Gibson highlighted that young stem tissue rooted faster than old stem tissue in some species (Dole and Gibson, 2006). This can be partly explained by the fact that endogenous auxin is produced in actively growing shoots including apical meristems, which plays a crucial role in the development of root primordia (Haissig, 1970; MacAdam, 2009).

Plant growth regulator. There was no significance in the type of rooting hormones applied in terms of the percent rooting, number of roots per cutting and length of the longest root formed (Table 3-4). Numerically, cuttings treated with Hormodin 2 tended to have greater rooting percentage (37.0%) and more roots (11.2) when compared with other hormones (Table 3-4). Similar results were observed previously in that Hormodin 2 produced more and longer roots in the *C. velutinus* cuttings (US Department of Agriculture, 2018). Conversely, it has been reported that high doses of auxin can lead to foliar senescence, chloroplast damage, destruction of membranes, and plant death (Blythe et al., 2007). In our study, we also observed leaf drooping while propagating the *C. velutinus* cuttings which may be due to high concentration of auxin. Further research is necessary to find out the right concentration of auxin for root formation in *C. velutinus* cuttings. Although not data-related, we observed that talc-based hormone was convenient to use and resulted in less stem rot problems.

Evaluation Series 2: *Cercocarpus montanus*

Stem and terminal cuttings. Cercocarpus montanus cuttings formed callus about 5 weeks after cuttings were treated and placed on the mist bench. On average, 50.8% and 23.8% of stem and terminal cuttings, respectively, formed callus (Table 3-5 and Fig. 3-5A and B), whereas 11.1% and 4.8% of the stem and terminal cuttings, respectively, developed roots. The number of roots and length of the longest root derived from stem cuttings were double that from terminal cuttings. Higher rooting response from stem cuttings may be due to higher levels of carbohydrate content. Dole and Gibson (2006) reported that high carbohydrate to nitrogen ratio favors adventitious root formation. In

addition, stem cuttings used in this study were semi-hardwood, while terminal cuttings were softwood. This result indicates that semi-hardwood cuttings are appropriate materials for propagating *C. montanus*.

Plant growth regulator. Hormodin 2 produced the highest rooting percentage at 37% and Hormodin 1 plus 3,000 $mg \cdot L^{-1}$ IBA and 1,500 $mg \cdot L^{-1}$ NAA had the lowest rooting percentage at 16.7% (Table 3-6 and Fig. 3-5C and D). Hormodin 2 treated cuttings also produced the highest number of roots (14.5) and the longest root (2.8 cm) when compared with cuttings treated with other rooting hormones. There was no significance for rooting percent, the number of roots formed, and the length of the longest root among rooting hormones. Based on a hierarchical cluster analysis, Hormodin 2 was a better plant growth regulator for root formation of *C. montanus* cuttings. Note: talc-based rooting hormone is easier to handle and use as compared with liquid-based rooting hormone products.

Overall rooting percentage for *C. montanus* was lower than 50%. Similarly, Rosner et al. (2000) reported less than 1% of rooting in *C. montanus* when cuttings of current season growth were used. Further research on the cutting propagation of *C. montanus* is necessary to produce acceptable propagation protocol for commercial application.

Wounding. Cercocarpus montanus cuttings formed callus about 4-5 weeks after cuttings were placed on the mist bench. Wounding significantly increased percent callus formation ($p = 0.003$), percent rooting ($p = 0.0009$), number of roots formed ($p = 0.006$) and length of the longest root ($p = 0.04$). Cuttings rooted at 38.9%, 52.8% and 86.1%,

respectively, when not wounded, cuts were made, or one side scraping was done (Table 3-7 and Fig. 3-6). Hartmann et al. (2002) reported that wounding positively affected root production on stem cuttings. In some hard-to-root species, adventitious root quantity and uniformity also increase when cuttings were wounded and treated with an auxin (Alsup et al., 2003, Griffin and Bassuk, 1996). Wounding increased cellular division near the vascular cambium and phloem, which promoted callus formation followed by adventitious roots (Pijut et al., 2011). The high rooting percent in this experiment indicates that timing of cutting collection may affect the rooting. Similarly, semi-hardwood and hardwood stem cuttings may have higher tendency toward root formation in *C. montanus*.

Table 3-1. Different rooting hormones and combinations for cutting propagation of *Cercocarpus montanus* ‘Coy’.

Treatment	Dip‘N Grow[z]	Hormodin
1	2,000 mg·L^{-1} IBA and 1,000 mg·L^{-1} NAA	-
2	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	-
3	4,000 mg·L^{-1} IBA and 2,000 mg·L^{-1} NAA	-
4	1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	1,000 mg·L^{-1} IBA as Hormodin 1
5	-	3,000 mg·L^{-1} IBA as Hormodin 2
6	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	1,000 mg·L^{-1} IBA as Hormodin 1

[z] IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

Table 3-2. Callus and root formation of terminal cuttings of *Ceanothus velutinus* collected in 2020 and treated with Hormodin 2[z].

Time	Callus (%)	Rooted cuttings (%)	No. roots per cutting	Length of longest root (cm)
15-June	40.3 b[y]	6.9 ab	5.2 a	4.4 a
1-July	38.9 b	13.9 ab	3.5 a	2.0 a
16-July	68.1 a	23.6 a	6.3 a	2.7 a
30-July	34.7 b	6.9 ab	3.0 a	2.0 a
15-August	38.9 b	5.6 b	2.5 a	1.3 a

[z] Hormodin 2: 3,000 mg·L^{-1} indole-3-butyric acid.

[y] Same letters within a column denote no significance among the time for cuttings collection as measured by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 3-3. Callus and root formation of stem and terminal cuttings of *Ceanothus velutinus* treated with plant growth regulators as liquid-based Dip'N Grow or talc-based Hormodin. Treatments were applied on 16 Aug., 2019.

Type of cuttings	Rooting hormone[z]	Callus (%)	Rooted cuttings (%)	No. of roots per cutting	Length of longest root (cm)
Stem	1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	37.4 ab[y]	0 a	0 a	0 a
	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	29.6 b	7.4 a	6.5 a	12 a
	1,000 mg·L^{-1} IBA as Hormodin 1	37 ab	0 a	0 a	0 a
	3,000 mg·L^{-1} IBA as Hormodin 2	55.5 ab	7.4 a	2.5 a	1.5 a
Terminal	1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	77.8 a	22.2 a	7.2 a	5.5 a
	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	63 ab	25.9 a	24.1 a	5.8 a
	1,000 mg·L^{-1} IBA as Hormodin 1	66.7 ab	3.7 a	2.5 a	1 a
	3,000 mg·L^{-1} IBA as Hormodin 2	63 ab	18.5 a	8.4 a	3.9 a

[z] IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

[y] Same letters within a column denote no significance among cutting-and-auxin treatments by Tukey's method for multiplicity at α = 0.05.

Table 3-4. Callus and root formation of terminal cuttings of *Ceanothus velutinus* treated with plant growth regulators formulated as liquid-based Dip‘N Grow or talc-based Hormodin. Cuttings were taken from greenhouse grown seedlings. Treatments were applied on 7 Aug., 2019.

Rooting hormone[z]	Rooted cuttings (%)	No. roots per cutting	Length of longest root (cm)
1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	22.2 a[y]	8.2 a	5.3 a
3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	25.9 a	5.9 a	3.1 a
1,000 mg·L^{-1} IBA as Hormodin 1	25.9 a	6.4 a	3.9 a
3,000 mg·L^{-1} IBA as Hormodin 2	37.0 a	11.2 a	5.2 a

[z] IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

[y] Same letters within a column denote no significance among auxin treatments as measured by Tukey’s method for multiplicity at $\alpha = 0.05$.

Table 3-5. Callus and root formation of stem and terminal cuttings of *Cercocarpus montanus* 'Coy'. Cuttings were treated with 3,000 mg·L^{-1} indole-3-butyric acid (IBA) and 1,500 mg·L^{-1} 1-naphthaleneacetic acid (NAA) as Dip'N Grow. This experiment was carried out on 11 July 2019.

Type of cuttings	Callus (%)	Rooted cuttings (%)	No. roots per cutting	Length of the longest root (cm)
Stem	50.8 a[z]	11.1 a	6.3 a	2.5 a
Terminal	23.8 b	4.8 a	3.0 a	1.1 b

[z] Values within a column accompanied by the same letters denote lack of significance between stem and terminal cutting by Tukey's method for multiplicity computed at α = 0.05.

Table 3-6. Root formation of *Cercocarpus montanus* 'Coy' terminal cuttings treated with liquid-based Dip'N Grow or talc-based Hormodin or a combination of both as treatments. This experiment was carried out on 11 July 2019.

Treatment	Dip'N Grow[z]	Hormodin	Rooted cuttings (%)	No. roots per cutting	Length of longest root (cm)
1	2,000 mg·L^{-1} IBA and 1,000 mg·L^{-1} NAA	-	24.1 a[y]	5.5 b	1.5 b
2	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	-	20.4 a	9.0 ab	2.0 ab
3	4,000 mg·L^{-1} IBA and 2,000 mg·L^{-1} NAA	-	25.9 a	8.3 ab	2.1 ab
4	1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	1,000 mg·L^{-1} IBA as Hormodin 1	18.5 a	9.9 ab	2.5 ab
5	-	3,000 mg·L^{-1} IBA as Hormodin 2	37.0 a	14.5 a	2.8 a
6	3,000 mg·L^{-1} IBA and 1,500 mg·L^{-1} NAA	1,000 mg·L^{-1} IBA as Hormodin 1	16.7 a	10.2 ab	2.6 ab

[z] IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

[y] Same letters within a column denote no significance among auxin treatments by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 3-7. Callus and root formation of stem cuttings without wounding, with cuts, and scraped on one side for *Cercocarpus montanus* 'Coy'. Cuttings were treated with Hormodin 2 (3,000 mg·L^{-1} indole-3-butyric acid).

Type of cuttings	Callus (%)	Rooted cuttings (%)	No. roots per cutting	Length of longest root (cm)
Control	55.6 b[z]	38.9 b	5 b	2.6 b
Cuts	80.6 ab	52.8 b	9.4 ab	4.4 ab
Scraping	91.7 a	86.1 a	11.1 a	4.4 a

[z] Same letters within a column denote lack of significance among wounding treatments as computed using Tukey's method for multiplicity at $\alpha = 0.05$.

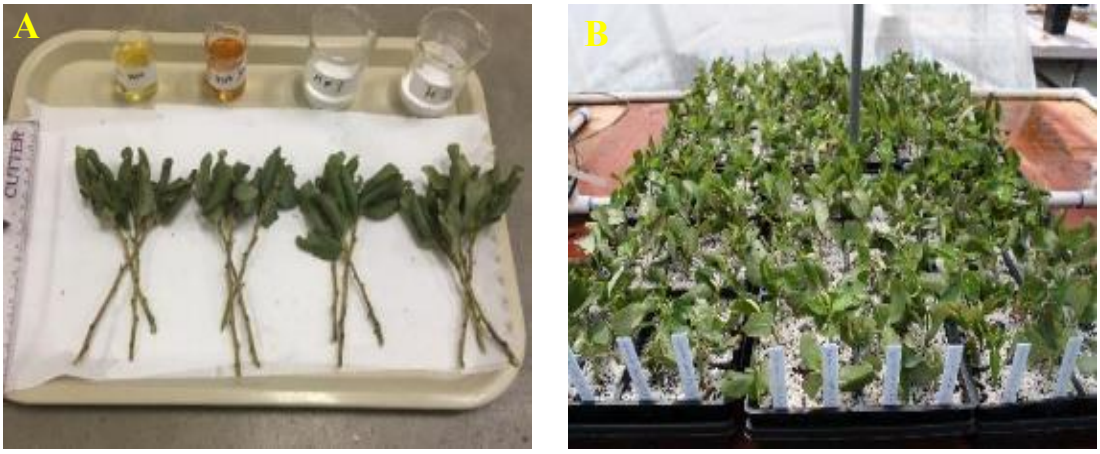

Fig. 3-1. *Ceanothus velutinus* cuttings treatments. (A) Cuttings treated with different rooting hormones and (B) placed on a mist bench.

Fig. 3-2. *Cercocarpus montanus* ‘Coy’ plants and cuttings. (A) Plants in the landscape and (B) cuttings on a mist bench.

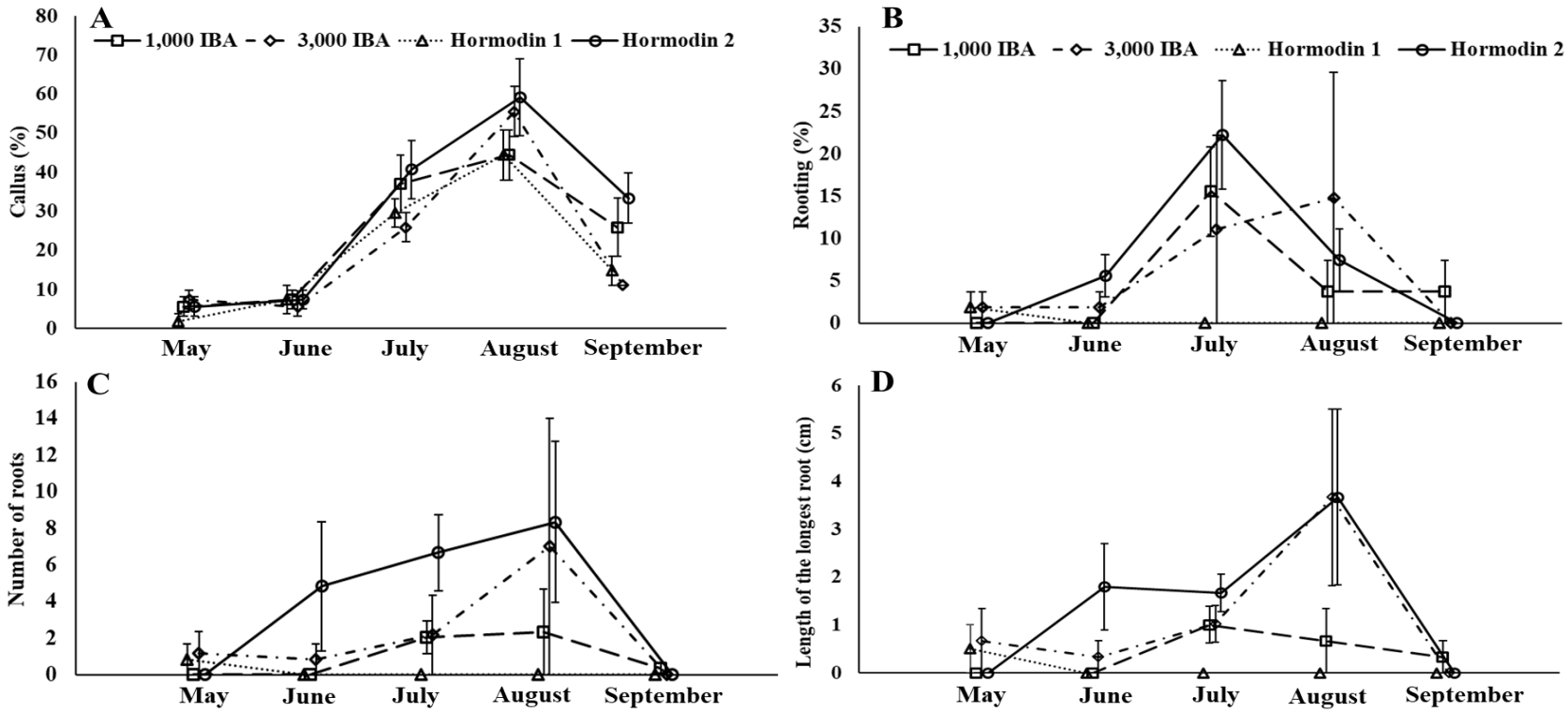

Fig. 3-3. Callus and root formation of *Ceanothus velutinus* terminal cuttings collected in May, June, July, Aug. and Sept. 2019 and treated with different rooting hormones. (A) Callus percentage, (B) rooting percentage, (C) number of roots, and (D) length of the longest root. Missing standard error bars represent no rooting.

IBA: indole-3-butyric acid; Hormodin 1: 1,000 mg·L^{-1} IBA; Hormodin 2: 3,000 mg·L^{-1} IBA

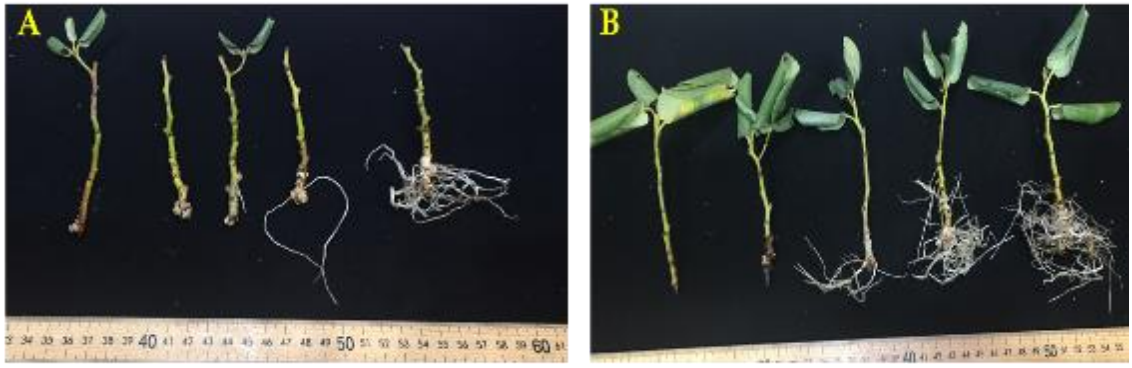

Fig. 3-4. Representative rooted *Ceanothus velutinus* cuttings. (A) Stem cuttings and (B) terminal cuttings.

Fig. 3-5. Representative photos of rooting on *Cercocarpus montanus* 'Coy' cuttings. (A) Rooted stem cuttings, and (B) terminal cuttings [both were treated with 3,000 mg·L^{-1} indole-3-butyric acid (IBA) and 1,500 mg·L^{-1} 1-naphthaleneacetic acid (NAA) (Dip'N Grow, 1% IBA and 0.5% NAA)], (C) rooted terminal cuttings [treated with Hormodin 2 (3,000 mg·L^{-1} IBA)] and (D) representative densely rooted cutting following treatment with Hormodin 2.

Fig. 3-6. *Cercocarpus montanus* ‘Coy’ rooted cuttings with or without wounding. Rooted cuttings: (A) without wounding, (B) with cuts on both stem sides, (C) scraping the bark on one stem side, and (D) closer view of rooted cutting with side scraped.

CHAPTER IV

MICROPROPAGATION OF *CEANOTHUS VELUTINUS* AND *CERCOCARPUS MONTANUS*

Abstract

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf or true mountain mahogany) are native species of Western United States. They have important role in soil building by nitrogen fixation. In addition, they have potential for water-efficient landscaping. However, research-based information on its propagation is limited. In this study, nodal segments of *C. velutinus* containing one or two axillary buds from greenhouse-grown seedlings were disinfected using 10% bleach and cultured on Gamborg's B-5 (B5), Murashige and Skoog (MS), Quoirin and Lepoivre (QL), Schenk and Hilderbrandt (SH), or Woody Plant Medium (WPM) supplemented with 1 $mg \cdot L^{-1}$ benzylaminopurine (BA), 30 $g \cdot L^{-1}$ sucrose, and 8 $g \cdot L^{-1}$ agar. Explants were incubated at 25 °C with a 16-hour photoperiod for 1 month. Based on cluster analyses, MS + 1 $mg \cdot L^{-1}$ BA and B5 + 1 $mg \cdot L^{-1}$ BA medium were better than other medium. Of the culture media evaluated, MS + 1 $mg \cdot L^{-1}$ BA medium produced more shoots (1.2) and leaves (4.2) and the longest shoot (1 cm). Application of rooting hormone [Hormodin 1 (1,000 $mg \cdot L^{-1}$ IBA), Hormodin 2, or Dip'N Grow at a concentration of 1,000 $mg \cdot L^{-1}$ IBA plus 500 $mg \cdot L^{-1}$ 1-napthaleneacetic acid (NAA)] enhanced the rooting percentage for *ex vitro* rooting of *C. velutinus*. For *C. montanus*, nodal segments containing one or two axillary buds were disinfected and cultured on MS medium. Microshoots induced after 1 month were subcultured for shoot proliferation on MS or WPM containing BA, kinetin (kin) or

zeatin (ZT) at 1 mg·L^{-1} for 1 month. Based on cluster analyses, MS + 1 mg·L^{-1} BA medium was better than other medium. On average, one new microshoot and four new leaves and longer microshoots (1.6) were produced on MS + 1 mg·L^{-1} BA.

Introduction

Micropropagation is one method for producing many identical plants from one original plant using cultured small pieces of plant shoots, roots, or reproductive structure. The success of micropropagation depends on explant type, culture media and growth regulators (Davies et al., 2018). Nodal stem cuttings or pieces from other plant parts like leaf, petiole, or root are used for micropropagation. Culture medium consisting agar, inorganic nutrient elements, sucrose, and vitamin supplements is used to grow tissue, and efficacious components of culture medium vary with plant species. As a rule of thumb, cytokinins are believed to enhance shoot growth and auxins in root growth.

Utah native plants have been successfully propagated by micropropagation. Pruski et al. (1990) successfully micropropagated four cultivars of *Amelanchier alnifolia (*saskatoon serviceberry) and found that shoot-tip explants harvested from actively growing plants worked better than dormant buds. Combination of benzylaminopurine (BA) and gibberellin (GA) as foliar spray was considered important in breaking dormancy and formation of axillary shoots in *A. alnifolia* plantlets. In addition, within the Pruski et al (1990) study, use of indole-3-acetic acid (IAA)/1-naphthaleneacetic acid (NAA) (2.8/1.1 µM) (≈0.5/0.2 mg·L^{-1}) mixture induced the best rooting response.

Propagation of *Populus tremuloides* (aspen) has been successful using micropropagation (Haapala et al., 2004). Moreover, nodal cuttings of *Acer grandidentatum* (bigtooth maple) were used for tissue culture with Driver-Kuniyuki Walnut (DKW) being the best medium for shoot multiplication and IAA being the best hormone for rooting microshoots (Bowen-O'Connor et al., 2007).

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf mountain mahogany) are two Utah native plants with potential for use in water-conserving landscapes. Very limited propagation research has been conducted on *C. velutinus* and *C. montanus*. *Ceanothus velutinus* is difficult to grow from seeds. No peer-reviewed literature is available related to tissue culture of *C. velutinus* and *C. montanus*. They are difficult to propagate by cuttings, and micropropgation may be another alternative for efficient propagation.

Materials and Methods

Preparation of explants. Healthy *C. velutinus* seedlings from plants growing in a research greenhouse and *C. montanus* plants from a landscape at Utah State University (Logan, UT) were chosen as subjects for micropropagation. Nodal cuttings from newly developed shoots were harvested and washed in running tap water for 15-30 minutes (Fig. 4-1). Cuttings were disinfected in 70% ethanol for 1 minute and rinsed 2-3 times in autoclaved distilled water. Cuttings were then soaked in 10% ultra-bleach (6% sodium hypochlorite, Sam's west, Bentonville, AR) plus five drops of Tween® 20 (Fisher

scientific, Fair Lawn, NJ) per 150 ml for 10 minutes and rinsed 3-5 times with autoclaved distilled water.

Culture conditions. All the apparatus involved in tissue culture procedures, such as scalpels, scissors and forceps were maintained as sterile to prevent contamination. UV light was switched on for 30 minutes to disinfect the laminar airflow hood. Surfaces were wiped with 70% ethanol before use. Aseptic cultures were maintained in a culture room with temperature being maintained at 25 °C and a 16-hour photoperiod under cool white fluorescent lamps (184.5 ± 9.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Fluorescent light intensity was measured using a Quantum Light Meter (MQ-100, Apogee Instrument, Logan, UT).

Establishment (Stage I). For *C. velutinus*, Murashige and Skoog (MS; Murashige and Skoog, 1962), Woody Plant Medium (WPM; Lloyd and McCown, 1980), Gamborg's B-5 (B5; Gamborg et al., 1968), Schenk and Hilderbrandt (SH; Schenk and Hildebrandt, 1972), or Quoirin and Lepoivre (QL; Quoirin and Lepoivre, 1977) were used as basal medium supplemented with 1 $mg \cdot L^{-1}$ benzylaminopurine (BA) as plant growth regulator, 30 $g \cdot L^{-1}$ sucrose as energy source, and 9 $g \cdot L^{-1}$ agar (Agar; Caisson laboratories, Smithfield, UT) as solidify agent. The pH of the medium was adjusted to 5.7 before being dispensed into 60-ml test tubes with 8 ml of growth medium in each tube.

For establishment of *C. montanus*, MS medium was supplemented with 30 $g \cdot L^{-1}$ sucrose, 9 $g \cdot L^{-1}$ agar, and 1 $mg \cdot L^{-1}$ BA. The disinfected nodal cuttings were dissected into segments containing 1-2 buds (Fig. 4-2) and planted proximal end down on the medium, and the culture tubes were sealed with parafilm (American National Can™, Menasha, WI). After 1 month, number of explants that survived without contamination

were recorded, and healthy shoots were used for multiplication stage studies. Number of shoots formed, and length of the longest shoot were recorded for *C. velutinus* as a way to determine the best medium for establishment.

Multiplication (Stage II). Microshoots of *C. velutinus* from stage I were transferred to MS medium supplemented with cytokinins for multiplication (Fig. 4-3A). Microshoots turned brown even after repeated subculture and dried over time.

Similary, microshoots of *C. montanus* from stage I were transferred to MS or WPM medium supplemented with cytokinins [BA, Zeatin (ZT), or Kinetin (kin)] at a concentration of 1 $mg \cdot L^{-1}$ and cultured for 1 month (Fig. 4-3B). Number of microshoots, number of leaves per microshoot, and the length of the longest microshoot were recorded.

An additional study on *C. montanus* to refine best conditions for shoot proliferation was performed using different GA_3 concentrations. MS as a base was supplemented with 1 $mg \cdot L^{-1}$ BA and 0.1 $mg \cdot L^{-1}$ NAA as well as 0, 0.5, or 1 $mg \cdot L^{-1}$ GA_3. Increase in the number of shoots, leaves and length of the longest shoot were recorded after 3 weeks.

Rooting (Stage III). Microshoots of *C. velutinus* and *C. montanus* were grown on full, half, or quarter-strength MS medium supplemented with 1 $mg \cdot L^{-1}$ or 2 $mg \cdot L^{-1}$ indole-3-butyric acid (IBA) for 1 month. Rooting was not observed *in vitro* on MS medium with IBA. Microshoots turned brown and dried over time. A study was performed on *ex vitro* rooting of *C. velutinus*. Microshoots were extracted from the tubes (Fig. 4-4A) and washed in tap water. Microshoots were treated with distilled water (without rooting hormone), talc-based rooting hormone Hormodin 1 (ai. 0.1%, 1,000

mg·L^{-1} IBA, OHP, Mainland, PA) or Hormodin 2 (3,000 mg·L^{-1} IBA) or liquid-based rooting hormone Dip‘N Grow (1% IBA, 0.5% 1-napthaleneacetic acid (NAA), Dip‘N Grow, Clackamas, OR) at a concentration of 1,000 mg·L^{-1} IBA plus 500 mg·L^{-1} NAA in 25% ethanol. Afterwards, the microshoots were laid on paper towels for about 1 minute and inserted vertically into inserts (180 ml, 8 cm depth, Landmark Plastic Corporation, Akron, OH) containing a moist medium of perlite (Expanded Perlite; Malad City, ID) and peat moss (100% Canadian Sphagnum peat moss, SunGro Horticulture, Agawam, MA) at a volumetric ratio of 4:1. Microshoots were placed in a greenhouse on a white-cloth-covered intermittent mist bench with bottom heat at 23 ^{o}C provided using a heating mat (Propagation mat, Grower’s Nursery Supply, Salem, OR) and a misting system set to maintain 60 Vapor pressure deficit (VPD) units using a Water Plus VPD mist controller (Phytotronics, Earth City, MO) (Fig. 4-4B).

Experimental design and data analyses. All experiments were conducted using a complete randomized design (CRD). Analysis of variance was calculated for all data. Rooting on microshoots were considered as a binary data (0, 1). Statistical analyses were performed with PROC GLIMMIX or PROC MIXED procedures using a Statistical Analysis Software (SAS) university edition (SAS University Edition, Cary, NC). Hierarchical cluster analyses were conducted in JMP 13.2.1 (SAS Institute, Cary, NC) for establishment and multiplication experiment with mean values of the number of shoots per node, length of the longest shoot, and number of leaves formed.

Results and Discussion

Evaluation Series 1: *Ceanothus velutinus*

Establishment. Within 3 to 4 weeks, microshoots of *C. velutinus* formed from cultured nodes. About 50% of the explants were free of contamination (data not shown). Contamination observed was primarily bacterial. *Ceanothus velutinus* is an actinorhizal plant (Binkley et al., 1982), and actinobacteria may exist in their tissues. Basal medium had a significant effect on the formation of shoots ($p < 0.002$). MS medium supplemented with 1 mg·L^{-1} BA produced the most shoots, about 1.2 per node (Table 4-1; Fig. 4-5). Shoots of at least 1 cm in length were formed on both MS and B5 medium supplemented with 1 mg·L^{-1} BA. More leaves were observed on MS and B5 medium when compared with WPM medium.

Protocols for new shoot growth and development vary among species, and specific basal salts and plant growth regulator combinations are necessary in order to create an efficacious procedure (Mackay et al., 1996; Rounsaville and Ranney, 2010). Based on these results, MS medium with 1 mg·L^{-1} BA proved to be the best of the basal medium evaluated for establishment or shoot initiation of *C. velutinus*. The MS salt formula is commonly used for plant regeneration via tissue culture (Davies et al., 2018). Single-node stem explants of *Epilobium canum* ssp. *garrettii* (Garrett's firechalice), although an herbaceous species, similarly produced more axillary shoots on MS medium when compared with WPM medium (Alosaimi et al., 2018).

Ex Vitro rooting. After 7 weeks in inserts with bottom heat, callus formed around the stems, and roots were observed on microshoots. Application of hormone enhanced the

rooting percentage ($p = 0.04$). Plantlets rooted at 11.1%, 50%, 55.6%, and 61.1% microshoots when dipped in distilled water as no hormone treatment, 1,000 $mg \cdot L^{-1}$ IBA and 500 $mg \cdot L^{-1}$ NAA, Hormodin 1, and Hormodin 2, respectively (Table. 4-2; Fig. 4-6). Number of roots and the length of the longest root formed lacked statistical significance among rooting hormones. For *ex vitro* rooting of *C. velutinus*, application of an auxin like IBA may be necessary. In addition to providing an effective alternative methodology to *in vitro* rooting, the *ex vitro* rooting procedure provided good transition to the environment and saved labor and time needed for micropropagation of this species.

Evaluation Series 2: *Cercocarpus montanus*

Establishment. Within three to four weeks microshoots of *C. montanus* formed from the explant nodes. About 75% of *C. montanus* explants were free of contamination (data not shown). Both bacterial and fungus contamination was observed. Bacterial contamination may have been due to actinorhizal nature of *C. montanus* (Paschke, 1997).

Multiplication. Formation of microshoots and increase in shoot length were significantly better on MS medium plus 1.0 $mg \cdot L^{-1}$ BA ($p = 0.01$ and $p = 0.02$, respectively). One new shoot and almost 4 new leaves formed on MS medium plus 1.0 $mg \cdot L^{-1}$ BA (Table 4-3). The length of the longest shoot was 1.6 cm. On WPM medium supplemented with 1.0 $mg \cdot L^{-1}$ BA, no multiple shoots formed, and leaf drop was common. MS supplemented with kin or ZT and WPM supplemented with kin or ZT had similar responses. MS medium supplemented with 1.0 $mg \cdot L^{-1}$ BA may be the best medium for shoot multiplication of *C. montanus*. In similar fashion, other researchers

observed more adventitious shoots produced on leaf, petiole or root explants of *Epilobium angustifolium* (fireweed) with BA supplemented medium compared with kin supplemented medium (Turker et al., 2008).

In order to increase the number of shoots the basal medium with MS plus BA was supplemented with NAA and GA_3. Higher concentrations of GA_3 increased formation of new leaves ($p = 0.02$) and shoots ($p = 0.02$) (Table 4-4; Fig 4-7). Approximately 3.7 new leaves and 1.1 new shoots formed at a concentration of 1.0 $mg \cdot L^{-1}$ GA_3 in three weeks. Gibberellic acid has ability to break the dormancy, promote bud growth, and help stem elongation (Pierik, 1997). The elongated shoots can be then further divided and serve as mother stock culture for multiplication phase.

Table 4-1. Establishment of *Ceanothus velutinus* cultured on different media[z].

Medium[y]	Cytokinin (1 mg·L^{-1})	Shoots (no.) per node	Length of the longest shoot (cm)	Leaves (no.)
B5	BA	1.0 b[x]	1.0 a	4.2 a
MS	BA	1.2 a	1.0 a	4.2 a
QL	BA	1.0 b	0.9 a	3.7 ab
SH	BA	1.0 b	0.9 a	3.9 ab
WPM	BA	1.0 b	0.8 a	3.5 b

[z] This experiment was repeated five times (n = 5, subsample = 10).

[y] Benzylaminopurine (BA), Gamborg's B-5 (B5), Murashige and Skoog (MS), Quoirin and Lepoivre (QL), Schenk and Hilderbrandt (SH), and Woody Plant Medium (WPM).

[x] Same letters within a column denote no significance among media as indicated by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 4-2. *Ex vitro* root formation for microshoots of *Ceanothus velutinus* treated with plant growth regulators applied as liquid-based Dip'N Grow or talc-based Hormodin. Cuttings were dipped in the distilled water as a treatment without rooting hormone. This experiment was carried out on 18 June 2020.

Treatments[z]	Rooted microshoots (%)	Roots (no.) per microshoot	Length of the longest root (cm)
Distilled water	11.1 b[y]	2 a	1 a
1,000 mg·L^{-1} IBA and 500 mg·L^{-1} NAA	50.0 ab	3.7 a	2.3 a
1,000 mg·L^{-1} IBA as Hormodin 1	55.6 ab	4.6 a	3.5 a
3,000 mg·L^{-1} IBA as Hormodin 2	61.1 a	3.7 a	1.9 a

[z] IBA: indole-3-butyric acid; NAA: 1-naphthaleneacetic acid.

[y] Same letters within a column denote no significance among auxin treatments indicated by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 4-3. Shoot proliferation of *Cercocarpus montanus* cultured on two different basal media and three different cytokinins[z].

Medium[y]	Cytokinin (1 mg·L^{-1})	New shoots (no.)	Length of the longest shoot (cm)	Leaves (no.)	Growth Index[w]
MS	BA	0.9 a [x]	1.6 a	3.8 a	11.6 a
MS	kin	0.3 ab	0.7 b	0.5 a	0.5 a
MS	ZT	0.3 ab	1.0 ab	3.3 a	4.3 a
WPM	BA	0.0 b	0.5 b	0.0 a	0.0 a
WPM	kin	0.4 ab	1.0 ab	3.0 a	4.2 a
WPM	ZT	0.3 ab	0.9 ab	2.1 a	2.5 a

[z] This experiment was repeated three times (n =3, subsample =10).

[y] Benzylaminopurine (BA), kinetin (kin), Murashige and Skoog (MS), Woody Plant Medium (WPM), and zeatin (ZT).

[x] Same letters within a column denote no significance among multiplication media as indicated by Tukey's method for multiplicity at $\alpha = 0.05$.

[w] Growth index = shoot number × shoot length × leaf number

Table 4-4. Shoot proliferation of *Cercocarpus montanus* cultured on Murashige and Skoog (MS) medium supplemented with 1 mg·L^{-1} BA, 0.1 mg·L^{-1} NAA and various concentrations of GA_3[z].

GA_3 (mg·L^{-1})	Increment in		
	Leaves (no.)	Length of shoot (cm)	New shoots (no.)
0	0.4 b[y]	0.2 a	0.4 b
0.5	0.3 b	0.6 a	0.4 ab
1	3.7 a	0.5 a	1.1 a

[z] This experiment was repeated twice (n=2, subsample =6).

Benzylaminopurine (BA), gibberellic acid (GA_3), and 1-naphthaleneacetic acid (NAA).

[y] Same letters within a column denote no significance among treatments as indicated by Tukey's method for multiplicity at $\alpha = 0.05$.

Fig. 4-1. Preparation of explants for micropropagation. (A) *Ceanothus velutinus* in a USU research greenhouse, (B) *Cercocarpus montanus* in USU landscape, (C) washing of cuttings in running tap water, and (D) disinfection of cuttings in a laminar flow hood using hypochlorite and Tween® 20.

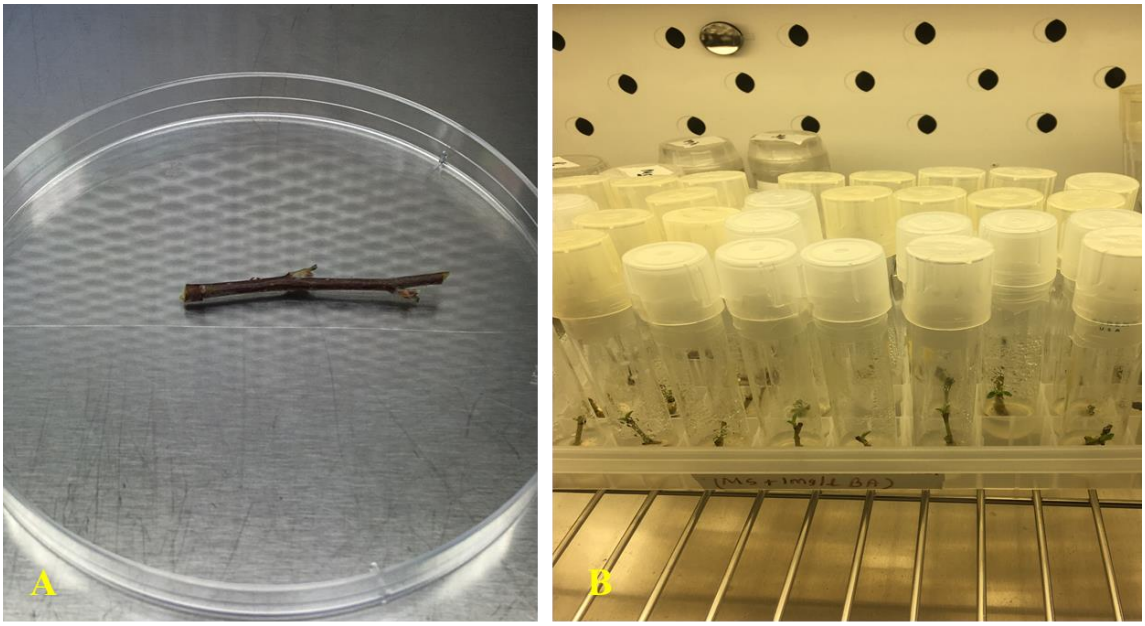

Fig. 4-2. Nodal cuttings for the establishment stage. (A) Prepared nodal cutting and (B) culture tubes under lights in a growth chamber.

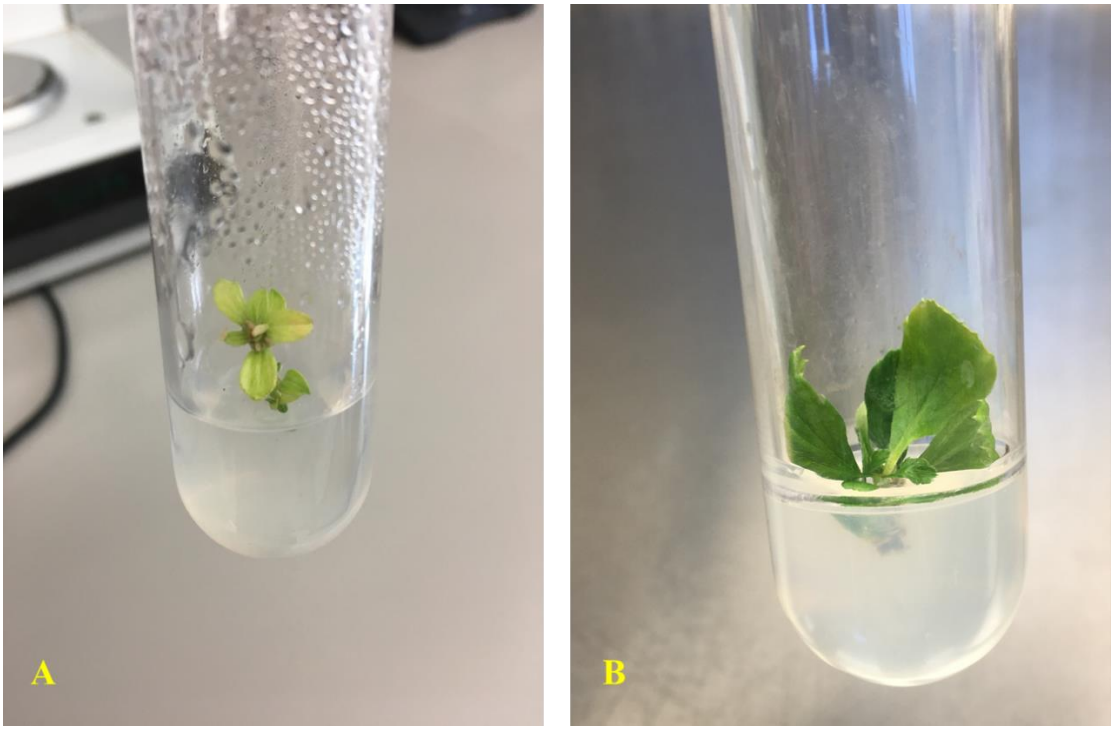

Fig. 4-3. Microshoots formed after the establishment stage. (A) Microshoots of *Ceanothus velutinus* and (B) *Cercocarpus montanus*.

Fig. 4-4. Photos for *ex vitro* rooting study. (A) Healthy microshoots of *Ceanothus velutinus* and (B) microshoots in a mist bench for *ex vitro* rooting.

Fig. 4-5. New microshoots from cultured nodes of *Ceanothus velutinus*. Microshoots were produced on Murashige and Skoog (MS) and Gamborg's B-5 (B5) medium supplemented with 1 mg·L^{-1} benzylaminopurine (BA).

Fig. 4-6. Rooted *Ceanothus velutinus* microshoots in *ex vitro* condition when treated with different auxin compounds. (A) Roots on microshoots without hormone treatment, (B) treated with 1,000 mg·L^{-1} indole-3-butyric acid (IBA) and 500 mg·L^{-1} 1-naphthaleneacetic acid (NAA) (Dip‘N Grow, 1% IBA and 0.5% NAA), (C) Hormodin 1 (1,000 mg·L^{-1} IBA), and (D) Hormodin 2 (3,000 mg·L^{-1} IBA).

Fig. 4-7. Multiple shoots of *Cercocarpus montanus* with addition of gibberellic acid. Murashige and Skoog (MS) medium supplemented with 1 mg·L^{-1} BA, 1 mg·L^{-1} gibberellic acid and 0.1 mg·L^{-1} 1-naphthaleneacetic acid (NAA) was used.

CHAPTER V

CONCLUSIONS

Ceanothus velutinus (snowbrush ceanothus) and *Cercocarpus montanus* (alder-leaf or true mountain mahogany) are two underutilized Utah native plants. Both *C. velutinus* and *C. montanus* are actinorhizal plants that form symbiosis with *Frankia* bacteria and have a soil-building role. In addition, they have high value in water-efficient landscapes. Therefore, the establishment of seed and vegetative propagation protocols for these native plants is crucial to introducing them in urban landscape.

Ceanothus velutinus seeds possess a hard seed coat and exhibit both physical and physiological dormancy. Seeds of *C. montanus* exhibit physiological dormancy. In order to break dormancy and enhance germination, pretreatment of seeds is necessary. Scarification in hot water at 90 ºC together with stratification for 2 or 3 months effectively broke the dormancy of *C. velutinus* seeds. In addition, gibberellic acid (GA_3) also helped to increase *C. velutinus* seed germination. For *C. montanus* seeds, a combination of stratification for 2 months and GA_3 treatment at 50 $mg \cdot L^{-1}$ effectively broke seed dormancy.

Various evaluated factors play important roles in the propagation of plants by stem cuttings. Timing of cutting harvest of *C. velutinus* positively influenced rooting percentage and quality. Late summer (July or August) was the best time for collecting *C. velutinus* cuttings from the wild at an elevation of 2010 m. Terminal cuttings were better for rooting when compared with interstem cuttings. Application of synthetic auxins, specifically indole-3-butyric acid (IBA) to the base of stem cuttings enhanced rooting rate

as well as increased the quantity of adventitious roots. From this experiment, we observed that talc-based Hormodin 2 (3,000 $mg \cdot L^{-1}$ IBA) was effective and easier to use than liquid formulations of auxin.

Hardwood or semihard wood cuttings of *C. montanus* were better for propagation when compared with softwood cuttings. Similarly, Hormodin 2 tended to be the most effective hormone for rooting *C. montanus*. Finally, wounding promoted greater adventitious rooting percentages and root quality.

In the micropropagation study, the growth of new shoots from nodal cuttings of *C. velutinus* and *C. montanus* was observed after 3 to 4 weeks of being cultured on Murashige and Skoog (MS) medium. The most efficient medium for micropropagating *C. velutinus* was MS medium supplemented with benzylaminopurine (BA). *Ex vitro* rooting was effectively performed for rooting microshoots of this species. Similarly, MS medium supplemented with BA was best for the multiplication of *C. montanus* shoots. Use of GA_3 supplemented medium led to increased number of shoots and stem elongation.

Continued micropropagation research will focus on increasing the number of microshoots and improving rooting response of *C. velutinus* and *C. montanus*. In the meantime, these protocols will provide a basis for successful *in vitro* propagation. Plant tissue culture is unique in mass propagating disease-free plants. However, further research is needed on all aspects of vegetative propagation of *C. velutinus* and *C. montanus* in order to develop a viable propagation system.

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APPENDIX

Table A-1. GPS coordinates and site elevations for *Ceanothus velutinus* plants collected within the area of Tony Grove Lake, Utah.

Plant	Coordinates
1	lat. 41°52'34"N, long. 111°34'21"W, elevation 2010 m
2	lat. 41°52'34"N, long. 111°34'20"W, elevation 2020 m
3	lat. 41°52'34"N, long. 111°34'19"W, elevation 2000 m
4	lat. 41°52'36"N, long. 111°34'16"W, elevation 1980 m
5	lat. 41°52'36"N, long. 111°34'16"W, elevation 2000 m
6	lat. 41°52'36"N, long. 111°34'16"W, elevation 2000 m
7	lat. 41°52'35"N, long. 111°34'16"W, elevation 2000 m
8	lat. 41°52'34"N, long. 111°34'20"W, elevation 2000 m
9	lat. 41°52'35"N, long. 111°34'20"W, elevation 2020 m

Fig. A-1. Seeds treatment. (A) Seeds soaked in gibberellic acid solution and (B) refrigerated to accomplish stratification.

Fig. A-2. Germinated seeds in Petri dishes. (A) *Ceanothus velutinus* and (B) *Cercocarpus montanus* seeds.

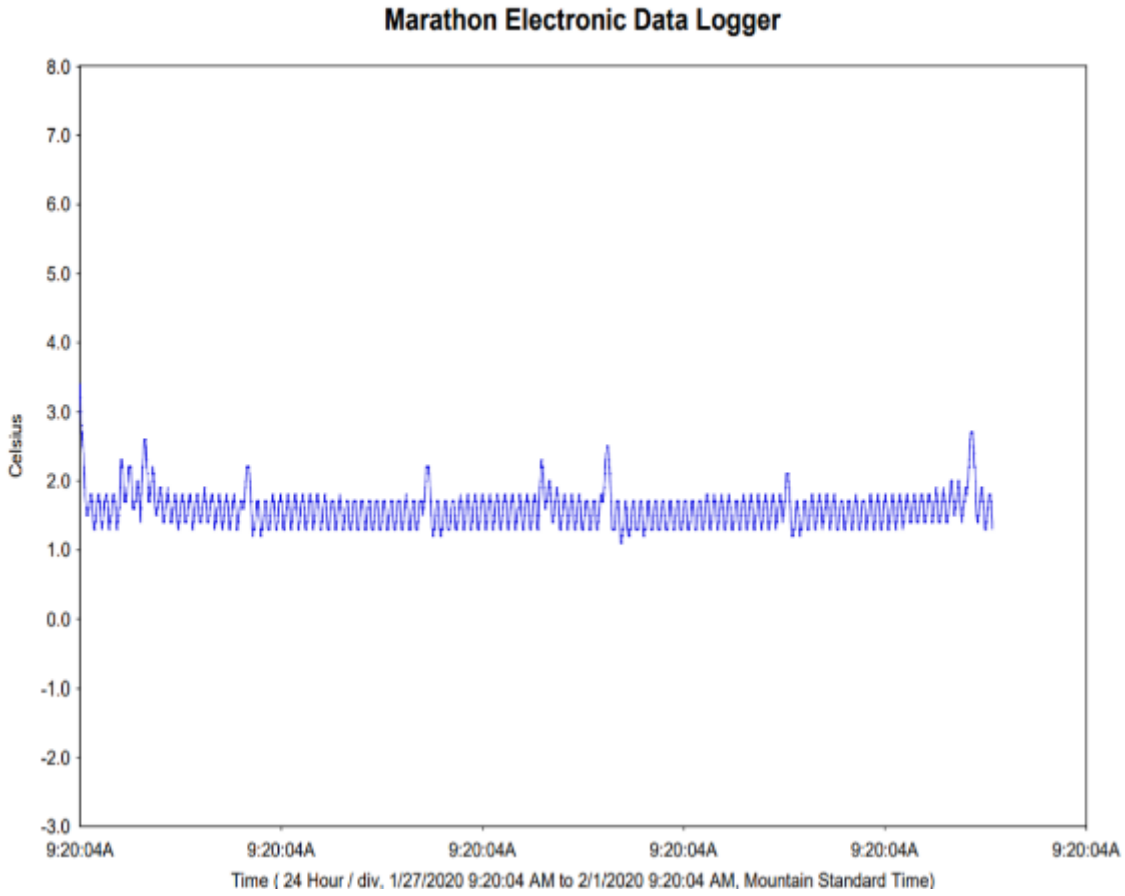

Fig. A-3. Temperature readings of the refrigerator where seeds were kept for stratification. An electronic data logger (Marathon, EDL, San Leandro, CA) was used to measure the temperature inside the refrigerator. Temperature reading were taken for 5 days with an interval of 12 seconds.

Fig. A-4. Aerial image of the site for collecting *Ceanothus velutinus* cuttings and wild populations of *Ceanothus velutinus* at Tony Grove Lake area, Utah.

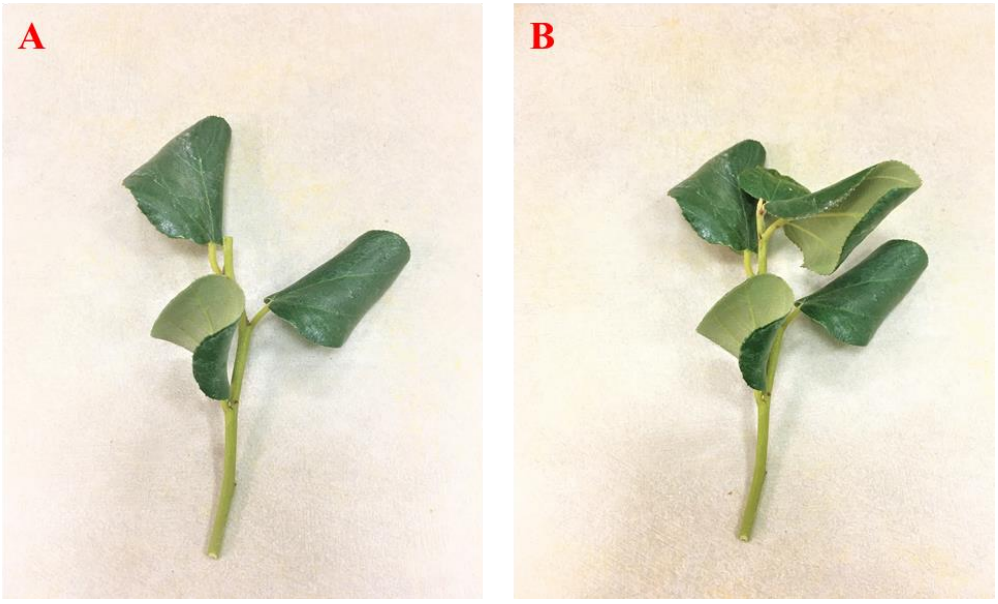

Fig. A-5. Stem and terminal cuttings of *Ceanothus velutinus*. (A) Stem and (B) terminal cuttings.

BIOGRAPHY

Asmita Paudel was born in Parbat, a hilly district of western region of Nepal on April 13, 1993. She was raised in Kathmandu, Nepal. She attended the Institute of Agriculture and Animal Science, Tribhuvan University, Chitwan, Nepal and graduated in 2016 with a Bachelor of Science degree in Agriculture. After graduation, she collected some professional experience while working on a United States Agency for International Development (USAID) funded project: Suaahara II.

In August, 2018, she enrolled in the Department of Plants, Soils and Climate at Utah State University as a Master's student. She presented her research work at different professional meetings. She is a member of the American Society for Horticulture Science, American Penstemon Society, and International Plant Propagators Society. She was awarded a Graduate Student Research Grant from American Penstemon Society. She won the AGRI Ambassador Ardeshir Zahedi International Endowment Scholarship, AGRI Elva Acklam and Arvil L. Stark Scholarship, Bruce Briggs Memorial Scholarship, and a Student Travel Grant by Research Graduate Studies at the Utah State University. She was awarded by the International Plant Propagator's Society with the First Place Award in the poster competition.

Asmita is a candidate for the Master of Plant Science degree from the Utah State University.