STEM CUTTING PROPAGATION OF THE ENDANGERED SPECIES

CLEMATIS SOCIALIS (KRAL)

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STEM CUTTING PROPAGATION OF THE ENDANGERED SPECIES *CLEMATIS SOCIALIS* (KRAL)

Connie Nicole Johnson

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STEM CUTTING PROPAGATION OF THE ENDANGERED SPECIES

CLEMATIS SOCIALIS (KRAL)

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Connie Nicole Johnson, daughter of Hattie Mae and Willie James Johnson, was born August 2, 1982 in Opelika, Alabama. She graduated from Beulah High School in Valley, Alabama in 2000 and entered Southern Union State Community College (SUSCC) in Opelika, Alabama. She received an Associate's of Science degree from SUSCC in 2002 before transferring into Auburn University's Department of Horticulture where she graduated summa cum laude with a Bachelor's of Science degree in Landscape Horticulture in 2004. She began her graduate study in Auburn University's Department of Horticulture in the summer of 2004. She was awarded a Presidential Graduate Opportunities Program Fellowship in the fall of 2004 which she held while serving as a graduate research assistant. She completed the Master's of Science degree December 15, 2006.

THESIS ABSTRACT

STEM CUTTING PROPAGATION OF THE ENDANGERED SPECIES

CLEMATIS SOCIALIS (KRAL)

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Clematis socialis (Kral), the Alabama leather flower, is a federally listed endangered species native to NE Alabama and NW Georgia. In addition to conservation and management practices of the few existing populations, knowledge of this species' reproduction is needed to aid in establishing additional populations needed to remove it from the endangered species list. Considering all methods used in clematis propagation, past and present, stem cuttings appear to be the most feasible method to obtain a large number of propagules in a relatively short amount of time without significantly disturbing native or established plants. However, there is little information about the cutting requirements of *C. socialis*. In one experiment, *C. socialis* stem cuttings were stuck in non-amended sand, perlite, vermiculite, and 1:1:1 (by volume) sphagnum peat moss: pine bark: sand (P:PB:S) to compare the efficiency of each substrate in rooting. Sand, the

industry standard, perlite and vermiculite all performed similarly and better than the P:PB:S substrate in 2000, 2004, and 2005. Rooting percent averages were highest in sand and perlite in all three years, and cuttings in these two substrates were the earliest to root in 2005; approximately two weeks earlier than those in vermiculite. Although vermiculite's root initiation was later than in sand and perlite, some of the highest root length, root number, and root rating averages were observed in it. Sand's consistent high performance, early root initiation, availability, and lower cost per cutting makes it the most suitable substrate for rooting *C. socialis* stem cuttings. In a second experiment, *C. socialis* stem cuttings were stuck in a 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite substrate amended with rates of dolomitic limestone ranging from 0.0 to 12.5 lbs/yd^3. Average root length, root number, and root rating increased linearly as dolomitic limestone amendment rate increased in all years, while rooting percent and cutting survival improved as lime rate increased only in 2004. Optimal rooting and root growth were obtained using lime rates between 10 to 12.5 lbs/yd^3. Since there were also quadratic responses in many instances, using rates higher than 12.5 lbs/yd^3 would not appear to yield better results. In a third experiment, *C. socialis* stem cuttings were stuck in 1:1:1 (by volume) sphagnum peat moss: pine bark: perlite amended with 10.0 lbs/yd^3 dolomitic limestone to compare the efficiency of growth regulator treatments from 0/0 ppm IBA/NAA [indole-3-butyric acid (IBA)/naphthalene acetic acid (NAA)] up to 7500/3750 ppm IBA/NAA on rooting. Results indicated that growth regulators were not necessary to initiate rooting but that rates between 4500/2250 ppm IBA/NAA and 6000/3000 ppm IBA/NAA initiate rooting earlier. Rooting percent and root growth was generally highest between 3000 to 4500 ppm IBA and 1500 to 2250 ppm NAA.

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CHAPTER I

LITERATURE REVIEW

Discovery and Description

Clematis socialis (Kral), Alabama leather flower, was discovered in St. Clair County, Alabama (33.66°N-86.35°W; USDA Hardiness Zone 7b) growing along a highway right-of-way by Robert Kral in 1980, and later described as a new species in 1982 (1). *C. socialis* is an erect, non-vining, deciduous, herbaceous perennial (11). Flowers emerge in late April through May with fruit set in June (2). The most distinctive feature of this species is the formation of dense clones by slender, horizontally spreading and branching rhizomes 0.08 to 0.12 inches (2 to 3 mm) thick (Figure 1). Stems are erect or ascending, solitary or in small clusters, and simple or sparingly branched at the uppermost nodes, usually 7.87 to 11.81 inches (20 to 30 cm) high. Stems have several (3 to 7) internodes, are 0.06 to 0.08 inches (1.5 to 2.0 mm) thick, and are bright green, sometimes with purplish tint, in color. The lower internodes are shorter than the upper or leafy internodes. Lower leaves are simple, sessile or short-petiolate, with blades mostly elliptic-linear. Upper leaves are pedately trilobate with segments narrow as in simple blades or pinnately compound, with petioles shorter than the 3 to 5 leaflets. The bright pale green leaves have an entire margin, are narrowly acute with a cuneate base, and slightly revolute (11, 12).

The flowers are urceolate to campanulate, spreading or nodding, and solitary at tips of erect, variably elongated peduncles (Figure 2). At anthesis, the four sepals are 0.79 to 0.98 inches (2.0 to 2.5 cm) long, lance-oblong, apically spreading or recurved, acute to acuminate, and thickest at the cupped base. The margins of the sepals are thin, dilated-cripate, medially and proximally entire towards the apex. The convex sepal backs are pale but bright blue-violet, sparingly puberulent and strigillose. The sepals' inner surface is smooth, except at the margin, proximally yellow and distally blue-violet. There are numerous yellow stamens 0.71 to 0.79 inches (1.8 to 2.0 cm) long and filaments 0.39 to 0.59 inches (1.0 to 1.5 cm) long. Anther connectives are pilose dorsally and apically apiculate. The carpels, lanceolate and 0.47 to 0.59 inches (1.2 to 1.5 cm) in length, taper gradually into elongate styles. The stigmas are 0.16 to 0.20 inches (4 to 5 mm) long and smooth except for the beveled, hairy ventral surface that is concealed by a dense, silvery subappressed tomentum. The achene or fruit measures 0.98 to 1.18 inches (2.5 to 3.0 cm) long including the style. The body of the achene is ovate, acute at the apex, flattened over the seed, and marginally thickened. The surface is appressed-strigillose-puberulous (11, 12).

Taxonomy

Clematis is a genus of the Ranunculaceae (Buttercup). *Clematis* is currently divided into nine subgenera including: *Clematis, Cheiropsis, Flammula, Archiclematis, Campanella, Atragene, Tubulosae, Pseudoanemone*, and *Viorna* (8). Kral (11) placed *Clematis socialis* in the subgenus *Viorna*. Subgenus *Viorna* is comprised of three sections including: *Viorna*, *Integrifoliae*, and *Hirsutissima*. Section *Viorna* consists of subsections *Viorna, Crispae*, and *Fuscae*. Section *Integrifoliae* consists of subsections

Integrifoliae and *Baldwinianae* (8). Narrow leaves and bractless peduncles made *C. socialis* more comparable to species *C. baldwinii* (subsection *Baldwinianae*) and *C. crispa* (subsection *Crispae*). However, *C. crispa* differs significantly from *C. socialis* in growth and flowering habit. The erect, low stem habit and blue-purple flower color range of *C. baldwinii* was most similar to that of *C. socialis* (11). Therefore, *C. socialis* is classified in the subsection *Baldwinianae* of section *Integrifoliae* in subgenera *Viorna* (8) (Figure 3).

Habitat

Clematis socialis populations occur in the Ridge and Valley physiographic region of northeast Alabama and northwest Georgia (USDA Hardiness Zones 6b – 7b) (2). Populations of *C. socialis* have been found growing in silty-clayey soils of the Conasuaga Series weathered from interbedded shale or shaley limestone of the Conasauga Formation. The soils are slightly neutral to slightly basic with a high hydroperiod (19). Plants occur in full sun to part shade in grass-sedge-rush communities and contiguous hardwood-forested edges (Figure 4). Sites where *C. socialis* have been found include highway rights-of-way, pasture land, and logged forests (2).

C. socialis is found in low areas associated with the following monocot and dicot prairie species:

Andropogon gerardi (big bluestem or turkey foot)
A. scoparius (little bluestem)
Asclepias viridis (green antelopehorn)
A. hirtella (green milkweed)
many *Carex sp.* (sedge and carices)
Cicuta maculate (water hemlock)
Eleocharis tenuis (slender spikerush)
E. compressa (flatstem spikerush)
Fimbristylis puberula (hairy fimbry)

Glyceria striata (fowl mannagrass)
Gratiola floridana (Florida hedgehyssop)
Juncus sp. (rush)
Liatris spicata (gayfeather or blazing star)
L. aspera (rough blazing star)
Panicum virgatum (switchgrass)
Penstemon laevigatus (eastern smooth beardtongue)
Phlox amoena (hairy phlox)
P. glaberrima (smooth phlox)
P. pilosa (downy phlox)
Poa sp.
Polygala sanguinea (purple milkwort)
Ptilimnium costatum (ribbed mock bishopweed)
Ranunculus fascicularis (early buttercup)
Rhynchospora caduca (anglestem beaksedge)
R. corniculata (shortbristle horned beaksedge)
Scirpus atrovirens (green bulrush)
Scirpus lineatus (drooping bulrush)
Silphium terebinthinaceum (praire dock)
S. laciniatum (compass plant)
Sisyrinchium atlanticum (eastern blue-eyed grass)
S. langloisii (roadside blue-eyed grass)
Thalictrum (meadow rue)
Tripsacum dactyloides (eastern gamma grass)
several *Viola sp.* (violets)

The surrounding forest is mostly pine hardwood, the dominant pine being *Pinus taeda* (loblolly pine). Lowland forest plants include the following:

<u>*Overstory trees*</u>
Acer rubrum (red maple)
Carya sp. (hickories)
Celtis laevigata (southern hackberry)
Diospyros virginiana (persimmon)
Fraxinus americana (white ash)
F. pennsylvanica (green ash)
Liquidambar styraciflua (sweet gum)
Nyssa sylvatica (black gum)
Quercus phellos (willow oak)
Q. lyrata (overcup oak)
Ulmus americana (American elm)

<u>*Understory trees*</u>
Cornus florida (flowering dogwood)

Ilex decidua (deciduous holly)
Rhamnus (buckthorn)
Sambucus canadensis (elderberry)
Sassafras albidum (sassafras)
Viburnum (viburnum) (11, 12).

Toxicodendron radicans (poison ivy) was also personally observed growing among the *Clematis socialis* colony located at the Cherokee County, Alabama site.

Current Status

Clematis socialis was designated an endangered species and published in the Federal Register by the United States Fish and Wildlife Service on September 26, 1986 (19). The endangered status of *C. socialis* occurred because of the small population numbers and distribution area, as well as its existence on sites subject to human disturbance (17). To date, there are only six reported populations of *C. socialis*. Five sites total have been reported in the St. Clair, Cherokee, and Etowah Counties of northeast Alabama, and one site in Floyd County of northwest Georgia (14, 16) (Figure 5).

The major threat to the survival of *C. socialis* is loss of habitat due to overgrowth of hardwood canopy, competition from surrounding vegetation, herbicide use, mowing and bush hogging, road maintenance, logging, and residential development (2, 19). Lack of information on or the knowledge of this species' life history is a major hindrance of habitat management and recovery work (19).

Efforts have been made to inform landowners of the importance of protecting inhabited sites and to establish recovery methods to save the existing populations. All landowners, personnel of the Alabama Highway Department, and Alabama Power Company have been notified of the sites on which this rare species occurs. Informal

agreements between the U.S. Fish and Wildlife Service and the Alabama Department of Transportation have been made to abolish herbicide use and exercise a special mowing schedule (late summer) on inhabited sites along rights-of-way. Mowing in late summer allows the plants to flower and set seed. The landowner of the St. Clair County site donated a 26-acre tract containing a major portion of a population and a sizeable buffer zone to The Nature Conservancy. The Alabama Forestry Commission has conducted prescribed burns at this site as a means of controlling competing vegetation. The landowner of the Cherokee County population also entered into a protection agreement with the U. S. Fish and Wildlife Service (19).

The U.S. Fish and Wildlife Service Recovery plan states that *C. socialis* will be reclassified from an endangered to a threatened status when ten geographically distinct, self-sustaining populations- each occupying a minimum of one acre of habitat- are known and protected from any foreseeable threats. Twenty such populations must be secured before the species is delisted and no longer considered threatened or endangered (19).

Significance

It has been estimated that 25% of the world's vascular plants will be threatened by extinction within the next 50 years (18). The state of Alabama has been ranked fourth in the continental U.S. in its number of federally-listed endemic plant species. Conservation and population management practices cannot be developed without at least some basic knowledge of the species' biology including reproduction. In the case of *C. socialis*, the limited seed or achene production reported by Timmerman-Erskine (17) would indicate that stem cutting propagation may be a more effective means of establishing additional

self-sustaining populations. However, there is little information about the requirements for stem cutting propagation of *C. socialis*.

Previous Research

Success of past studies of stem propagation of *Clematis* sp. has varied depending on the species or cultivar, size of flower, technique, and substrate used. Small-flowered species *C. alpine* (alpine clematis), *C. macropetala* (downy clematis), *C. Montana* (anemone clematis), *C. tangutica* (golden clematis), and *C. viticella* (Italian clematis) and its cultivars, are fairly easy to propagate from internodal cuttings. Cuttings of large-flowered *C. armandii* (Armand's clematis), American pitcher-shaped species, and the cultivar *C. florida* 'Sieboldii' (passionflower clematis) are difficult to root. As a general rule, the larger the flower, the more difficult the cuttings will be to root (5). Cultivated clematis is traditionally propagated in sand-based substrates without an indole-3-butyric acid (IBA) rooting compound. Placed in a humidity tent, cuttings generally take 6 weeks to root (3). With bottom heat of approximately 23°C (73.4˚F), rooting can begin within 14 days (9).

In the 1990s, Erwin et al. (4) looked at how cultivar, substrate type, IBA application, and node position from which the cuttings were taken influenced the rooting of some cultivated clematis species. In 1991, Erwin and Schwarze (3) stuck single-node cuttings of *Clematis* cultivars 'Jackmani', 'Contesse de Bouchard', 'Gypsy Queen', and *Clematis purpurea plena elegans* in the following substrates:

- 100% washed sand
- 100% perlite
- 50% washed sand and 50% sphagnum peat (by volume)

- 50% sphagnum peat and 50% perlite (by volume)
- 50% sphagnum peat, 25% perlite and 25% vermiculite (by volume).

The cuttings were collected from different positions on the stem and assigned node numbers from 1 (at the base of the plant) up to 5 (at the tip of the stem). Cuttings were treated with or without 0.1% IBA before being stuck and placed in a humidity chamber. Erwin and Schwarze (3) reported earlier and more rooting on single node cuttings stuck in sand and perlite than in the other three substrates. However, the degree of rooting observed seemed to be more related to substrate pH rather than substrate structure. Sand and perlite had the highest pH values, 8.2 and 8.6, respectively, nearly twice that of the peat based mixes (4.4 to 4.6). Regression analysis indicated a decrease in time of rooting and an increase in primary root number and root dry weight as substrate pH increased. Time to rooting (when roots were ≥0.5 cm long), primary root number, and root dry weight also varied depending on cultivar. For instance, the easily rooted cultivar 'Gypsy Queen' rooted after 38 days whereas the difficult-to-root 'Jackmani' rooted later at 48 days after sticking. It was also observed that cuttings taken from the distal end of the stem were less likely to root than those made from the proximal end. Time of rooting decreased for proximal cuttings taken between nodes 1 and 2 but increased as the node position became more distal (from nodes 2 to 5). In 1992, Erwin and Schwarze also reported that the 0.1 % IBA application used (Hormex 1; Brooker Chemical, Chatsworth, CA) was not necessary because it had no effect on rooting. In 1997, IBA application was only effective at increasing cutting survival of the cultivar 'Jackmani' compared to other cultivars tested (4).

Evison (6) suggested using internodal cuttings stuck in compost consisting of 1 part loam, 1 part peat, 2 parts grit, and 2 parts screened sand to propagate clematis. Evison (6) also recommended using the bottom-most nodes of the stem first and handling cuttings by the stem only. Futhermore, he suggested the propagation house environment have an air temperature between 15-31^{o}C (60-90^{o}F), 40-75% shade depending on the time of year, and a 15-20 second duration of fine mist once each hour.

Hatch (10) has had up to a 95% success rate rooting cuttings of New Zealand clematis species, such as *C. paniculata* (sweet autumn clematis), *C. hookeriana*, *C. forsteri* (puataua), and *C. afoliata* (leafless clematis), in pumice sand. Hatch suggested using one- to two-node internodal cuttings. Single-node cuttings stuck with the node just above the medium surface were most recommended for propagating clematis, especially climbing clematis species (9). A disadvantage of single-node cuttings is their inability to survive *Botrytis* (grey mold). Clematis cuttings are prone to fungal diseases such as *Botrytis,* and when infected with *Botrytis*, the fungus destroys the buds on or above the surface of the substrate. If using a two-node cutting, there are buds below the surface of the substrate that can restart the cutting and continue to grow. Two-node cuttings also develop a better crown with more shoots coming from below the ground which means a greater chance of recovering from an infection of *Phoma clematidina* (clematis wilt). Using two-node cuttings does result in fewer cuttings, and cutting length can be a problem with cultivars with long internodes. However, over-wintering losses of two-node cuttings are lower than with single-node cuttings. Two-node cuttings are more robust and better-prepared for over-wintering because they have more carbohydrate reserves. Single-node cuttings must have a root system thoroughly developed to pot up

in summer to over-winter. Whether using single- or two-node cuttings, removing or reducing the size of one of the two leaves at the node above the surface is recommended to reduce transpiration and prevent overcrowding, especially for larger-leaved types. Both leaves on the bottom node of two-node cuttings should be removed. Also, two-node cuttings should be taken from slightly harder material than that used for single-node cuttings. Flowering shoots should be avoided in both cases, because flowering shoots typically do not root successfully. Cuttings should be taken using a sharp, clean blade and prepared in a shady, humid environment to prevent drying out. Some specialists recommend taking cuttings only on cooler days or early in the morning before sunrise (9).

Erwin et al. (4) used 0.1% IBA (Hormex 1; Brooker Chemical, Chatsworth, CA) but in the end determined that the IBA application was not necessary to root their clematis cuttings. Both Evison (6) and Hatch (10) used the rooting powder Seradix No. 2 (indole-butyric acid) (Certis UK; Amesbury, Wiltshire, UK). Rooting powder incorporated with a fungicide could help inhibit mold and rot development along with proper sanitation, spacing and good air circulation. Fungicide spray treatments on the stock plants could provide higher quality disease-free cutting material to start with, thus decreasing the chance of a disease outbreak. Evison (6, 9) also recommends fungicide drenches (Captan) and dusts (Botrilex) for clematis stem cuttings at two week intervals (9, 6). Clematis will root without wounding the stem, but doing so could encourage callusing and stimulate natural auxin production. Since wounding is unnecessary and time-consuming, most professional growers avoid it (9).

Kreen et al. (13) have shown that micropropagation can be a successful means of propagating commercial clematis cultivars, even more successful than stem cutting propagation. Micropropagation also makes it possible to free stock material of viruses, bacteria, and plant borne fungi thus producing healthy plant material. Protection from disease is especially important in propagating early, large-flowering cultivars and cultivars with *Clematis lanuginosa* parentage, both of which seem to be more susceptible to *Phoma clematidina* (clematis wilt) (13). Kreen et al. stuck softwood, one-node stem cuttings of the cultivars 'Columella', 'Propertius', 'Ballet Skirt', 'Tage Lundell', and 'Riga' from section *Atragene* in 1:1 sphagnum peat moss: perlite, 1:1 coarse washed quartz sand: perlite, and 100% pure perlite. The substrates were irrigated with nematodes, *Steinernema feltiae*, as a preventative against Sciaridae larvae and the cuttings sprayed with Rovral Aqua^{TM} 0.05% and Topsin^{TM} 0.10% as a preventative against *Botrytis* (grey mold). The stem cuttings, which required a relative humidity of 80-90%, were more susceptible to grey mold because they were kept in the same humidity tent as the microshoots which required 100% humidity. Exposure to the higher humidity and an attack of Sciaridae larvae, despite the preventative nematode drench, biased and probably reduced stem cutting rooting percentage. Rooting percentage may have also been influenced by the cultivars' susceptibility to fungi, especially the *Botrytis* susceptible 'Ballet Skirt'. Kreen et al. (13) stated that fungal problems were, in general, less severe for the microshoots which rooted rapidly in comparison to the stem cuttings. Rooting occurred quicker and primary root number and root dry mass were higher in perlite and sand-perlite than in the peat-perlite substrate. Microshoots of all five cultivars rooted equally well independent of the substrate, but there were interactions between

substrate and cultivars which might suggest that the propagation method could be optimized for the specific needs of each cultivar (13).

Spring is considered the optimal time of year for taking clematis cuttings. Nau (15) recommended taking cuttings in March in the southern United States and a month later in the North but did not indicate whether indoor and/or outdoor stock plants were used. Gunn (9) stated that cuttings could be taken in late April if stock plants are indoors. Cuttings from outdoor stock plants could be taken in May in warmer climates, but in colder climates it is advised to wait at least a month longer (9). Erwin et al. (3, 4) collected cuttings from indoor stock plants in early March to early May, and Hatch (10) collected cuttings of outdoor stock plants in late March to early April.

There are several methods that can be used to propagate cultivated clematis including seeds, layering, division, grafting, and stem cuttings. Seed is not the most common method because germination times vary from species to species. Germination can occur in as quickly as 2 to 3 weeks to as long as twelve months depending on species. Seed is also not guaranteed to be true to its parent. This can be good for new cultivar development or improvements of old species/cultivars but not if characteristics of the original species/cultivar are trying to be maintained. Layering is the easiest method of propagating clematis but requires a longer period of time. In spring, one-year old growth is gently bent down and pegged down to soil. A slit is cut in the stem, and a rooting compound can be used. The layer should be rooted by autumn when it can be severed from the mother plant (8). Division is primarily used for herbaceous clematis such as *C. recta*, and *C. integrifolia*. The root and crown are dug up and divided with each division having roots to sufficient supply the crown. Division is best done from mid-autumn to

early spring (5). Grafting was very common until the mid-1900s, especially for large-flowering clematis, but its use as a means of propagating clematis has declined (13). Grafting is labor intensive and requires skill for success. Clematis rootstock differs from other plant rootstocks in that it is only a nurse stock to start the scion off. The rootstock dies away once the scion develops its own roots (7). These methods, though effective for most cultivated species, are not practical choices for propagating *C. socialis*. For instance, seed propagation would be limited due to the reportedly low seed production of *C. socialis* (17). A large supply of seeds would be difficult to obtain but required for the quantity of seedlings needed to establish all additional populations needed to remove *C. socialis* from the endangered species list. It is also not known how genetically true future *C. socialis* seedlings will be to the original seed parent. The endangered status of *C. socialis* would also hinder methods such as layering and division which would require significant disturbance of native sites and established plants.

Objectives

Twenty self-sustaining populations are required before *C. socialis* will be considered for removal from the endangered species list. Some form of propagation will have to be employed to establish those 20 populations. Limited seed production hinders seed propagation (17). The federally protected and limited plant material prevents its propagation using division. Stem tissue collected for micropropagation or stem cutting propagation would result in the least amount of damage to native sites. Micropropagation has been shown to be as successful as stem cutting propagation in propagating commercial clematis species (13), but not all growers will have the skill, equipment, or

facilities required for this procedure. Therefore, stem cutting propagation seems to be the most feasible method to propagate and eventually increase the number of *C. socialis* populations. Most clematis cutting propagation studies and information available are relevant to commercially cultivated clematis species and cultivars. Since there is little to no cutting requirement information on *Clematis socialis*, this study was conducted to accumulate data on the substrate chemical and physical properties and growth regulators that affect the rooting of its stem cuttings. This research could aid in establishing additional self-sustaining populations, provide genetic material for future hybridization, and possibly promote *C. socialis* as an ornamental horticultural commodity, especially to growers who specialize in native species.

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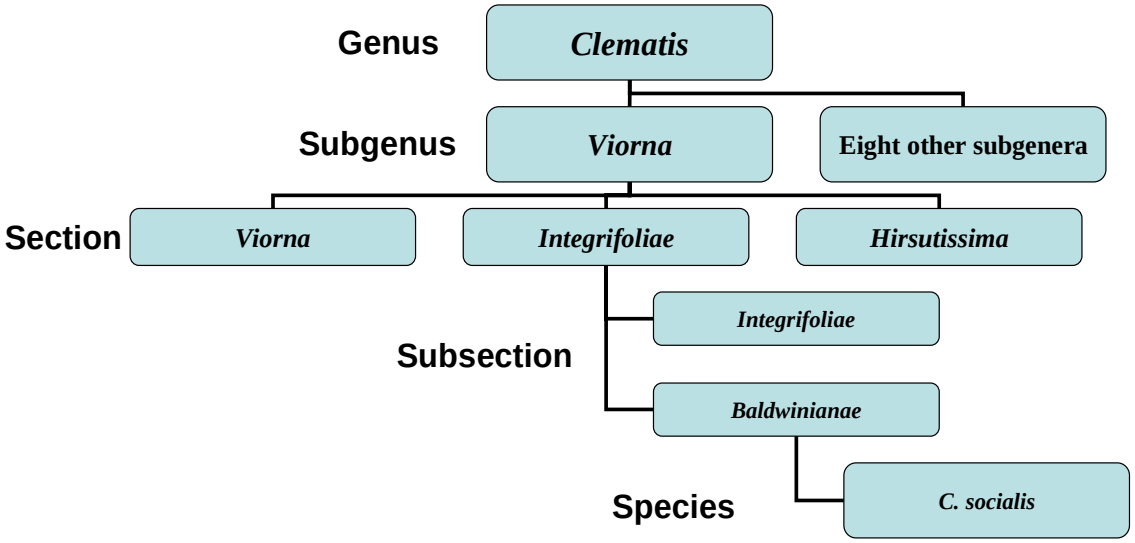
Figure 1. Dense colony of *Clematis socialis* (Kral).

Figure 2. Blue-violet campanulate or bell-shaped flowers of *Clematis socialis* (Kral).

Figure 3. Taxonomic tree of *Clematis socialis* (Kral).



Clematis, The Genus. Christopher Grey-Wilson, 2000.

Figure 4. Habitat of *Clematis socialis* (Kral) population located in Cherokee County, Alabama.

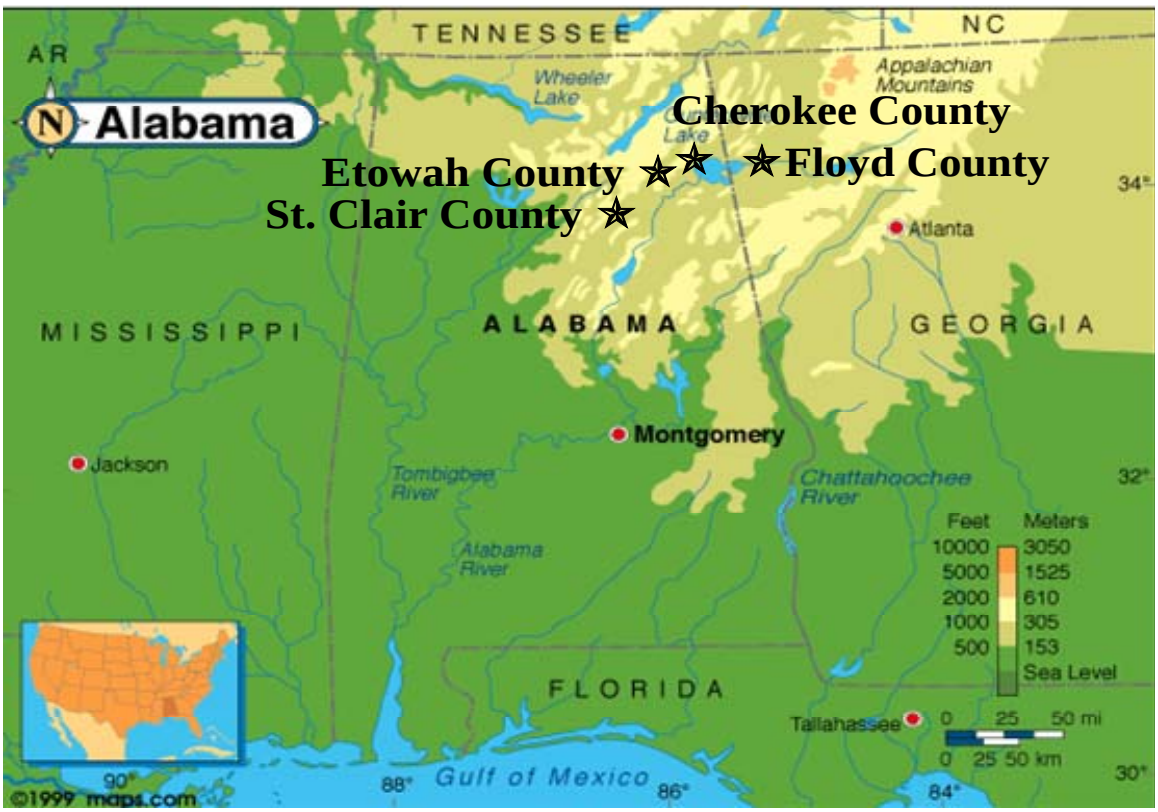


Figure 5. Locations of naturally occurring populations of *Clematis socialis* (Kral).

(Alabama physical map by Maps.com.)

CHAPTER II

SUBSTRATE AND PH INFLUENCE ON THE ROOTING OF *CLEMATIS SOCIALIS* (KRAL) STEM CUTTINGS

Abstract

Alabama leather flower, *Clematis socialis* (Kral), stem cuttings were stuck in non-amended sand, perlite, vermiculite, or 1:1:1 (by volume) sphagnum peat moss: pine bark: sand (P:PB:S) in 2000, 2004, and 2005 to compare efficiency of rooting this endangered species. Sand, the industry standard, perlite, and vermiculite all performed better than the P:PB:S substrate in all years. Cuttings rooted earlier in sand and perlite at 42 days after sticking (DAS), but cuttings in vermiculite rooted shortly after and equaled sand and perlite in percent rooting and root growth by the final harvest date, 70 DAS. The poor rooting percent and root growth results observed in the un-amended P:PB:S substrate was likely due to low pH rather than substrate physical properties. In a separate study, results showed that increasing dolomitic limestone amendment rate in 2:1:1 (by volume) sphagnum peat moss: pine bark: perlite linearly increased root growth in all years and rooting percent in 2005. An amendment rate range of 10.0 to 12.5 lbs/yd^3 would be recommended to provide the adequate substrate pH range required for rooting *C. socialis* cuttings in a peat and/or pine bark based substrate. High performance, availability, and lower cost per cutting ($0.002) would make sand the more suitable choice of the non-amended substrates in rooting *C. socialis* stem cuttings.

Index words: Alabama leather flower, rooting medium, endangered species, propagation

Species used in this study: *Clematis socialis* (Kral)

Significance to the Nursery Industry

Establishing requirements for stem cutting propagation of *Clematis socialis* can produce propagules for conservation purposes and also for commercial horticultural purposes. Resulting propagules can provide genetic material for hybridization. *C. socialis'* flower color and spreading growth habit are desirable characteristics that could be bred into commercial species/cultivars currently produced. Results show that sand, along with perlite and vermiculite, were among the highest performing substrates tested for rooting *C. socialis* stem cuttings. Sand, however, was the most readily available and least expensive (approx. $0.002) per cutting and therefore recommended for rooting *C. socialis* stem cuttings. If a substrate including organic components such as peat or pine bark is to be used, a dolomitic limestone amendment rate of 10 to 12.5 lbs/yd^3 would be recommended to acquire a pH adequate for rooting clematis cuttings.

Introduction

Clematis socialis (Kral), also known as the Alabama leather flower, is an endangered species with only six reported populations in northeast Alabama and northwest Georgia (9, 11). *C. socialis* is an erect, non-vining, herbaceous perennial with blue-violet urn shaped flowers that are 0.79 to 0.98 inches (2.0 to 2.5 cm) long and occur

in late April through May. The bright green foliage has an entire margin and is simple to pinnately compound. This clematis species differs from most in that it forms dense clones by underground rhizomes (7, 8, 1).

C. socialis was federally listed as an endangered species by the United States Fish and Wildlife Service in September of 1986 because of small population numbers and limited natural distribution, as well as known habitat on sites subject to human disturbance (13, 15). Twenty geographically distinct, self-sustaining populations- one acre each- that are known and protected must be secured before the species is delisted and no longer endangered (15). Propagation of this species could aid in conserving existing populations and establishing additional self-sustaining populations, provide genetic material for future hybridization and genetic preservation, and potentially introduce the Alabama leather flower as a new ornamental crop, especially to native plant growers.

The limited seed production of *C. socialis* reported by Timmerman-Erskine and Boyd in 1999 suggests that seed propagation would not be the most reliable or efficient source of future plants (14). The 1996 Ball perennial propagation manual stated that stem cuttings are the most commonly used method in cultivated clematis propagation (10). However, the success of stem cutting propagation can also vary dependent upon technique, rooting substrate, and the species or cultivar being propagated**.** Erwin and Schwarze (2) reported earlier and greater rooting on cuttings of clematis cultivars such as 'Gypsy Queen', 'Jackmani', 'Contesse de Bouchard',and the species *C. purpurea plena elegans* in sand and perlite than in other substrates evaluated. The cultivars propagated varied in time to rooting, root number, and root dry weight. For instance, the easily

rooted cultivar 'Gypsy Queen' rooted 10 days before the more difficult-to-root 'Jackmani' cultivar. The location on the stem from which the cuttings were taken also played a role in rooting. The cuttings made from the distal end were less likely to root than those made from the proximal end of the stem. In 1992, Erwin and Schwarze also reported that IBA (indole-3-butyric acid) application was not necessary because it had no effect on the rooting of the cuttings tested. In 1997, they reported that IBA application was only effective at increasing cutting survival of the more difficult-to-root cultivar 'Jackmani' compared to other cultivars tested (3).

In the United States, sand is currently the primary substrate used in commercial clematis stem cutting propagation (3). Some growers use sand only; others use mixtures of peat and sand or vermiculite and peat moss. Regardless of the substrate used, it is recommended that it have a pH 7.0 to 7.5 for optimal rooting performance (10). The pH of native soil collected from the *C. socialis* population located in Cherokee county, Alabama ranged from 6.8 to 7.3. The objectives of this study were to evaluate the influences of four non-amended substrates and their physical properties as well as the chemical properties, specifically pH, of dolomitic limestone-amended substrates on root initiation, root length, root number, root quality, and survival of *C. socialis* stem cuttings.

Materials and Methods

Stem cuttings of *C. socialis* were taken May 26th in 2000, June 11th in 2004, and June 2nd in 2005 from a roadside population located in Cherokee County, Alabama (34.2°N-85.67°W). The cuttings were placed in plastic bags, placed on ice, and transported back to Auburn University's Paterson Greenhouse Complex. Later in the

day, cuttings were prepared with stems being re-cut to leave one inch (2.54 cm) of stem below the bottom-most node. Each cutting received a basal application of Dip 'N Grow (Astoria-Pacific, Inc., Clackamas, Oregon) rooting solution with the bottom-most node submerged for approximately five seconds. The 2000 study used 2-node cuttings treated with 1,000 ppm of indole-3-butyric acid (IBA) and 500 ppm of naphthalene acetic acid (NAA). Terminal 3-node cuttings treated with 1,500 ppm IBA and 750 ppm NAA were used in 2004. In 2005, 2-node cuttings were treated with similar auxin concentrations to those in 2004.

Two experiments were completed each year of this study. In one experiment, cuttings were stuck in 606 cell packs [10 in^3 per cell (163 cm^3)] containing the following non-amended media: sand, perlite, vermiculite, and 1:1:1 (by volume) sphagnum peat moss, pine bark, and sand (P:PB:S).

In a second experiment, treated cuttings were stuck into 606 cell packs containing a 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) mix amended with varying rates of dolomitic limestone. The following dolomitc limestone rates were used: control or 0.0 lbs/yd^3 (0.0 kg/m^3), 2.5 lbs/yd^3 (1.48 kg/m^3), 5.0 lbs/yd^3 (2.9 kg/m^3), 7.5 lbs/yd^3 (4.5 kg/m^3), 10.0 lbs/yd^3 (5.9 kg/m^3), and 12.5 lbs/yd^3 (7.4 kg/m^3). The Virginia Tech Extraction Method (VTEM) was used to collect leachate and monitor pH of samples of the amended substrate mixes throughout the experiment at two week intervals (16). There were interactions for pH between dolomitic limestone rate and date of extraction at the 0.01% level in 2004 and 2005.

Trays containing the cell packs from both experiments were placed under an intermittent mist system that cycled from 7:30 A.M. to 6:30 P.M. In 2000, the cuttings

were kept in a full sun double-poly greenhouse and misted for 6 seconds every 5 minutes. In a separate study, better cutting survival was observed among cuttings in shade versus cuttings in sun (data not shown). Cuttings in shade also required less misting which is why cuttings in 2004 and 2005 were kept in a shaded glass greenhouse and misted for 5 seconds at 15 minute intervals.

The experiments were arranged in a randomized complete block design using four replications (six cuttings per replication) of each substrate in 2000 and six replications (four cuttings per replication) of each substrate in 2004. In 2005, there were four replications (4 cuttings per replication) of each substrate for each of three harvest dates [42, 56, and 70 days after sticking (DAS)]. Data were collected only once at 70 DAS in 2000 and 2004. Data collected included subjective root ratings (0 to 5, with 5 representing the best root system) (Figure 1), root number (roots > 5 mm (0.197 in) in length), average root length of the three longest roots, cutting survival, and rooting. A cutting was considered alive if green foliage was present. A cutting was rooted if a new root measured greater than 5 mm (0.197 in) in length. In 2004, physical properties data including porosity, air space, water holding capacity, and particle size distribution were also collected on the non-amended substrates (4, 5).

All data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test ($P = 0.05$) (12). Data in 2005 were collected on three harvest dates to determine whether there were greater initial differences among substrate types and dolomitic limestone amendment rates than observed in having only one harvest date in 2000 and 2004. The 2005 results will be presented and discussed as main effects only because there were no interactions for pH between substrate type or dolomitic limestone

rate and days after sticking at the 5% level using ANOVA for any of the growth parameters measured.

Results and Discussion

Substrate Type

In 2000, stem cuttings in sand, perlite, and vermiculite had higher root length, root numbers, root ratings, cutting survival, and percent rooting overall than those in the 1:1:1 P:PB:S substrate (Table 1). Among the three best substrates, average root length was similar and ranged from 15.0 mm (0.591 in) in perlite to 28.8 mm (1.134 in) in sand. Root number was highest in sand and perlite, both averaging 4.2 roots per cutting. Average root rating was as high as 2.2 in sand and 1.4 in the vermiculite substrate. Average root length, number, and rating were all less than 1.0 in 1:1:1 P:PB:S. Less than 13% of the cuttings survived or rooted in the 1:1:1 P:PB:S substrate compared with 58 to 71 percent of the cuttings in the other substrates.

There were more differences in root growth data among substrate types in 2004 than in 2000. Vermiculite had the highest average root length [133.6 mm (5.260 in)], root number (102.8), and rating (4.4) (Table 2). In fact, vermiculite's averaged root length and root number was more than two and half times that of the second highest averages of sand. Sand, perlite, and vermiculite were all similar and highest in percent cutting survival and rooting with over 90% of cuttings surviving and rooting in these three substrates. Only about 33% of the 1:1:1 P:PB:S cuttings survived or rooted. The 1:1:1 P:PB:S substrate was lowest in all measured parameters except average root length in which it was similar to perlite.

In 2005, sand, perlite, and vermiculite were similar within all measured parameters except rooting percent. Among these three substrates, average root lengths ranged from 5.8 (0.228 in) to 9.6 mm (0.378 in), average root number ranged from 1.4 to 3.2, and percent cutting survival ranged from 52.1 to 60.4% (Table 3). Average root rating was less than 1.0 in all substrates due to low cutting survival and rooting percentages. Percent rooting was highest in sand and perlite. Although over 35% of the 1:1:1 P:PB:S cuttings survived, none actually rooted or had any root growth.

Average root number did not change significantly, but average root length, rating, cutting survival, and rooting percent responded linearly over time (Table 3). The means of all growth parameters except cutting survival increased from 42 DAS to 56 DAS then decreased at 70 DAS, but only the trends of average root length and root rating were best fit by a quadratic model at the 0.1% level. Percent cutting survival was the only parameter with a negative linear response over time decreasing from 62.5% at 42 DAS to 29.7% at 70 DAS (Table 3). Over time, the number of cuttings that survived decreased, but there was an increase in the percent of those cuttings that rooted. Only 9.4 % of cuttings stuck were rooted at 42 DAS but that number more than doubled to 21.9% at 70 DAS.

It was also observed in 2005 that sand and perlite were the only substrates with rooted cuttings at 42 DAS; cuttings in vermiculite had root growth by 56 DAS (data not shown). Although vermiculite rooted slower than sand and perlite, its average root length, number, and rating were equal to that of sand and perlite by the end of the study.

Substrate Physical Properties

In 2004, particle size distribution of the four non-amended substrates was determined by placing multiple 500 cc samples of each substrate on a Soil Test shaker (Soil Test, Inc. Model # CL – 340) using sieve pans with openings that ranged from 0.25 (0.009 in) to 4.75 mm (0.187 in) in diameter and a closed pan to collect particles less than 0.25 mm (0.009 in) in diameter. The 1:1:1 P:PB:S substrate had the largest percent of particles higher than 4.75 mm (0.187 in) in diameter (5.8%) due to the pine bark component of the mixture (Figure 2). Less than 0.1% of sand, perlite, and vermiculite particles were as large. Perlite had the highest percent of particles 1.69 (0.067 in) to 4.75 mm (0.187 in) in diameter (Figure 2). In fact, the largest percent (26.7%) of perlite particles were in the 2.01 (0.079 in) to 3.35 mm (0.132 in) diameter range. The percent of sand particles was similar to 1:1:1 P:PB:S and vermiculite at the 2.01 (0.079 in) to 3.35 mm (0.132 in) diameter range and similar only to 1:1:1 P:PB:S at the 1.69 (0.067 in) to 2.0 mm (0.078 in) diameter range. Perlite and 1:1:1 P:PB:S were similar in the percent of their particles, about 20%, that were 0.86 (0.034 in) to 1.68 mm (0.066 in) in diameter (Figure 2). The largest percent of vermiculite's particles were in the 0.44 (0.017 in) to 0.85 mm (0.033 in) (26.9%) and 0.86 (0.034 in) to 1.68 mm (0.066 in) (52.2%) diameter ranges (Figure 2). The largest percent (28.9%) of 1:1:1 P:PB:S particles were in the 0.44 (0.017 in) to 0.85 mm (0.033 in) diameter range (Figure 2). Just over 33%, the largest percent, of sand's particles were 0.44 (0.017 in) to 0.85 mm (0.033 in) in diameter (Figure 2). There was also a large percent of sand particles in the 0.25 (0.009 in) to 0.43 mm (0.016 in) (19.1%) and 0.86 (0.034 in) to 1.68 mm (0.066 in) (21.7%) diameter

ranges as well. At almost 18%, the largest percent of particles less than 0.25 mm (0.009 in) in diameter were perlite particles (Figure 2).

The four non-amended substrates were also evaluated for percent porosity, air space, and water holding capacity (WHC) in 2004 using methods by Gessert (4). Percent porosity was highest in vermiculite (69.8%) and lowest in sand (39.8%) (Table 4). Vermiculite was also highest in percent air space (20.3%). Percent air space was similar among sand, perlite, and 1:1:1 P:PB:S. Vermiculite and perlite had the highest water holding capacities of about 50%. Sand had the lowest percent porosity and WHC when compared to the other substrates.

Dolomitic Limestone Amendment

In the 2000 dolomitic lime study, there were linear responses in average root length, number, and rating as dolomotic lime rate increased in the 2:1:1 P:PB:PT substrate (Table 5, Figures 10, 11, and 12). Compared to the control, average root length in the treatment groups increased 35 to 1188%, root number increased 40 to 420%, and root rating increased 33 to 367%. Percent rooting was highest (45.8%) at the rates of 10.0 and 12.5 lbs/yd^3, but percent cuttings survival was 1.3 times higher at 10.0 lbs/yd^3 than at 12.5 lbs/yd^3.

There were linear responses in all measured rooting parameters as dolomitic limestone rate increased in 2004 (Table 6, Figures 13, 14, 15, 16, and 17). There was as much as a 28,433% increase in average root length, 53,100% in root number, and 7400% in root rating as dolomitic lime rate increased. The amendment rate of 10.0 lbs/yd^3 yielded the highest increase in root growth, cutting survival, and rooting percent means as

compared to the control but was statistically similar to 5.0, 7.5, and 12.5 lbs/yd^3. Similar high cutting survival and root percent at 10 and 12.5 lbs/yd^3 were also observed in 2000.

As in 2000 and 2004, there were linear responses in average root length, number, and rating as dolomitic limestone amendment rate increased in the 2:1:1 P:PB:PT substrate in 2005 (Table 7, Figures 18, 19, and 20). Nearly 17% of cuttings survived in the 0.0 lbs/yd^3 amended substrate, but none rooted. Percent cutting survival was not different based on lime rate, but there was a quadratic response in percent rooting (Table 7, Figure 21). Percent rooting increased as dolomitic lime rate increased to 5.0 lbs/yd^3 and then decreased. There was also a linear trend in average root length, number, and rating and a quadratic trend in percent rooting as days after sticking increased (Table 7). Average root length and root number increased from less than 1.0 at 42 DAS to 9.7 mm (0.382 in) (length) and 3.5 (root number) at 70 DAS. As with dolomitic lime rate, all ratings were less than 1.0 but did increase over time. Percent cutting survival was similar among days after sticking, but percent rooting increased from 2.8% at 42 DAS to 19% at 56 DAS before decreasing to about 13% at 70 DAS.

At 42 DAS, only dolomitic limestone rates 2.5 and 5.0 lbs/yd^3 had rooted cuttings (data not shown). All rates except the control (0.0 lbs/yd^3) had rooted cuttings by 56 DAS. Cuttings in the control group survived, but none rooted.

Although cuttings performed better in sand, perlite, and vermiculite across all years, these three substrates were not similar in percent porosity, air space, WHC, or particle size distribution. This inconsistence in the physical property data collected may suggest that the higher rooting, survival, and root growth observed in sand, perlite, and vermiculite might be attributed to chemical properties instead. Erwin et al. (2) also

believed that the degree of rooting observed in their study was more related to the pH of the substrates than the structure of the substrates. The pH of sand, perlite, and vermiculite were most likely influenced by the pH of the greenhouse irrigation water which was approximately 6.4 to 6.5 in 2000 and 7.0 to 7.1 in 2004 and 2005. Sand and perlite both have no buffering capacity or cation exchange capacity (CEC) (6). Although vermiculite has a high CEC and buffering properties, it is neutral in reaction.

Clematis socialis is primarily found growing in slightly neutral to slightly basic soils (14). Laboratory analyses of soil samples collected from the Cherokee County site in Alabama, where the cuttings used in these studies were collected, indicated a soil pH range of 6.8 to 7.3. The irrigation water pH range in this study was comparable to the pH range of the species' native soil. The 1:1:1 P:PB:S substrate was much more acidic with a pH of 4.9 which might explain why the survival rate and root growth in this substrate was so poor. The success of the 1:1:1 P:PB:S substrate could possibly be increased if amended with dolomitic limestone to raise the pH. Results in 2000 indicated a linear relationship between increasing dolomitic limestone amendment rate in the 2:1:1 P:PB:PT substrate and average root length, root number, and root quality ratings (Table 5). The mean pH of the dolomitic limestone-amended 2:1:1 P:PB:PT substrate ranged from 4.8 [0.0 lbs/yd^3 (0.0 kg/m^3)] to 6.9 [7.5 lbs/yd^3 (4.5 kg/m^3)] in 2000 (data not shown) and about 4.3 [0.0 lbs/yd^3 (0.0 kg/m3)] to 7.8 [12.5 lbs/yd^3 (7.4 kg/m^3)] in 2004 and 2005 (Tables 8 and 9). There was a similar linear relationship between dolomitic limestone rate and cutting survival, rooting percent, and root growth in 2004 (Table 6), and between average root length, number, and rating and increasing dolomitic limestone rate in 2005 (Table 7).

Consideration of the data in 2000, 2004, and 2005, reveals that the highest survival, rooting, and root growth means were generally observed at the dolomitic limestone amendment rate range of 5 to 12.5 lbs/yd^3. Using the lower half of this range would yield good results, but better results would be obtained using 10 to 12.5 lbs/yd^3. This higher amendment rate range would certainly be recommended in order to markedly increase the pH of a peat and/or pine bark based substrate such as was used in this study. Although there were positive growth and rooting responses with increasing lime rate observed in this study, rates much higher than 12.5 lbs/yd^3 could be excessive. One must be careful and not over-amend because using excessive amounts of lime to raise the substrate pH could result in nutrient deficiencies or toxicities once cuttings are rooted.

Sand, perlite, and vermiculite performed similarly and far better overall than the 1:1:1 P:PB:S substrate. Sand and perlite have been shown to provide good rooting results and earlier rooting than many other substrates in this study and in studies conducted by others involving commercially cultivated clematis species (2, 3). Results also show that, although vermiculite is slower to root, it can yield results similar to sand and perlite if given time. Sand, perlite, and vermiculite did yield similar root growth results but these substrates do differ in cost. The estimated cost of sand per cutting (per cell in 606 cell pack) was approximately $0.002. Perlite and vermiculite each cost about $0.013 per cutting. The cost of 1:1:1 P:PB:S per cutting was intermediate at about $0.007 but this cost would increase slightly once dolomitic limestone amendment expenses are factored in to make the substrate's pH more suitable for rooting clematis cuttings. The extra expense and labor time to prepare the mixture would also make the 1:1:1 P:PB:S substrate a less desirable substrate to use. Sand, perlite, and vermiculite are more pH

stable and much less likely to require amending. Sand is the least expensive of the three, but if weight is a concern, perlite and vermiculite would be more suitable choices. In terms of substrate pH, affordability, and availability, sand would be the most appropriate choice of the un-amended substrates for rooting *Clematis socialis* stem cuttings.

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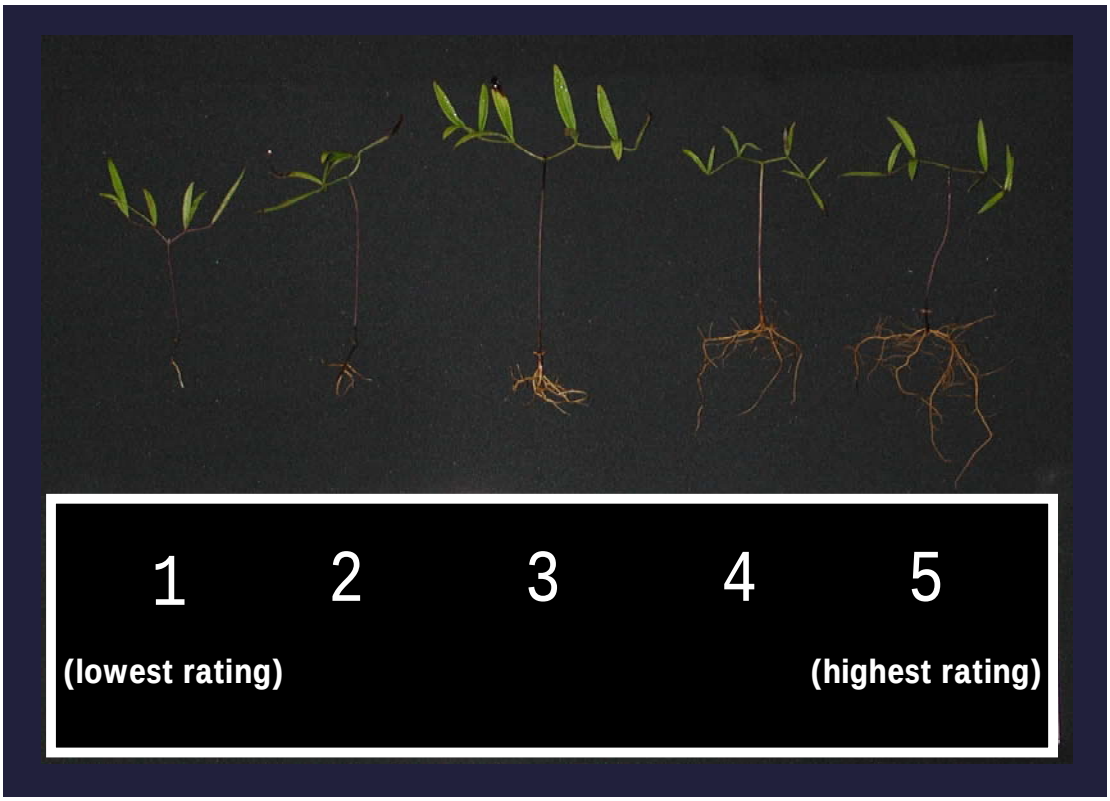
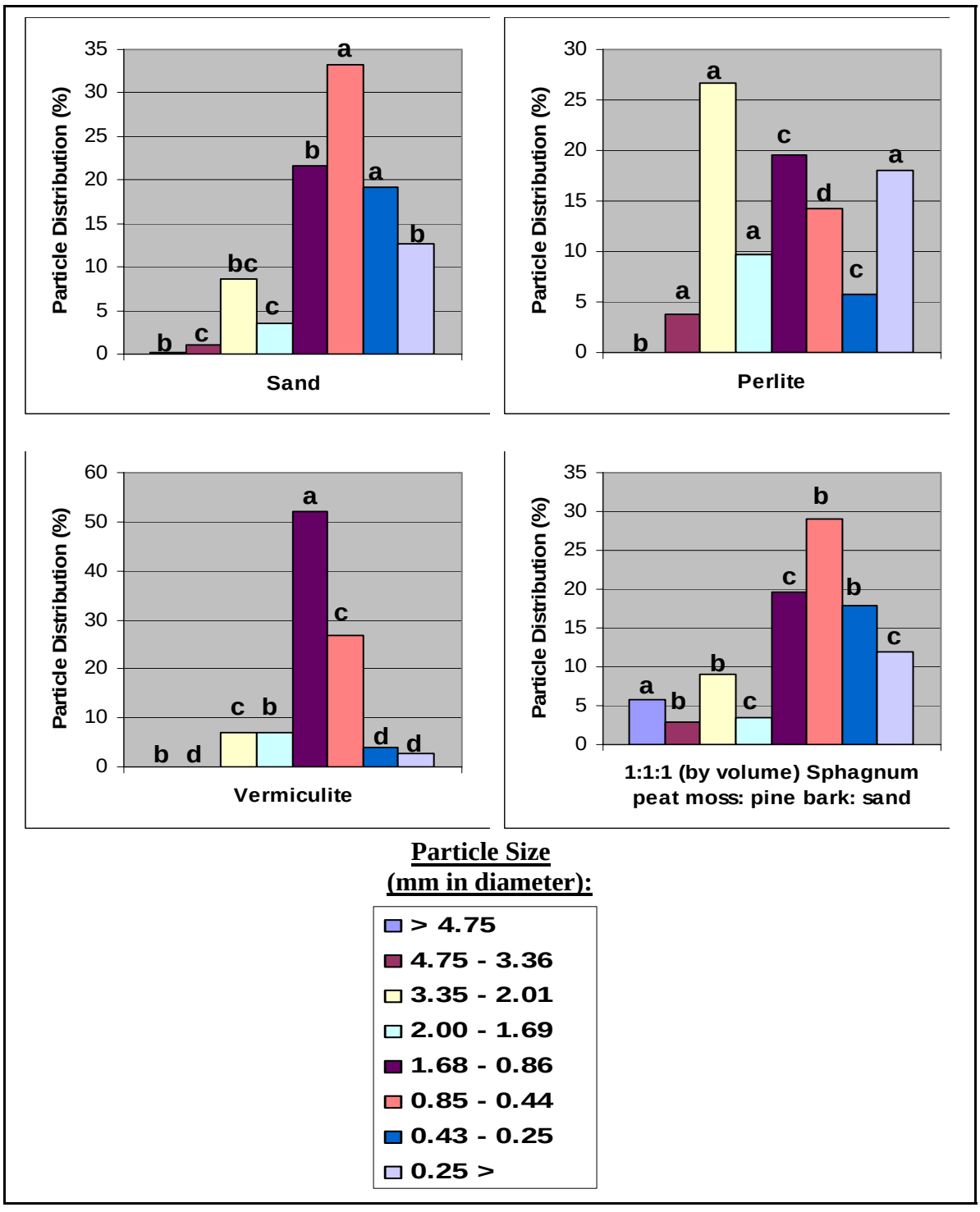


Figure 1. Example of visual rating scale used for root evaluation of *Clematis socialis* (Kral) stem cuttings.

Rating	Root Development
0	No root growth; may or may not have callus tissue (Not shown).
1	One to very few primary roots that are short.
2	Several primary roots that are short.
3	Numerous primary roots but short; rarely any secondary roots.
4	Numerous primary roots with some secondary roots; roots longer.
5	Numerous primary and secondary roots with some tertiary roots; longest roots; root system most proportional to cutting size.

Figure 2. Percent particle distribution[Z] in each of four non-amended substrates.



[Z]Substrates with same letters for same particle size distribution range were similar at *P* = 0.05 using Duncan's Multiple Range Test.

Figure 3. Regression analysis of average root length for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2000.

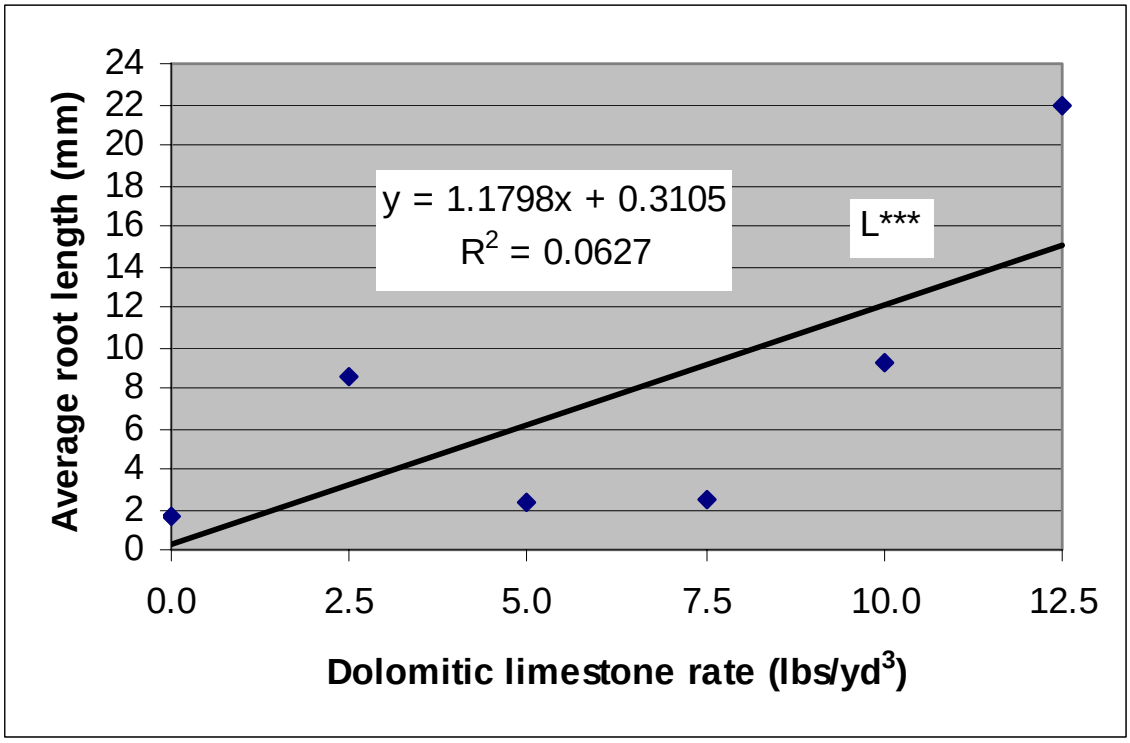


Figure 4. Regression analysis of average root number for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2000.

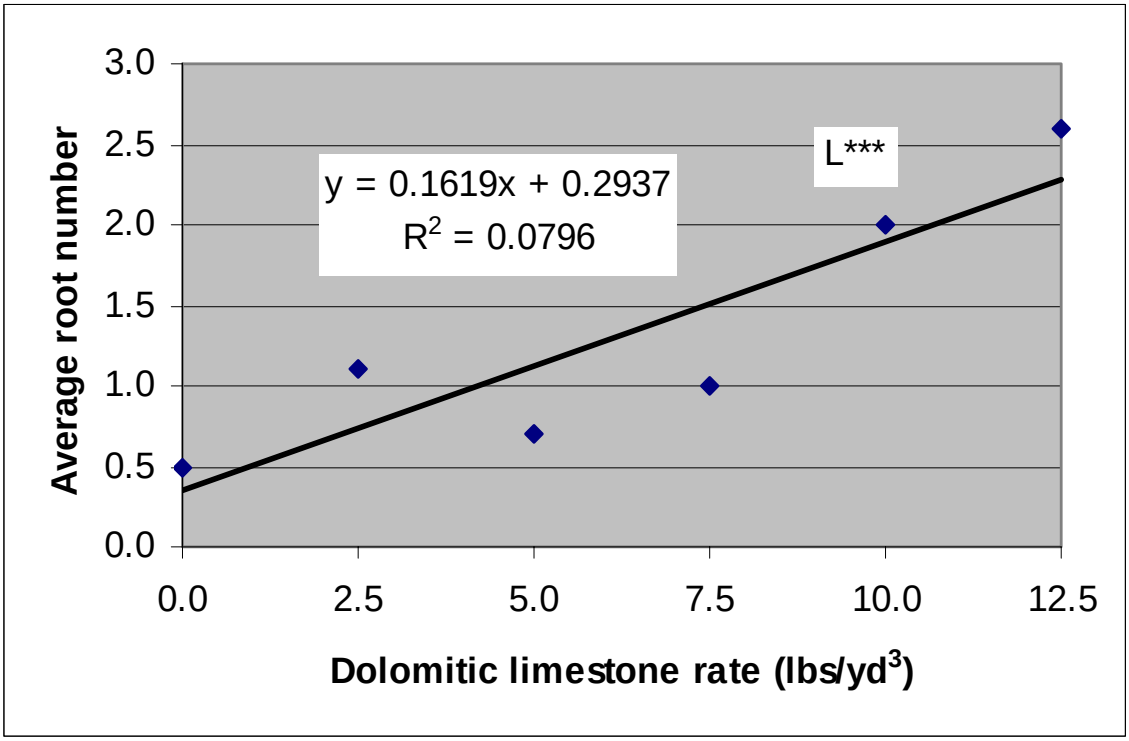


Figure 5. Regression analysis of average root rating for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2000.

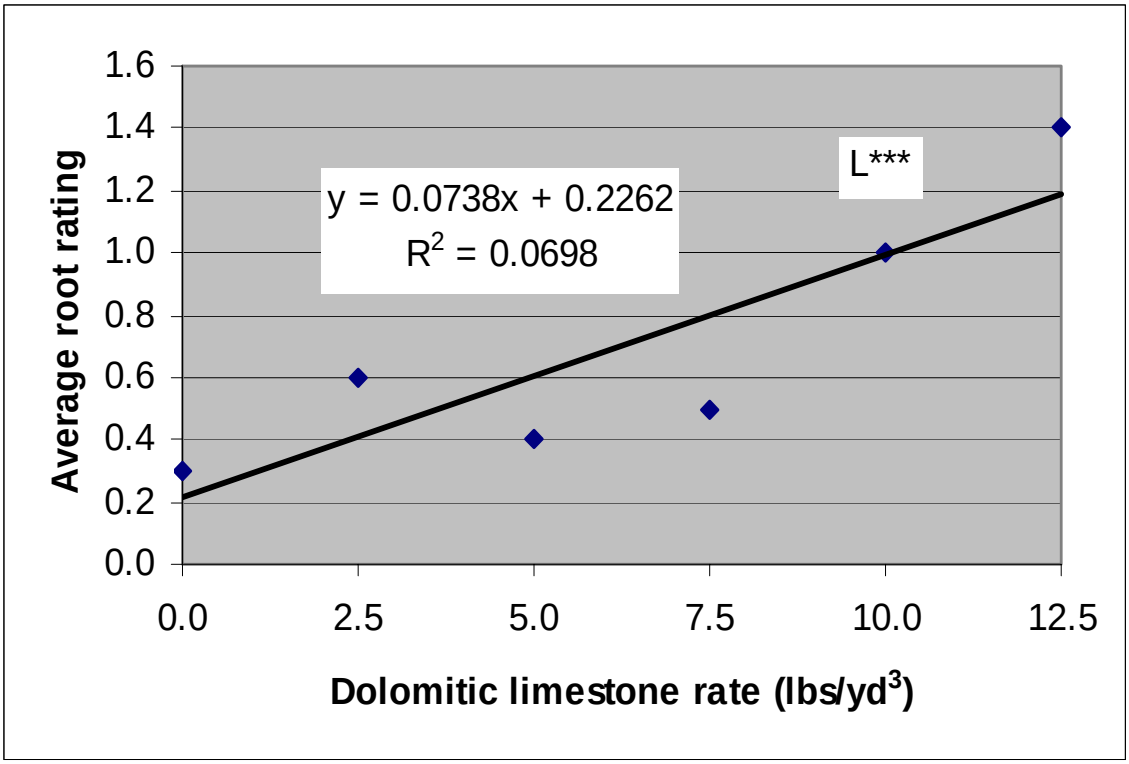


Figure 6. Regression analysis of average root length for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

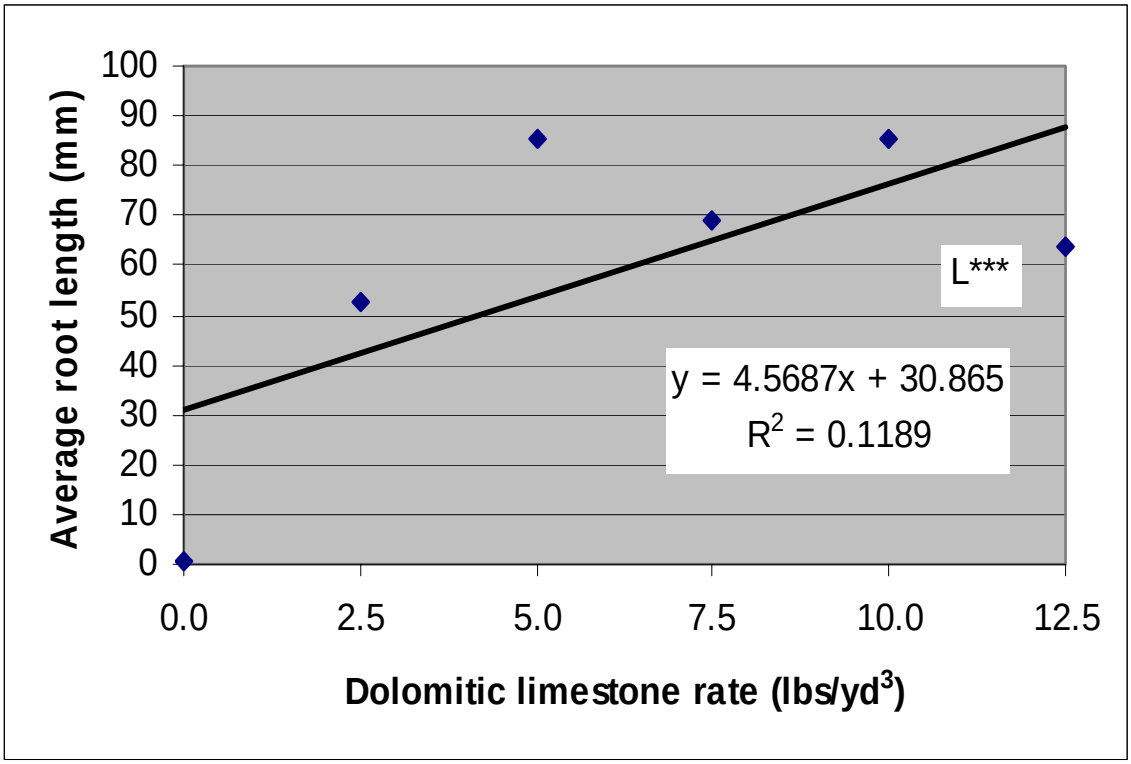


Figure 7. Regression analysis of average root number for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

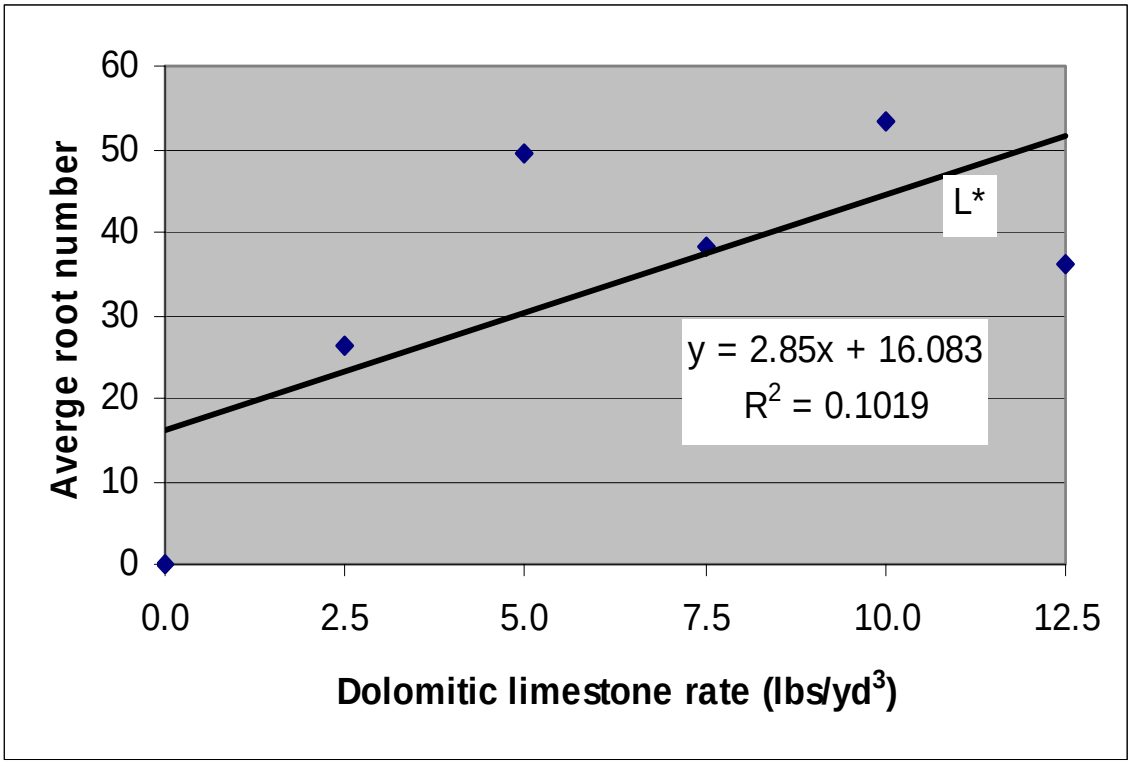


Figure 8. Regression analysis of average root rating for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

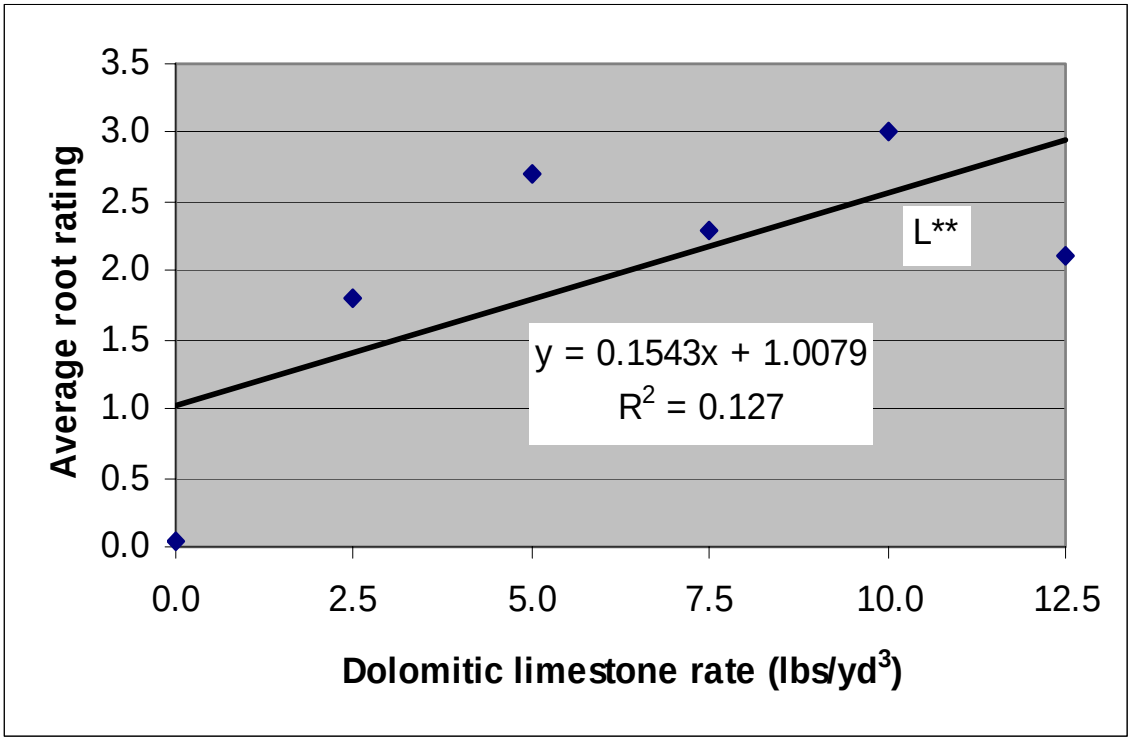


Figure 9. Regression analysis of average percent cutting survival for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

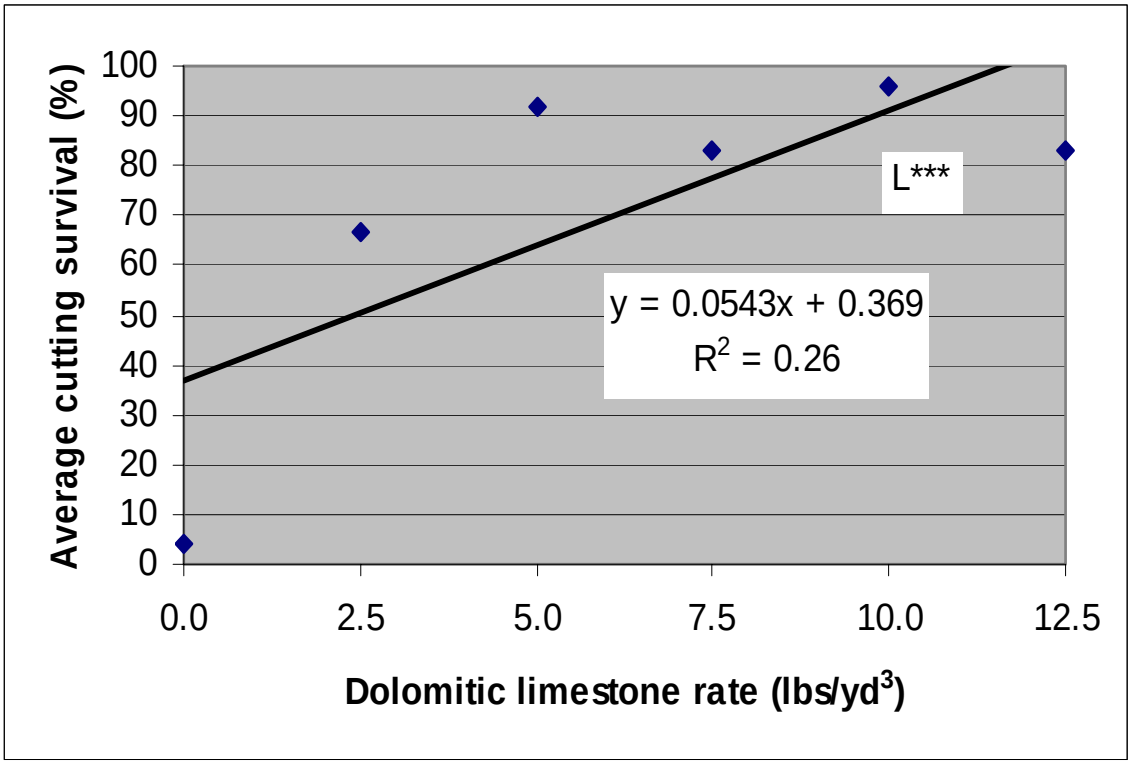


Figure 10. Regression analysis of average rooting percent for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

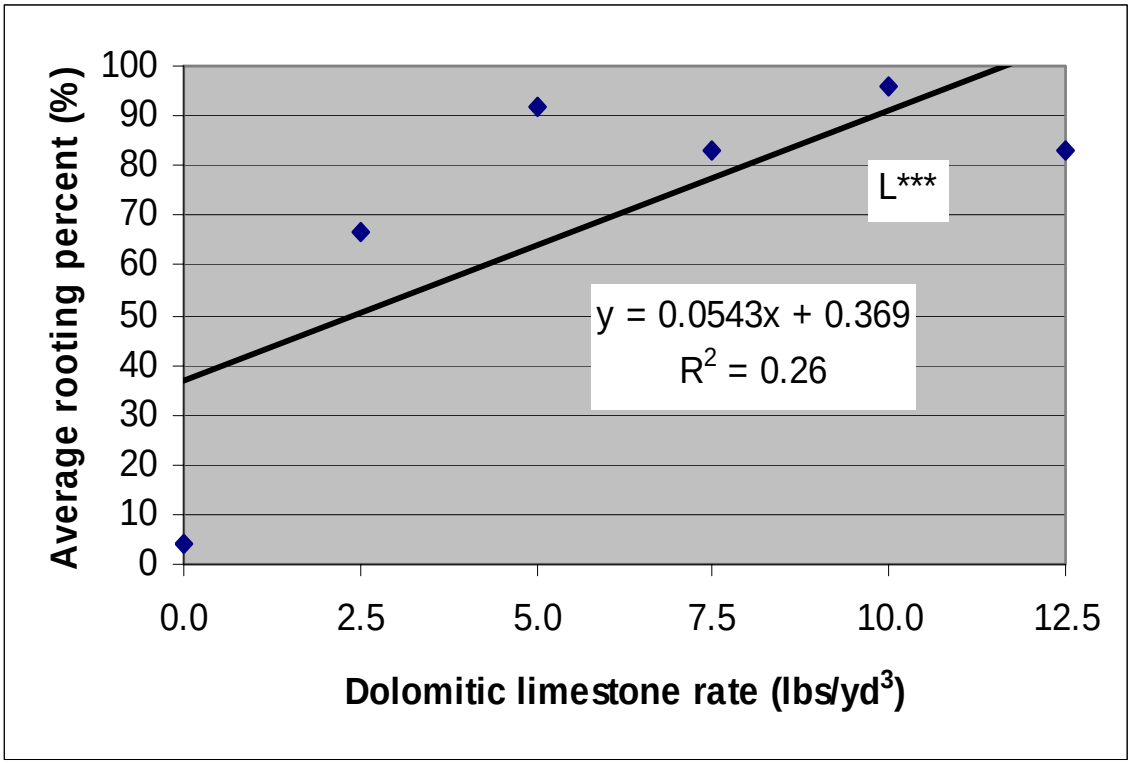


Figure 11. Regression analysis of average root length for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

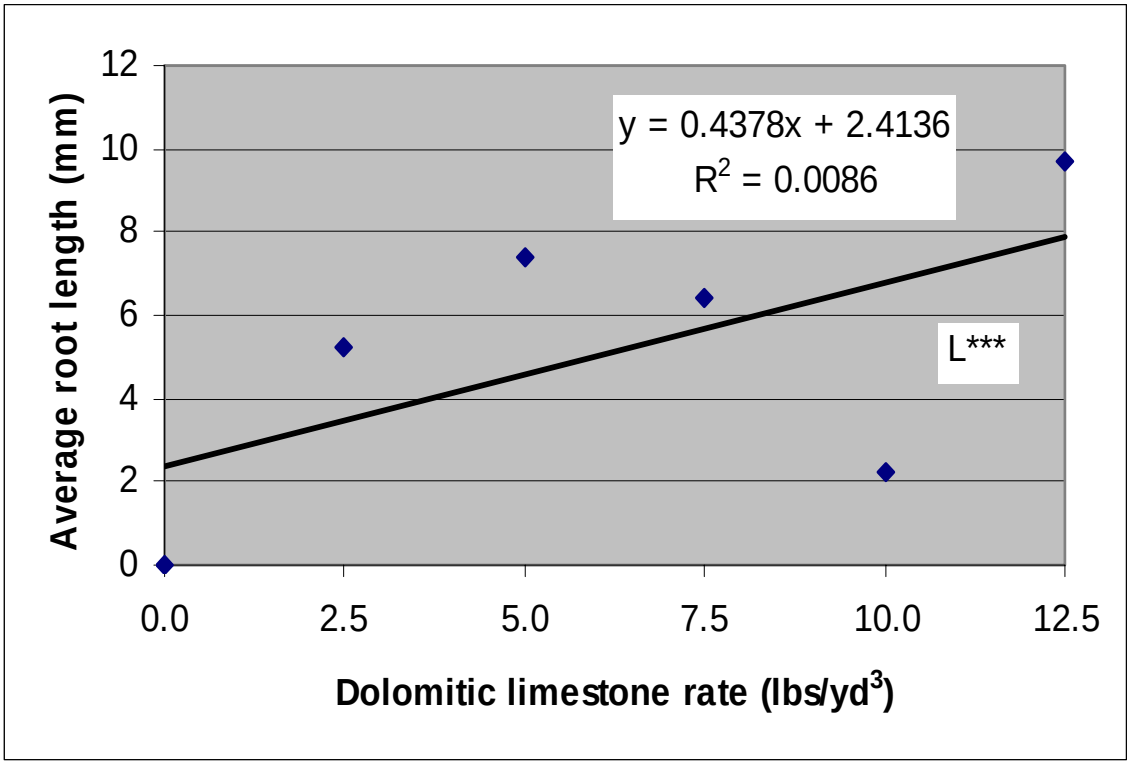


Figure 12. Regression analysis of average root number for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

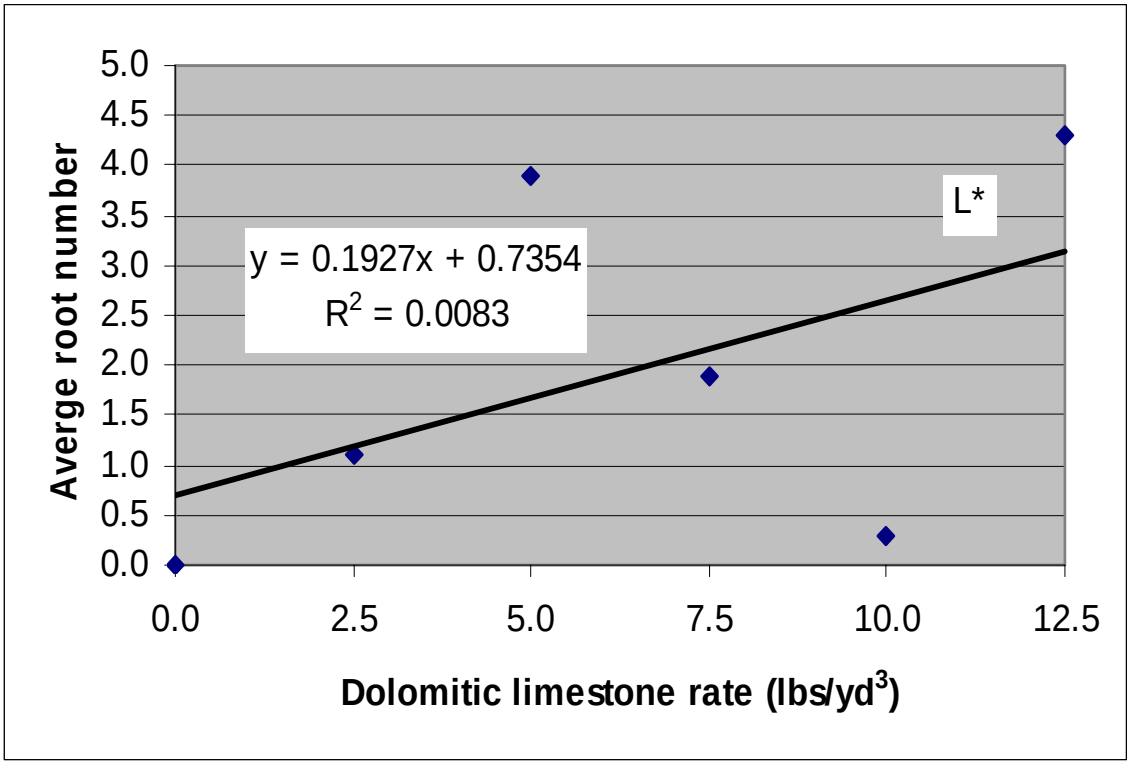


Figure 13. Regression analysis of average root rating for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

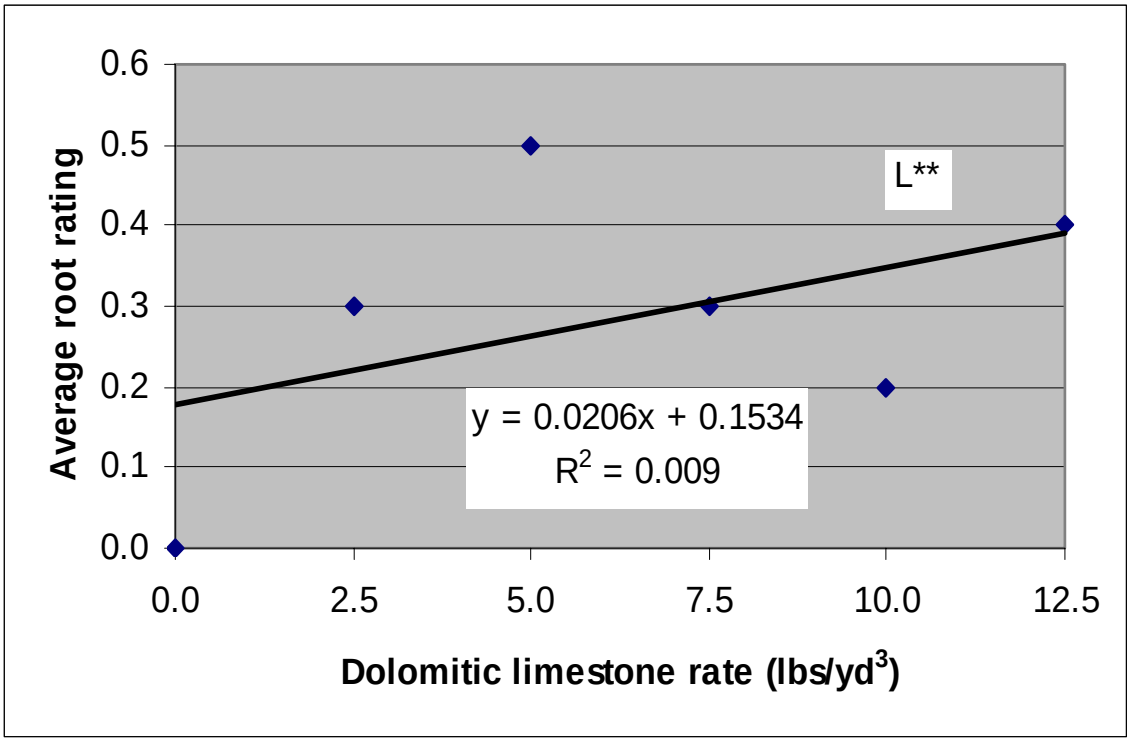


Figure 14. Regression analysis of average rooting percent for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

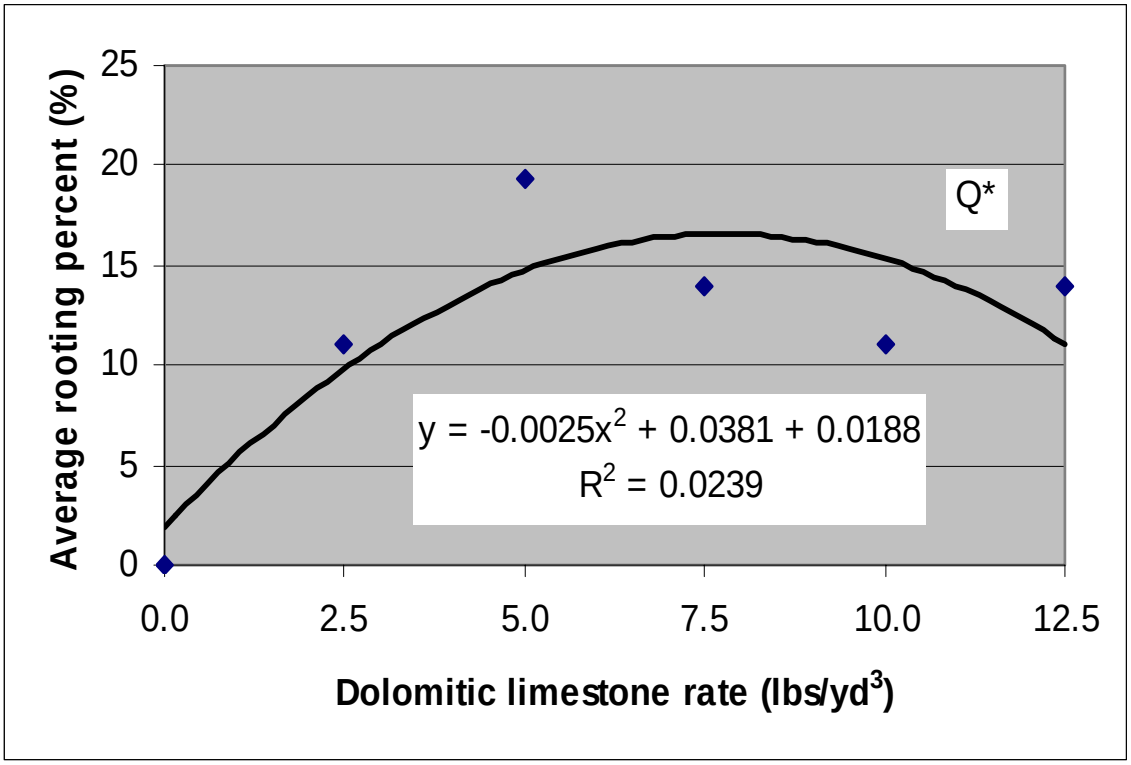


Table 1. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings rooted in four non-amended substrates 70 days after sticking in 2000.

Substrate	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
Sand	28.8 a[W]	4.2 a	2.2 a	70.8 a	70.8 a
Perlite	15.0 a	4.2 a	1.5 ab	66.7 ab	66.7 a
Vermiculite	23.9 a	1.7 b	1.4 b	58.3 ab	58.3 a
1:1:1 (by volume) peat/pine bark/sand	0.1 b	0.2 b	0.1 c	12.5 b	12.5 b

[Z]Roots were rated on a scale 0 to 5, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at $P = 0.05$ using Duncan's Multiple RangeTest.

Table 2. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings rooted in four non-amended substrates 70 days after sticking in 2004.

Substrate	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
Sand	49.1 b[W]	39.6 b	3.5 b	100.0 a	100.0 a
Perlite	25.7 c	21.2 b	1.5 c	95.8 a	95.8 a
Vermiculite	133.6 a	102.8 a	4.4 a	91.7 a	91.7 a
1:1:1 (by volume) peat/pine bark/sand	6.0 c	2.1 c	0.3 d	33.3 b	33.3 b

[Z]Roots were rated on a 0 to 5 scale, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at $P = 0.05$ using Duncan's Multiple Range Test.

Table 3. Average root length, number, rating[Z], cutting survival and, rooting percent for *Clematis socialis* stem cuttings rooted in four non-amended substrates in 2005.

Substrate	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
Sand	5.8 a[W]	1.4 ab	0.5 a	52.1 ab	20.8 a
Perlite	9.5 a	3.2 a	0.7 a	60.4 a	37.5 a
Vermiculite	9.6 a	2.9 a	0.5 a	52.1 ab	18.8 b
1:1:1 (by volume) peat/pine bark/sand	0.0 b	0.0 b	0.0 b	35.4 b	0.0 c
Days After Sticking[V]					
42	2.0 b[W]	0.6 a	0.1 b	62.5 a	9.4 b
56	9.7 a	3.0 a	0.7 a	57.8 a	26.6 a
70	7.1 a	2.0 a	0.5 ab	29.7 b	21.9 a
Significance[U]	L***,Q***	NS	Q***,L***	L***	L**

[Z]Roots were rated on a scale 0 to 5, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Values within columns followed by same letter are similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]No interaction between substrate and days after sticking at 5% level.

[U]Nonsignificant (NS) at the 0.05 level, Quadratic (Q) or Linear (L) response at the 0.001 (***) or 0.01 (**) level within columns.

Table 4. Percent porosity, air space, and water holding capacity (WHC) of four non- amended substrates in 2004.

Substrate	Porosity (%)[Z]	Air space (%)[Y]	WHC (%)[X]
Sand	39.8 d[W]	13.5 b	26.3 c
Perlite	66.3 b	16.3 b	49.8 a
Vermiculite	69.8 a	20.3 a	49.5 a
1:1:1 (by volume) peat/pine bark/sand	49.3 c	13.5 b	35.8 b

[Z]Percent porosity = (Total volume of water required to saturate substrate ÷ total Volume of container) **x** 100.

[Y]Percent air space = (Volume of drained water ÷ total volume of container) **x** 100.

[X]Percent WHC = (Percent porosity **-** percent air space) **x** 100.

[W]Means followed by similar letters within columns were similar at *P* = 0.05 using Duncan's Multiple Range Test.

Table 5. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2000.

Rate (lbs/yd^3)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
0.0	1.7 b[W]	0.5 c	0.3 c	25.0 c	16.7 b
2.5	8.5 b	1.1 bc	0.6 bc	41.5 bc	25.0 ab
5.0	2.3 b	0.7 c	0.4 bc	41.5 bc	20.8 b
7.5	2.5 b	1.0 bc	0.5 bc	33.3 bc	25.0 ab
10.0	9.3 b	2.0 ab	1.0 ab	70.5 a	45.8 a
12.5	21.9 a	2.6 a	1.4 a	54.0 ab	45.8 a
Significance[V]	L***,Q***	L***	L***	NS	NS

[Z]Roots were rated on a scale 0 to 5, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at *P* = 0.05 using Duncan's Multiple Range Test.

[V]Nonsignificant (NS) at 0.05 level, Quadratic (Q) or Linear (L) response at the 0.001 (***) level within columns.

Table 6. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone 70 days after sticking in 2004.

Rate (lbs/yd^3)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
0.0	0.3 c[W]	0.1 c	0.04 c	4.2 c	4.2 c
2.5	52.5 b	26.4 b	1.8 b	66.7 b	66.7 b
5.0	85.4 a	49.4 a	2.7 ab	91.7 a	91.7 a
7.5	69.2 ab	38.1 ab	2.3 ab	83.3 ab	83.3 ab
10.0	85.6 a	53.2 a	3.0 a	95.8 a	95.8 a
12.5	63.6 ab	36.2 ab	2.1 ab	83.3 ab	83.3 ab
Significance[V]	L***,Q***	L*, Q*	L**,Q**	L***,Q***	L***,Q***

[Z]Roots were rated on a 0 to 5 scale, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]Linear (L) or Quadratic (Q) response at the 0.05 (*), 0.01 (**), or 0.001 (***) level within columns.

Table 7. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings rooted in 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

Dolomitic limestone rate (lbs/yd^3)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
0.0	0.0 c[W]	0.0 a	0.0 b	16.7 a	0.0 b
2.5	5.2 abc	1.1 a	0.3 ab	27.8 a	11.1 ab
5.0	7.4 ab	3.9 a	0.5 a	33.3 a	19.4 a
7.5	6.4 ab	1.9 a	0.3 ab	30.6 a	13.9 ab
10.0	2.2 bc	0.3 a	0.2 ab	27.8 a	11.1 ab
12.5	9.7 a	4.3 a	0.4 ab	30.6 a	13.9 ab
Significance[V]	L***	L*	L**	NS	Q*
Days After Sticking[U]					
42	0.14 c[W]	0.03 b	0.03 b	31.9 a	2.8 b
56	5.6 b	2.3 ab	0.4 a	30.6 a	19.4 a
70	9.7 a	3.5 a	0.4 a	20.8 a	12.5 ab
Significance[V]	L***	L*	L**	NS	Q*

[Z]Roots were rated on a 0 to 5 scale, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within rows were similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]Nonsignificant (NS) at the 0.05 level, Quadratic (Q) or Linear (L) response at the 0.001 (***), 0.01 (**), or 0.05 (*) level to increasing dolomitic limestone rate.

[U]No interaction between dolomitic limestone rate and days after sticking at 5% level.

Table 8. Virginia Tech Extraction Method (VTEM) results for a 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2004.

	Leachate pH					
	Dolomitic limestone amendment rate					
Extraction[Z]	0.0 lbs/yd^3	2.5 lbs/yd^3	5.0 lbs/yd^3	7.5 lbs/yd^3	10.0 lbs/yd^3	12.5 lbs/yd^3
1	4.0[Y]	4.6	5.4	6.3	6.7	7.0
2	4.1	5.0	6.6	7.4	7.8	7.9
3	4.3	5.2	6.6	7.3	7.6	7.7
4	4.7	5.6	7.1	7.8	8.0	8.2
5	4.5	5.7	7.0	7.6	8.0	8.2
Average	4.3	5.2	6.5	7.2	7.6	7.8

[Z]Extractions conducted at two week intervals.

[Y]Linear response in pH within rows and columns at the 0.01% level.

Table 9. Virginia Tech Extraction Method (VTEM) results for a 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate amended with varying rates of dolomitic limestone in 2005.

	Leachate pH					
	Dolomitic limestone amendment rate					
Extraction[Z]	0.0 lbs/yd^3	2.5 lbs/yd^3	5.0 lbs/yd^3	7.5 lbs/yd^3	10.0 lbs/yd^3	12.5 lbs/yd^3
1	4.8[Y]	5.9	6.7	7.0	7.5	7.6
2	4.9	6.1	7.4	7.5	7.8	8.0
3	4.9	6.4	7.3	7.5	7.9	7.9
4	4.7	6.3	7.1	7.4	7.7	7.9
5	4.6	6.2	7.0	7.7	7.9	7.9
6	4.8	6.3	7.0	7.3	7.4	7.6
Average	4.8	6.2	7.1	7.4	7.7	7.8

[Z]Extractions conducted at two week intervals.

[Y]Linear response in pH within rows at 0.1% level and quadratic response within columns at the 0.01% level.

CHAPTER III

INFLUENCE OF GROWTH REGULATOR ON THE ROOTING OF *CLEMATIS SOCIALIS* (KRAL) STEM CUTTINGS

Abstract

Alabama leather flower, *Clematis socialis* (Kral), stem cuttings were stuck in a 2:1:1 (by volume) sphagnum peat moss moss: pine bark: perlite substrate amended with dolomitic limestone to compare the efficiency of increasing growth regulator concentration on rooting this endangered species. Results showed that cuttings could root without growth regulator treatment and that the IBA/NAA growth regulator applications used were not necessary but did improve rooting. Certain concentrations did however initiate rooting earlier than the control and resulted in more root growth. Average root length, number, rating, cutting survival, and percent rooting data observed was generally higher and earlier at concentrations between 3000/1500 ppm and 4500/2250 ppm IBA/NAA.

Index words: Alabama leather flower, indole-3-butyric acid, naphthalene acetic acid, endangered species, propagation

Species used in this study: *Clematis socialis* (Kral)

Significance to the Nursery Industry

Establishing cutting requirements of *Clematis socialis* stem cuttings could be used for producing propagules for conservational and commercial horticultural purposes. This research could help promote *C. socialis'* attractive flower color and spreading growth habit as desirable characteristics that could be bred into commercial species/cultivars currently produced to clematis hybridizers and native plant growers. Results show that IBA/NAA growth regulator application is not necessary for rooting *C. socialis* stem cuttings because some cuttings rooted without receiving a growth regulator treatment. Concentrations of IBA/NAA growth regulator between 3000/1500 ppm and 4500/2250 ppm did, however, initiate rooting quicker and resulted in root growth greater than if cuttings had not been treated.

Introduction

Clematis socialis (Kral), also known as the Alabama leather flower, is an endangered species with only six reported populations in northeast Alabama and northwest Georgia (6, 8). *C. socialis* is an erect, non-vining, herbaceous perennial with blue-violet urn shaped flowers 0.79 to 0.98 inches (2.0 to 2.5 cm) long occurring in late April through May. The bright green foliage has an entire leaf margin and has a simple to pinnately compound leaf. Unlike many other clematis species, *C. socialis* forms dense clones by underground rhizomes (4, 5, 1).

C. socialis was federally listed as an endangered species by the United States Fish and Wildlife Service in September of 1986 because of small population numbers and limited natural distribution, as well as known habitat on sites subject to human

disturbance (9, 10). Propagation research for this species will aid in conserving its existing populations and establishing additional self-sustaining populations, provide genetic material for future hybridization and genetic preservation, and potentially introduce the Alabama leather flower as a new ornamental crop, especially to native plant nurseries.

In 1992, Erwin and Schwarze reported that the indole-3-butyric acid (IBA) application used (0.1% Hormex 1) in their cutting propagation study regarding commercially produced species/cultivars was not necessary because it did not improve rooting for any species/cultivar tested (2). In later work, IBA application was reportedly only effective at increasing cutting survival of the cultivar 'Jackmani' (3). Other than the articles by Erwin and others, there is little published research on the influence of IBA and NAA growth regulators on rooting clematis stem cuttings, cultivated or native. There were sources from which general IBA concentration recommendations could be obtained, but usually varied from source to source and species to species. The 1996 Ball perennial propagation manual stated that cuttings are the most common clematis propagation method used and that propagators who use growth regulators often use a concentration of 2,000 to 3000 ppm IBA in a liquid quick dip for approximately 3 to 5 seconds (7). The objective of this study was to evaluate the effects of increasing rates of the growth regulators, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), treatments on root initiation, root length, root number, root quality, and survival of *C. socialis* stem cuttings.

Materials and Methods

Stem cuttings of *C. socialis* were taken May 26th in 2000, June 11th in 2004, and June 2nd in 2005 from a roadside population located in Cherokee County, Alabama (34.2°N-85.67°W). The cuttings were placed in plastic bags on ice and transported back to Auburn University's Paterson Greenhouse Complex. Later in the day, two-node cuttings in 2000 and 2005, and three-node cuttings in 2004 were prepared and re-cut to leave one inch (2.54 cm) of stem below the bottom-most node before receiving a liquid basal application of Dip 'N Grow (Astoria-Pacific, Inc., Clackamas, Oregon) with the bottom-most node submerged for approximately five seconds. The following IBA and NAA concentrations were used in the years noted:

Table 1. Indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) growth regulator concentrations used in 2000, 2004, and 2005.

IBA/NAA concentration (ppm)		
2000	**2004**	**2005**
0/0 (control)	0/0 (control)	0/0 (control)
250/125	1500/750	1500/750
500/250	3000/1500	3000/1500
1000/500	4500/2250	4500/2250
1500/750	6000/3000	6000/3000
3000/1500	7500/3750	7500/3750
5000/2500		

The treated cuttings were stuck in 606 cell packs with a volume of 10 in^3 (163 cm^3) per cell containing a 1:1:1 (by volume) sphagnum peat moss: pine bark: perlite substrate amended with dolomitic limestone at a rate of 10 lbs/yd^3 (5.9 kg/m^3). The

Virginia Tech Extraction Method (VTEM) (11) was used to monitor pH of the amended substrate mix at the beginning and end of the experiment.

Trays containing the cell packs were placed under an intermittent mist system that cycled from 7:30 A.M. to 6:30 P.M. In 2000, the cuttings were kept in a full sun double-poly greenhouse and misted for 6 seconds every 5 minutes. In studies additional to the growth regulator study in 2000, cutting survival was higher in shade versus sun (data not shown). With shade, cuttings also required less frequent misting which is why cuttings in 2004 and 2005 were kept in a shaded glass greenhouse and misted for 5 seconds at 15 minute intervals.

The experiments were arranged in a randomized complete block design using four replications (six cuttings per replication) of each substrate in 2000 and six replications (four cuttings per replication) of each substrate in 2004. In 2005, there were four replications (4 cuttings per replication) of each substrate for each of three harvest dates [42, 56, and 70 days after sticking (DAS)]. Data were collected only once at 70 DAS in 2000 and 2004. Data collected included subjective root ratings [0 to 5, with 5 representing the best root system (Figure 1)], root number [roots > 5 mm (0.197 in) in length)], average root length of the three longest roots, and cutting survival. A cutting was considered alive if green foliage was present. All data were evaluated using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test ($P = 0.05$). The 2005 data were also evaluated using regression analysis. The 2005 IBA/NAA growth regulator concentration and days after sticking results will be discussed as main effects only because there were no interactions between the two at the 5% level using ANOVA for any of the growth parameters measured.

Results and Discussion

In 2000, average root length, number, rating, cutting survival, and rooting percent changed linearly as IBA and NAA growth regulator concentration increased (Table 2, Figures 2, 3, 4, 5, and 6). Average root length ranged from 20.4 mm (0.803 in) at 0/0ppm IBA/NAA to 77.4 mm (3.047 in) at 5000 IBA/2500 NAA ppm. Average root number was as high as 5.3 at the highest IBA/NAA concentration. Average root rating ranged from 1.0 (0/0 ppm IBA/NAA), having one to few primary roots, to 3.0 (5000/2500 ppm IBA/NAA), having numerous primary roots with some secondary roots on many cuttings. The IBA/NAA concentrations of 1000/500 ppm, 1500/750 ppm, 3000/1500 ppm, and 5000/2500 ppm were all similar in their percent cutting survival which varied from 79.2% at 1500/750 ppm IBA/NAA up to 95.8% at 3000/1500 ppm IBA/NAA. About half as many survived and rooted in the control group (0/0 ppm IBA/NAA). Percent rooting was as high as 91.7% at 3000/1500 ppm but was similar to 5000/2500 ppm IBA/NAA with 87.5% rooting. In fact, the 3000/1500 ppm and 5000/2500 ppm IBA/NAA concentrations were similar in all growth parameters except average root length in 2000.

Since there was a linear relationship among growth parameters and increasing IBA/NAA growth regulator concentration in 2000, the IBA/NAA concentration range used in 2000 (0/0 ppm to 5000/2500 ppm) was increased in 2004 to range from 0/0 ppm to 7500/3750 ppm. Unlike in 2000, there were no linear or significant growth responses to the increasing IBA/NAA concentrations in 2004 (Table 3). Cuttings in 2004 were, however, much better developed than those in 2000. Average root length in 2000 ranged from 19.1 mm (0.752 in) at 250/125 ppm IBA/NAA to 77.4 mm (3.047 in) at 5000/2500

ppm IBA/NAA. Average root length in 2004 ranged from 68.2 mm (2.685 in) at the control to as high as 92.6 mm (3.646 in) at 3000/1500 ppm IBA/NAA. Average root number was never more than 6.0 in 2000 but averaged about 30 to 50 in 2004. Average root rating ranged from 1.0 to 3.0 in 2000 and 2.4 to 3.3 in 2004. Cutting survival and rooting percent were also generally higher in 2004 than in 2000.

There were no differences among any of the IBA/NAA concentrations evaluated in any growth parameters measured in 2004 even though higher IBA/NAA concentrations and a larger range than 2000 were used. There could possibly have been differences between concentrations earlier in the study, but because there was only one harvest date at 70 DAS, this could not be determined. There were three harvest dates when the study was repeated in 2005 in the hopes of finding any initial differences in rooting and root development that might have been lost over time in the 2004 study. The same IBA and NAA concentrations used in 2004 were used in 2005, and only quadratic responses in average root length and rating were observed over IBA/NAA concentrations (Table 4, Figures 7 and 8). Regression analysis of growth data over the three harvest dates in 2005 did indicate linear responses in average root length and rating over time (Table 4). All growth parameters other than average root length and rating were not significant in 2005.

In 2005, average root length was highest at 3000/1500 ppm [10.7 mm (0.421 in)], 4500/2250 ppm [10.7 mm (0.421 in)], and similar to 6000/3000 ppm [6.3 mm (0.248 in)] IBA/NAA (Table 4). Average root rating was less than 1.0 in all IBA/NAA concentrations, but was due to the low rooting percent averages. There were cuttings rooted, about 3.0%, as early as 42 DAS but root growth was lower than that observed at

70 DAS. Also, at 42 DAS, only cuttings treated with 4500/2250 ppm and 6000/3000 ppm IBA/NAA were rooted; all treatments had rooted cuttings by 56 DAS (data not shown). Average root length, number, and rating were all less than 1.0 at 42 DAS (Table 4). Average root rating at 70 DAS was also less than 1.0, but average root length was 15.7 mm (0.618 in) and average root number was 4.7.

Rooting in the control group indicated that the IBA/NAA growth regulator application was not required to initiate rooting of some *C. socialis* stem cuttings, but more rooting and root growth in the groups that did receive applications show improved overall root development. At 42 DAS, no cuttings in the control group were rooted, but there were rooted cuttings in the some of the treated groups. The higher root growth numbers observed in the IBA/NAA treatment groups were a result of earlier rooting which allowed for a longer period of time than the control group had to develop root growth.

In the observed data it appears that the more optimal IBA/NAA concentration range, in terms of root growth, cutting survival, and rooting, is 3000/1500 ppm to 4500/2250 ppm. In all three years, root growth, cutting survival, and rooting percent means were generally higher between 3000/1500 ppm and 4500/2250 ppm IBA/NAA. In 2005, cuttings rooted earlier when treated at 4500/2250 ppm and 6000/3000 ppm. In 2004 and 2005, there was a general decrease in root growth, cutting survival, and rooting percent at IBA/NAA concentrations above 6000/3000 ppm, which is why a concentration this high or higher would not be recommended. A rate as low as 1500/750 ppm IBA/NAA could yield reasonably good rooting results but for better and earlier rooting

and root growth of *Clematis socialis* stem cuttings, an IBA/NAA growth regulator concentration within the range of 3000/1500 to 6000/3000 ppm would be recommended.

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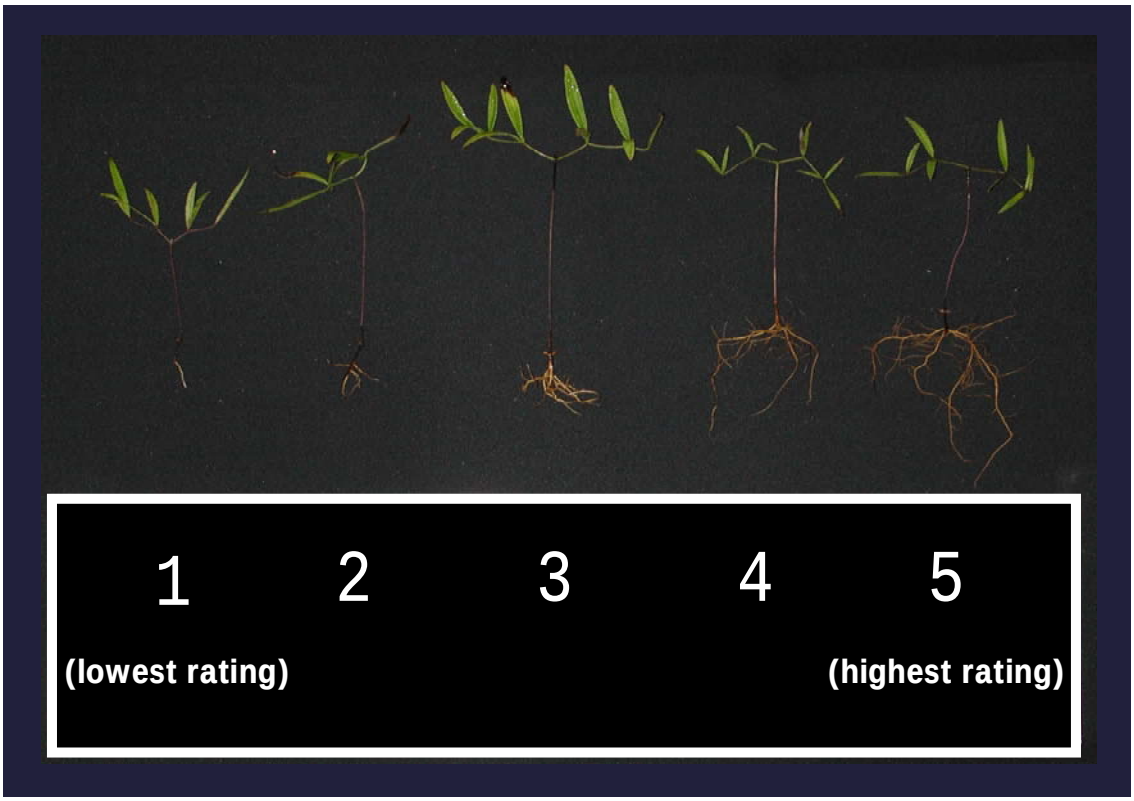
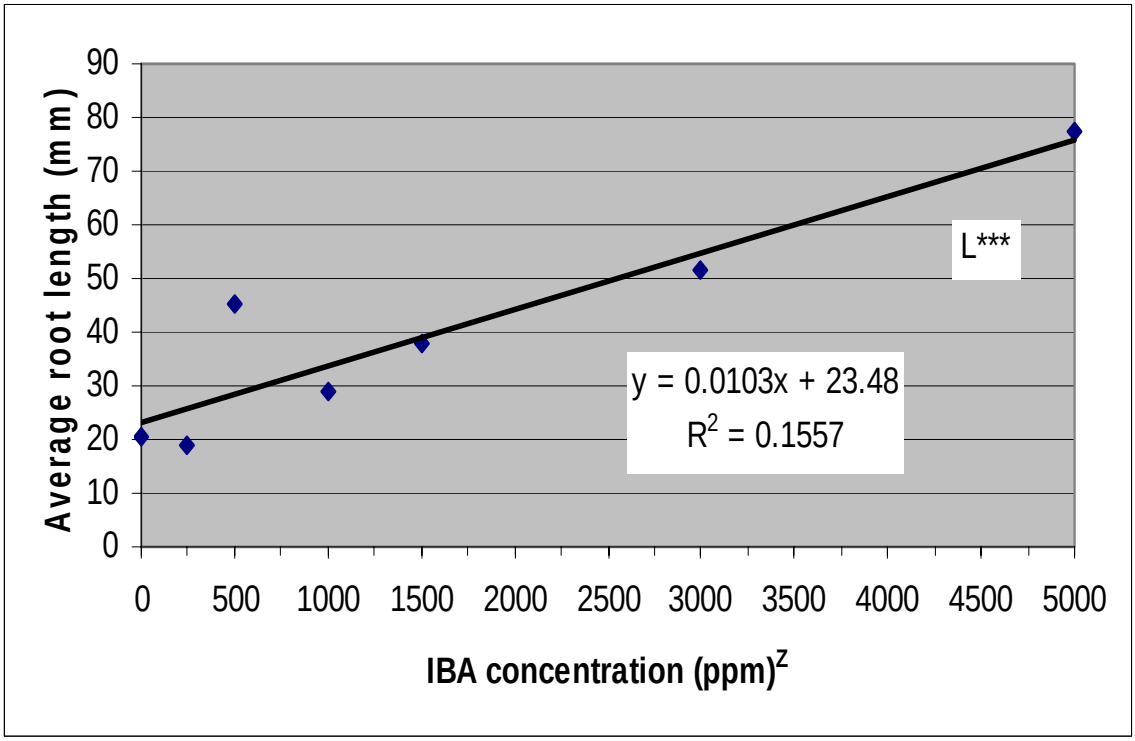


Figure 1. Example of visual rating scale used for root evaluation of *Clematis socialis* (Kral) stem cuttings.

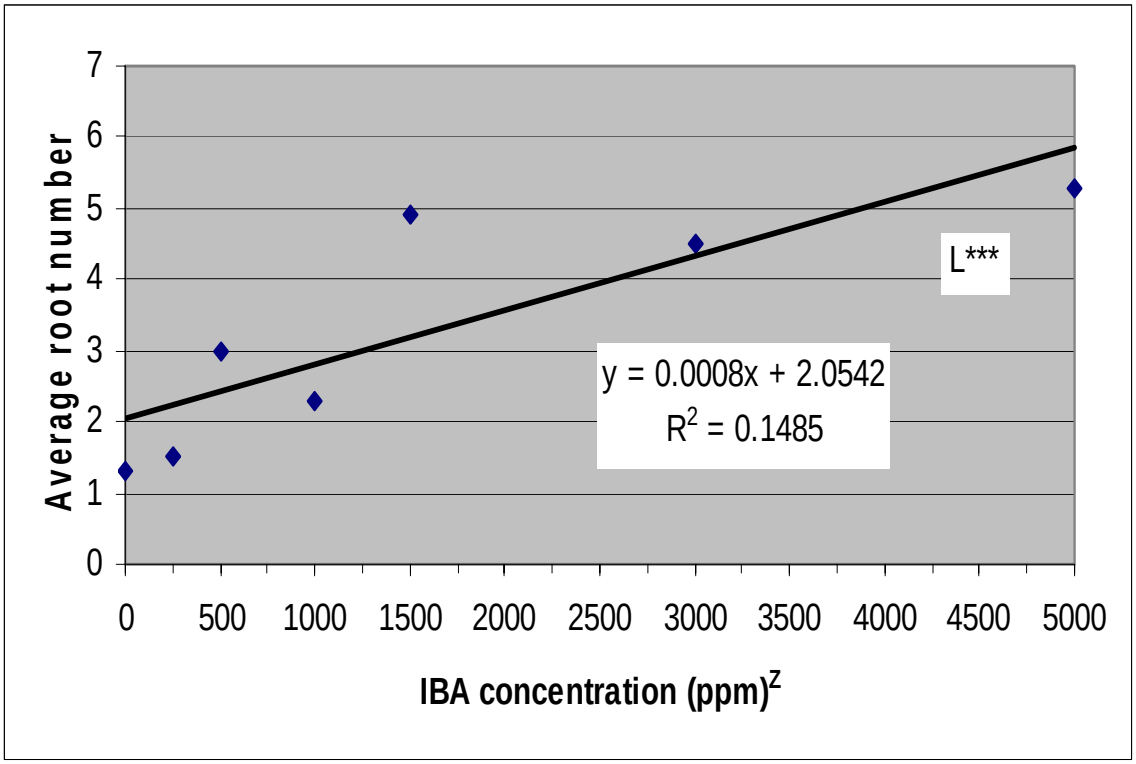
Rating	Root Development
0	No root growth; may or may not have callus tissue (Not shown).
1	One to very few primary roots that are short.
2	Several primary roots that are short.
3	Numerous primary roots but short; rarely any secondary roots.
4	Numerous primary roots with some secondary roots; roots longer.
5	Numerous primary and secondary roots with some tertiary roots; longest roots; root system most proportional to cutting size.

Figure 2. Regression analysis of average root length for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) 70 days after sticking in 2000.



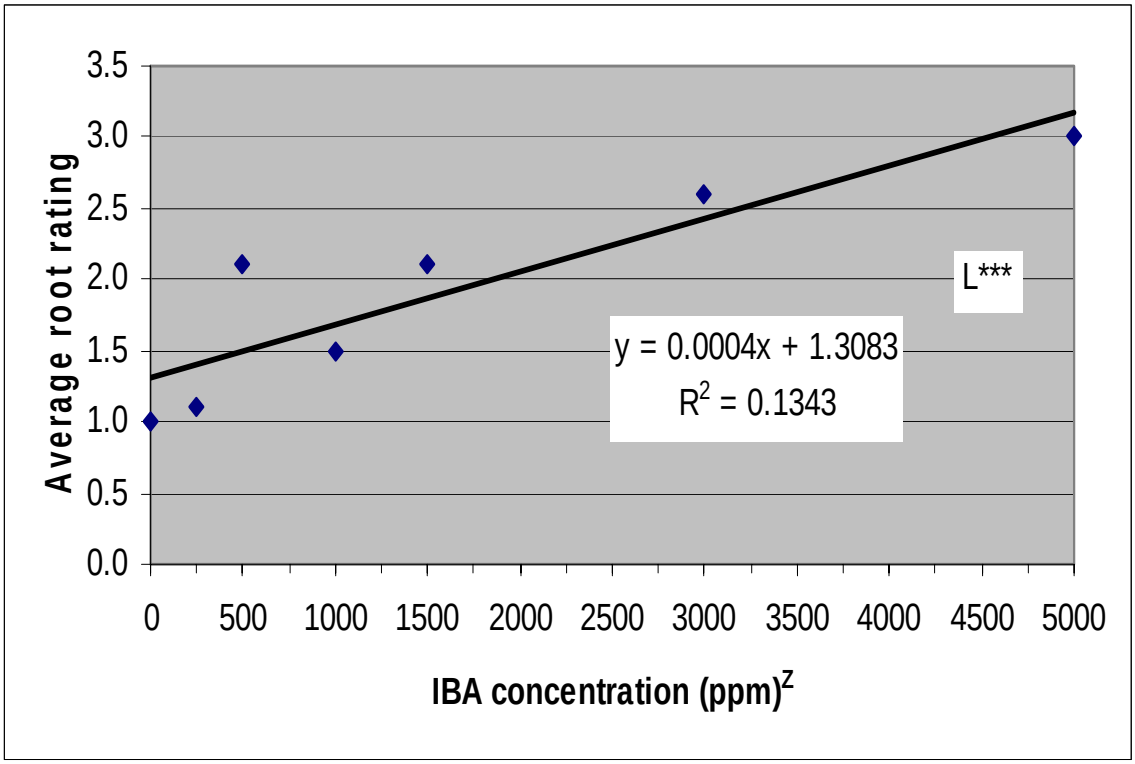
[Z]Average root length response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 3. Regression analysis of average root number for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) 70 days after sticking in 2000.



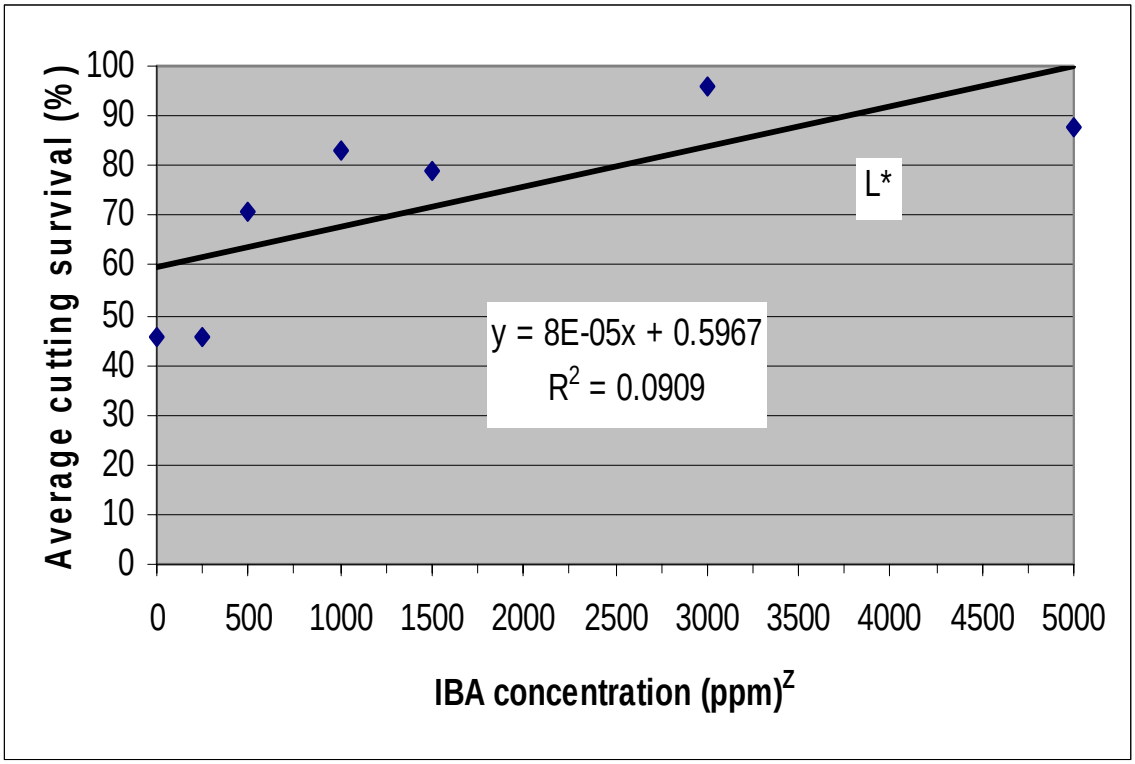
[Z]Average root number response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 4. Regression analysis for average root rating for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) 70 days after sticking in 2000.



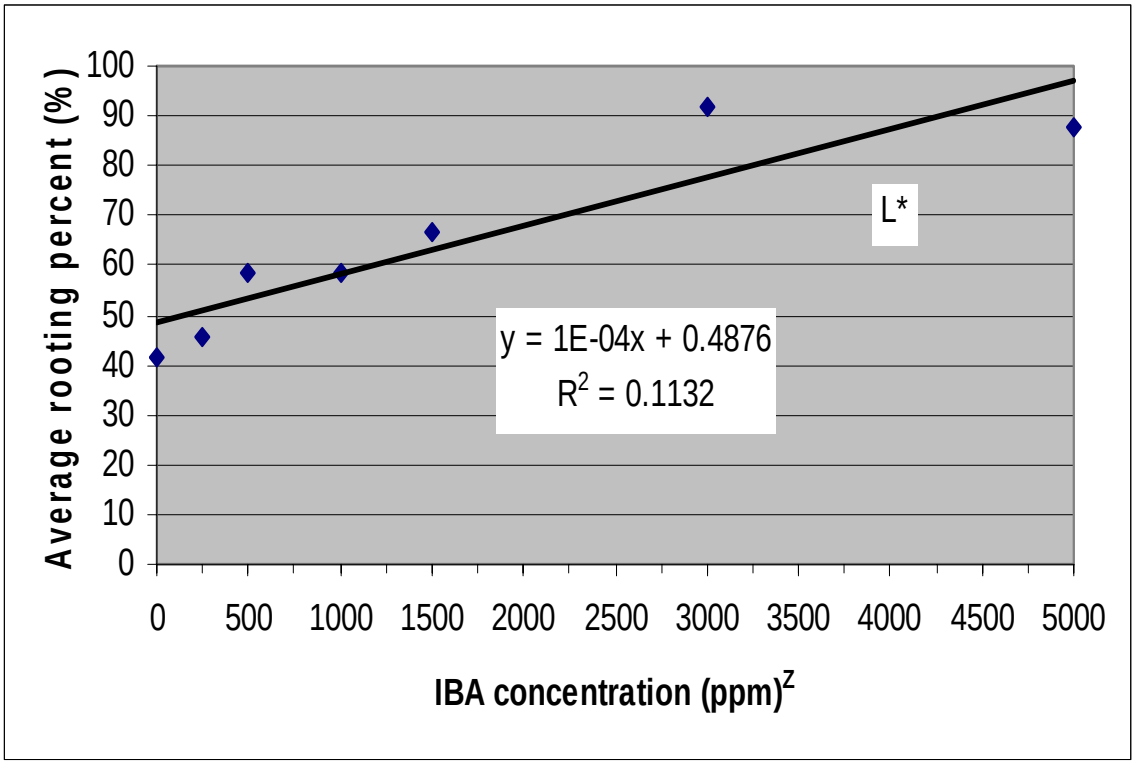
[Z]Average root rating response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 5. Regression analysis of average percent cutting survival for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) 70 days after sticking in 2000.



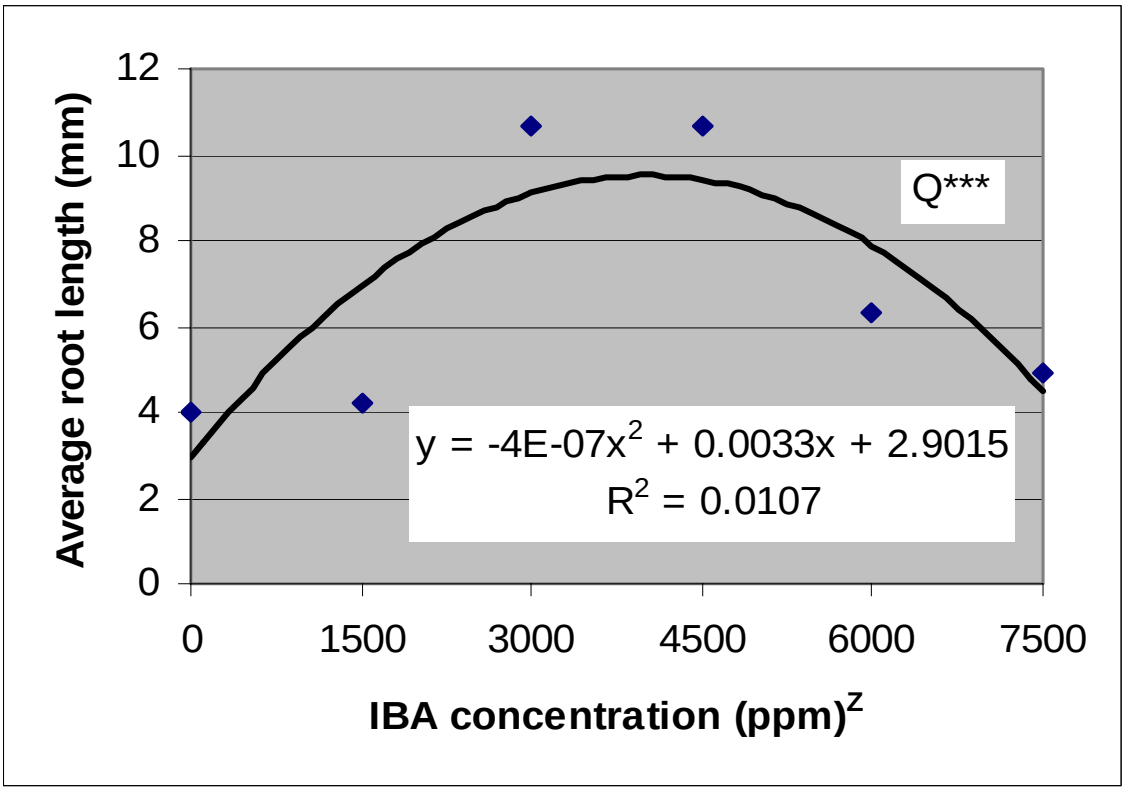
[Z]Average cutting survival response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 6. Regression analysis of average rooting percent for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) 70 days after sticking in 2000.



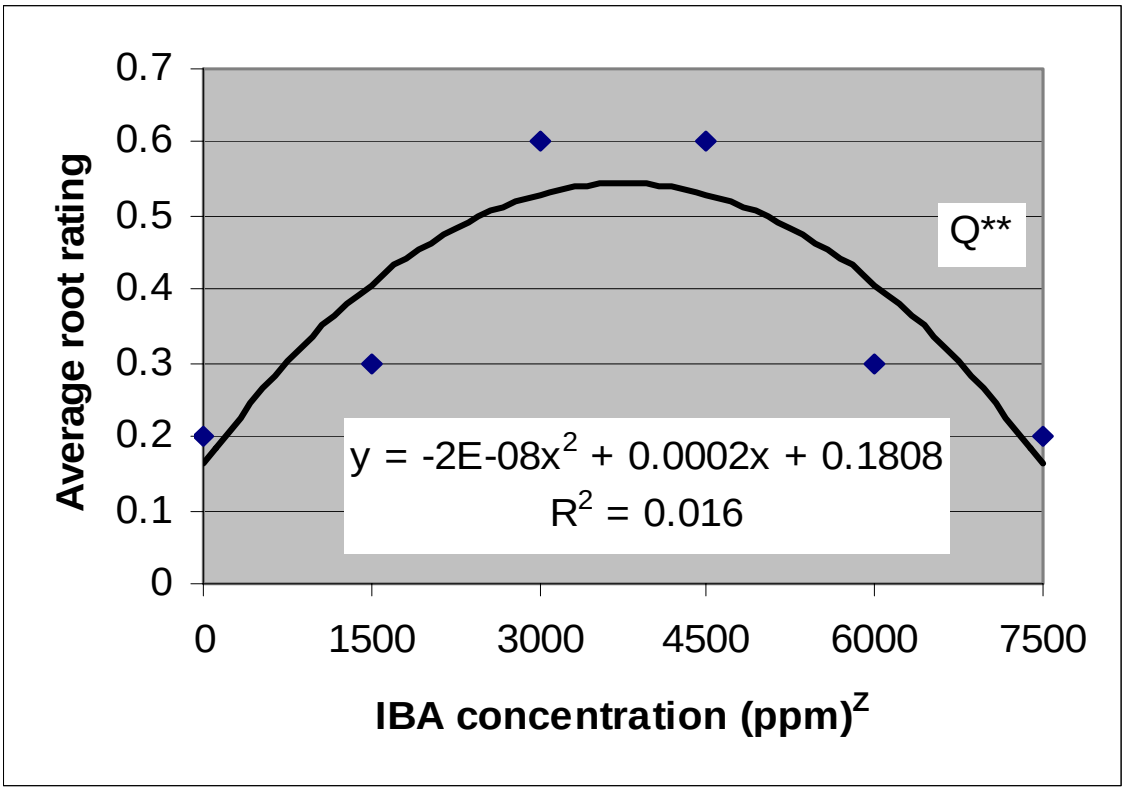
[Z]Average rooting percent response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 7. Regression analysis of average root length for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) in 2005.



[z]Average root length response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Figure 8. Regression analysis of average root rating for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) in 2005.



[z]Average root rating response to Dip 'N Grow with 2:1 ratio of IBA to NAA was the same for both auxins.

Table 2. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) as Dip 'N Grow 70 days after sticking in 2000.

IBA/NAA concentration (ppm)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting (%)[X]
0/0	20.4 d[W]	1.3 c	1.0 d	45.8 c	41.7 c
250/125	19.1 d	1.5 c	1.1 d	45.8 c	45.8 bc
500/250	45.3 bc	3.0 b	2.1 bc	70.8 b	58.3 bc
1000/500	29.2 dc	2.3 bc	1.5 cd	83.3 ab	58.3 bc
1500/750	37.9 bc	4.9 a	2.1 bc	79.2 ab	66.7 b
3000/1500	51.7 b	4.5 a	2.6 ab	95.8 a	91.7 a
5000/2500	77.4 a	5.3 a	3.0 a	87.5 ab	87.5 a
Significance[V]	L***,Q***	L***	L***	L*	L*

[Z]Roots were rated on a scale 0 to 5, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]Linear (L) or Quadratic (Q) response at the 0.001 (***) or 0.05 (*) level within columns.

Table 3. Average root length, number, rating[Z], cutting survival, and rooting percent for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) as Dip 'N Grow 70 days after sticking in 2004.

IBA/NAA concentration (ppm)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting (%)[X]
0/0	68.2 a[W]	29.9 a	2.4 a	75.0 a	75.0 a
1500/750	82.3 a	40.3 a	2.7 a	87.5 a	83.3 a
3000/1500	92.6 a	50.3 a	3.3 a	91.7 a	91.7 a
4500/2250	69.4 a	44.9 a	2.7 a	87.5 a	87.5 a
6000/3000	76.5 a	50.1 a	2.9 a	83.3 a	83.3 a
7500/3750	72.4 a	47.1 a	2.8 a	75.0 a	75.0 a
Significance[V]	NS	NS	NS	NS	NS

[Z]Roots were rated on a scale 0 to 5, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within columns were similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]Nonsignificant (NS) at the 0.05 level within columns.

Table 4. Average root length, number, rating[Z], cutting survival and rooting percent for *Clematis socialis* stem cuttings treated with increasing concentrations of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) as Dip 'N Grow at 42, 56, & 70 days after sticking in 2005.

IBA/NAA concentration (ppm)	Length (mm)	Number	Rating	Cutting survival[Y] (%)	Rooting[X] (%)
0/0	4.0 b[W]	1.1 a	0.2 a	20.8 bc	10.4 ab
1500/750	4.2 b	1.2 a	0.3 a	20.8 bc	12.5 ab
3000/1500	10.7 a	2.9 a	0.6 a	37.5 ab	25.0 a
4500/2250	10.7 a	3.5 a	0.6 a	39.6 a	20.8 ab
6000/3000	6.3 ab	2.0 a	0.3 a	29.2 ab	14.6 ab
7500/3750	4.9 b	1.4 a	0.2 a	8.3 c	8.3 b
Significance[V]	Q***	NS	Q**	NS	NS
Days After Sticking[U]					
42	0.1 c[W]	0.05 b	0.03 c	14.6 b	3.1 b
56	4.6 b	1.3 b	0.3 b	35.4 a	16.7 a
70	15.7 a	4.7 a	0.8 a	28.1 a	26.0 a
Significance[V]	L***,Q***	NS	L**	NS	NS

[Z]Roots were rated on a 0 to 5 scale, with 5 being best (Figure 1).

[Y]Cutting considered alive if foliage was still green.

[X]Cutting considered rooted when new root measured > 5.0 mm (0.197 in.) in length.

[W]Means followed by similar letters within rows were similar at $P = 0.05$ using Duncan's Multiple Range Test.

[V]Nonsignificant (NS) at the 0.05 level, Quadratic (Q) or Linear (L) response at the 0.001 (***) or 0.01 (**) level to increasing IBA/NAA concentration.

[U]No interaction between IBA/NAA concentration and days after sticking at 5% level.

CHAPTER IV

FINAL DISCUSSION

Clematis socialis (Kral), Alabama leather flower, has been federally listed as an endangered species for 20 years due to small population numbers, limited natural distribution, and loss or disturbance of habitat caused by humans. Twenty geographically distinct, self-sustaining populations- one acre each- that are known and protected must be secured before the species can be delisted. It is unlikely that twenty such populations will be found in the wild, so some form of propagation will have to be employed to establish them. Stem cutting propagation would appear to be the most efficient method and would cause little disturbance to native and established plants. This study was conducted to accumulate information on rooting stem cuttings of *C. socialis* to help establish cutting requirements of this species in the future. In addition to establishing additional populations, this research could also aid in providing genetic material for genetic preservation, future hybridization, and possibly promote *C. socialis* to the ornamental trade. Due to its low population numbers, there is concern over the possible limited variability in this species gene pool. Increasing the population numbers could possibly increase genetic variability and thus increase this species' adaptability to the changing conditions of its habitat. The attractive masses of bell-shaped, blue-violet flowers atop dense, low growing, bright-green foliage are characteristics that make this species an

excellent choice in clematis breeding. The attractive flowers and growth habit, along with its rarity, would also appeal to plant collectors and homeowners with native-plant landscapes.

Data on substrate type, substrate chemical and physical properties, and dolomitic limestone amendment rate were collected. In chapter 2, sand, perlite, and vermiculite were found to be similar in rooting and root growth, and were far better than the 1:1:1 (by volume) sphagnum peat moss, pine bark, and sand (P:PB:S) substrate in all years of the study. Cuttings in sand and perlite were the earliest to root but the root growth of cuttings in vermiculite, though slower to initiate roots, were equal to sand and perlite by the end of the study in 2005. Sand and perlite have no cation exchange capacity (CEC), no buffering capacity, and contain no mineral nutrients, but vermiculite has a high CEC, good buffering capacity, and retains nutrients well. These differences might explain why rooting and root growth in vermiculite continued to increase between 56 and 70 days after sticking (DAS), but began to decrease in sand and perlite. Adding nutrition to cuttings in sand and perlite may cause them to develop root systems far beyond those observed in vermiculite if the fertilizer is applied as the cuttings develop roots. Fertigation was not used in this study, but many growers may choose to do so to supply nutrients for growth when cuttings begin to root. Sand is the most commonly use substrate for cultivated clematis propagation and it was the most consistent in its performance across all years in this study. Sand is also more readily available and was the least expensive per cutting which is why it was recommended for rooting *C. socialis* stem cuttings. Rooting and root growth in the 1:1:1 P:PB:S substrate or any other peat or pine bark based substrate could be more successful if amended with dolomitic limestone to raise the substrate pH. Results showed improved rooting and root

growth in a 2:1:1 (by volume) sphagnum peat moss, pine bark, and perlite (P:PB:PT) substrate as dolomitic limestone amendment rate increased. Results of the Virginia Tech Extraction Method (VTEM) in 2005 did indicate that pH would only improve up to a certain point as dolomitic limestone amendment rate increased, meaning rates higher than 12.5 lbs/yd^3 would not be more effective. The optimal pH range for rooting clematis, 7.0 – 7.5, was obtained when a lime amendment rate of 5.0 to 12.5 lbs/yd^3 were used, however, the best rooting and root growth data was observed at rates of 10 to 12.5lbs/yd^3.

Data on the influence of growth regulators on rooting *C. socialis* stem cuttings was also collected. In chapter 3, IBA (indole-3-butyric acid) and NAA (naphthalene acetic acid) growth regulator treatments were not found essential in rooting *C. socialis* stem cuttings but concentrations between 4500/2250 ppm IBA/NAA and 6000/3000 ppm IBA/NAA were shown to initiate rooting earlier in 2005. Results showed that rooting and root growth began to decline at concentrations higher than 6000/3000 ppm IBA/NAA. The best root growth data was observed at 3000/1500 ppm to 4500/2250 ppm IBA/NAA. For the best combination of good rooting, root growth, and early rooting, a concentration of about 4500/2250 ppm IBA/NAA is recommended.

Although there were cuttings in the control groups that rooted without the use of dolomitic limestone amendment and growth regulators, there was improved rooting and root growth in those groups receiving treatment. Growth regulator applications and dolomitic limestone amendment require extra labor, but the extra cost per cutting for the actual compounds is minimal.

This study was just one step in the process of establishing the cutting requirements of *C. socialis*. Further evaluations, such as trials performed by growers interested in producing the species, of techniques used in this study would be helpful to ensure practicality and consistency in results. There is other information not obtained in this study that is also needed to help growers properly produce this species in the industry. Questions on proper hardening-off once cuttings are rooted, whether pinching or pruning will be required to produce a compact plant or to control runners, production time for 4" pot or 1-gallon container, how long before flowering plant produced, etc. still need to be addressed. There is also the question of establishment in the landscape or in the wild. Trials that evaluate establishment technique, site location, culture requirements, etc. should be performed for establishment in landscape and natural settings.

A general observation made in this study included the sensitivity of *C. socialis'* stem cuttings to moisture. It was very important to keep the cuttings thoroughly hydrated immediately after taking the cuttings, and while preparing and sticking the cuttings. Wrapping cuttings with wet paper towels, placing them on ice, and preparing the cuttings under mist did help prevent wilting. The rooting substrate had to be thoroughly moistened before sticking as well, especially the peat-based substrates. This is true of many plant species, but especially so for *C. socialis* which grows in moist soils natively. Cuttings in this study received intermittent mist several hours a day, during sunlight hours, for the entirety of the experiment to provide adequate moisture for the cuttings and to mimic the soil moisture of the native location from which the cuttings were taken. Shutting off the intermittent mist system for only a couple of hours on a warm day, even though the substrate was thoroughly saturated and the cuttings were shaded, resulted in

some wilting. It was also observed that cuttings kept in the cooler for more than a day, were not likely to root. Extra cuttings that were left in the cooler for more than one day were stuck in the same manner and substrate as those used for data collection in the study, but none of them survived. Cuttings in 2004 were also observed to have yielded much higher rooting percentages and root growth averages than those in 2000 and 2005. Cuttings from all years were taken between late-May and early-June, and early in the morning, so time of cutting collection is not likely to have made a significant difference in cutting performance. Cuttings in 2004, however, were made entirely from new growth, the result of a recent mowing, where as the cutting material in 2000 and 2005 did not look as though it had been mown yet that year and was more mature. No data was collected in this study to support any of these general observations, but they may warrant future studies or consideration from growers/propagators that choose to propagate this species in the future.