Induction of adventitious rooting in stem cuttings of eastern redcedar (*Juniperus virginiana* L.)

by

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Abstract

Eastern redcedar (*Juniperus virginiana* L.) is a vital component of windbreaks and conservation plantings across the Great Plains. Lack of management in rangeland has contributed to an exponential increase in establishment of eastern redcedar seedlings and conversion of land cover, decreasing productivity of rangeland while increasing fire risk. Eastern redcedar is dioecious (male and female individuals) so the selection and planting of seedless male specimens would be preferable to continuing mixed-sex plantings.

Eastern redcedar does not reveal male or female cones until 10-15 years of age, making selection at the nursery stage (1-2 year-old seedlings) impossible. Grafted male specimens are available, but are not economical for low-cost conservation plant material use due to the comparatively high cost of grafting. If the rooting of stem cuttings from male specimens was made economically viable by increasing the success rate, then male eastern redcedars would be available for conservation purposes while reducing the threat of seedling establishment and vegetation cover conversion in adjacent rangelands.

This study's results indicate that stem cuttings of eastern redcedar can be induced to form adventitious roots using bottom heat and treatment with concentrated auxins (indole-3-butryic acid, IBA; and 1-napthaleneacetic acid, NAA), plant growth regulators that induce differentiation of plant tissue as roots. Rooting percentage was low (15-50%) compared to generally accepted rates for other horticultural species in the trade, but can potentially be improved through control of various environmental (i.e. water status, soil-induced stress) and genetic (i.e. provenance, form) factors in the stock plants from which cuttings are removed.

Objectives for this research project were to: 1) Study the influence that root-promoting auxins have on stem cuttings of eastern redcedar, 2) Assess the establishment of eastern redcedars produced from rooted cuttings, as compared to traditionally-produced seedlings; and 3) Assess the growth form of rooted eastern redcedars for windbreak suitability.

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Dedication

This report is dedicated to Bob Henrickson and the late Harlan Hamernik, exceptional and practical plantsmen who were always quick to remind that not every plant read the textbook; it's critical to try new things and expect failure, because there's always a good chance you might learn something from the plants.

Preface

Eastern redcedar (*Juniperus virginiana* L.) is a vital component of windbreaks and conservation plantings across the Great Plains. Lack of management in rangeland has caused an exponential increase in establishment of seedlings and conversion of land cover. Eastern redcedar, as a dioecious species, has male and female individuals, so the selection of only seedless male specimens for planting would be preferable to reduce species spread. Eastern redcedar does not differentiate male from female until 10-15 years of age, making selection at the nursery stage impossible. Grafted specimens would be a viable solution, if not an economical one due to the cost of grafting. If the rooting of male cuttings was to be made economically viable by increasing the percentage to an acceptable level, then male eastern redcedars would remain available for conservation purposes without presenting a resource concern (threat of seedling establishment and vegetation cover conversion) for adjacent rangelands.

Chapter 1 - Review of the Literature

Since the discovery, in 1934, that plant hormones known as auxins can initiate cell dedifferentiation and subsequent rooting in vegetative plant tissue, many plant species have been propagated by this method (Loach 1988). However, stem cuttings of conifers are generally more difficult to root and are often propagated from seed, or the more labor-intensive methods of grafting. For conifers, the cost of producing rooted cuttings exceeds the comparable cost of producing seedlings of the same species in a nursery setting (Gill, 1983; Frampton et al. 1989).

Rooting of conifers has been attempted for many years, but the information is sporadic and inconsistent (Ragonezi et al. 2010). Reliable protocols for rooting of conifers exist for only a few species, i.e., *J. chinensis, J. communis, J. horizontalis,* and *J. sabina*, and cultivars such as 'Hetzii', 'Pfitzer', 'Ramlosa', or 'Skyrocket' (Hill 1962, Gil-Albert 1978, Chong 2003, Ragonezi et al 2010, Kentelky 2011).

Groundcover junipers are commonly propagated from stem cuttings, but species that grow upright are considered more difficult to root than prostrate forms (Banko 1981, Hartmann et al. 2011). It has long been recognized that *Juniperus virginiana* (eastern redcedar) is difficult to root, and rarely produces a suitable, vigorous, fibrous root system from cuttings (Wagner 1967).

Many conifers are plagiotropic, with lateral shoots continuing "programmed" lateral growth instead of resuming upright growth after rooting or grafting. Younger parent trees exhibit less plagiotropism than 40-year-old specimens (Edson et al. 1996). Upright growth of eastern redcedar is critical for landscape and windbreak/conservation purposes (Brandle et al. 1991). Lateral, plagiotropic growth is unacceptable in most cases.

When junipers have been reported as being successfully rooted, it has been demonstrated that longer 10-inch tip cuttings root better than shorter 5-inch cuttings (Edson et al. 1996). Terminal cuttings have long been recommended for all species of *Juniperus*, especially upright tree form species (Bogdany 1954).

Relatively higher auxin levels (10,000 ppm and above) applied directly to vegetative cuttings, result in better rooting (Edson et al. 1996) than lower levels (less than 10,000 ppm). Indole-3-butryic acid (IBA) is the most common auxin used in asexual propagation by stem cuttings, but 1-napthaleneacetic acid (NAA) has been shown to promote increased rooting as well (Ragonezi et al. 2010). A combination and comparison of these two synthetic growth regulators, also known as "rooting hormones," has not been thoroughly studied on stem cuttings of eastern redcedar.

Auxins delivered in a liquid formulation have been shown to increase rooting when compared to powder formulations (Chong and Hamersma 1995) potentially due to increased uptake of the auxin by the plant tissue (Loach 1988).

Some sources show that *Juniperus* cuttings started in October root better than ones started in January (Edson et al. 1996) but some report later cutting dates in the winter months (Wagner 1967, Banko 1981) such as November (Zorg 1953) or January (Bogdany 1954) root better. Because of long rooting periods, it has been suggested that taking cuttings as early as possible after hard dormancy (cessation of active growth in winter) is beneficial (Duer 1981).

Banko (1981) reported that the time of year cuttings are taken contributes more to overall rooting success than the concentration of rooting hormone. For *Juniperus* spp., it has been reported that varying auxin concentration may have no influence on rooting percentage (Chong and Hamersma 1995), although other studies (Henry et al. 1992) found significant differences in rooting percentage dependent on auxin concentration.

In addition, the condition or health of the tree that the cuttings are taken from will have an effect on the rooting success of the cutting, with nitrogen, potassium, and stored carbohydrate levels impacting rooting percentage (Henry et al. 1992). There is variance in the genotype that affects rooting potential, as well. This wide variation in genotype across provenances (particular place of origin of a population of tree species) in the Great Plains is well-known in the field (Van Haverbeke et al. 1990, Cunningham and King, 2000) and commonly exploited to produce locally-adapted seedlings from local seed sources.

Formation of callus (undifferentiated plant cells) as a wound response suggests that stripping needles or otherwise creating a wound on the stem tissue could initiate callus tissue formation (Ikeuchi et al. 2017) which can then be induced to differentiate into root tissue by presence of auxins.

Bottom heat, or supplemental heat applied only to the media in which cuttings are placed, is essential to inducing root growth from callus for junipers (Wagner 1967, Duer 1981). However, some studies have determined that higher bottom heat of 70 F actually retards rooting, as compared to 54 F (Chong 2003).

Cuttings taken from young (2-4 years) stock plants of eastern redcedar rooted better than cuttings from 40-year-old specimens (Edson et al. 1996). However, young eastern redcedar trees cannot be differentiated between male and female, as they often remain juvenile (lacking reproductive cones) until around 10-15 years of age (Lawson 1990). For conservation purposes, an ability to root differentiated-sex, or mature male, cuttings is needed.

The closely related species *Juniperus scopulorum* (rocky mountain juniper), also difficult to root, has been shown to respond significantly to relatively high levels of IBA, as high as 4.5% or 45,000 ppm (Duer 1981). Other sources cite damage or mortality due to phytotoxicity at concentrations of IBA above 8,000 ppm (Loach 1988).

For *Juniperus virginiana,* rooting rates have varied significantly in various studies which varied in timing and geographic location (or provenance) of the parent trees.

Zorg (1953) found rooting of 53% of Pennsylvania *Juniperus virginiana* stem cuttings of 6-7 inches taken in September and treated with 8,000 ppm IBA.

Bogdany (1954) described a method of rooting multiple sizes of terminal stem cuttings taken in January, in Connecticut, and treated with NAA at 10,000 ppm, resulting in 40% rooting rate, and unspecified concentrations of IBA resulting in 0-20% success rates.

Box and Beech (1968) treated 5-9 inch stem cuttings, taken in November in Louisiana, with 10,000 ppm IBA and found 82% produced roots.

Gil-Abert and Boix (1978) found that stem cuttings taken in November and December and treated with 4,000 ppm and 8,000 ppm IBA resulted in 10-20% and 10% rooting, respectively. This study occurred in Spain, with *Juniperus virginiana* from an undescribed (American) provenance.

Loach (1988) treated stem cuttings of eastern redcedar with unspecified concentrations of NAA and IBA, resulting in 73-86% rooting success with NAA and 50-91% success for IBA. This study occurred in Holland, from an undescribed (American) provenance.

Henry et al. (1992) described an 87% rooting rate for 15 cm hardwood stem cuttings taken in January in North Carolina, and treated with 5,000 ppm IBA.

Some commercial cultivars of *Juniperus virginiana* may root at a higher rate than the species, such as Hetz, Burk, and Koster, at rates from 33 to 75 percent (Westervelt, 1959), in a study done in Kansas.

Due to many factors such as water quality, day length, relative humidity, local and regional variation in genetics, and unreported yet important environmental factors, replication of prior work is often highly inconsistent (Loach 1988).

The literature, as a whole, does not provide a clear protocol for the process of rooting eastern redcedar, and reports inconsistent success within many variables. Controlling for these variables should provide clarity on the significantly contributing factors that must be understood in order to succeed and improve the rooting of male eastern redcedar plants that meet conservation purposes.

Objectives for this research project were to: 1) Identify the relevant factors influencing the rooting response of eastern redcedar, primarily the concentration of auxin at which eastern redcedar produces the highest rooting percentage, 2) Assess the establishment of rooted eastern redcedars as compared to traditionally-produced seedlings; and 3) Assess the growth form of rooted eastern redcedars for windbreak suitability.

Chapter 2 - Rooting of Stem Cuttings

BACKGROUND

In the series of experiments included within the scope of this project, several methods for producing roots on stem cuttings of eastern redcedar (*Juniperus virginiana* L.) are presented. These experiments were done in series, to build on lessons learned and increase likelihood of success, but also out of necessity as complications in early iterations resulted in high or total cutting mortality with little to no rooting.

In early spring of 2014, temporary space in a Kansas State University glass greenhouse (Manhattan, KS) was secured for this project. Cuttings were taken from parent trees located at the Howe Conservation Education Area just outside Manhattan, KS, treated with a variation on the treatments detailed below, and placed under a mist bench. Due to an inadequate circuit capacity in this greenhouse space, no bottom heat for these cuttings was available. There was no rooting, and no callus tissue was present. The experiment was terminated in summer 2014.

A second attempt was made in the spring of 2015, in a different greenhouse space within the same structure. No attempt was made to distinguish between male and female stock plants, therefore the experiment likely included cuttings from female trees. This time, the electrical service was sufficient to support heat mats, but the long rooting period and constant foliar wetness created a situation where foliar fungus compromised the experiment, with few cuttings surviving to produce callus and roots. This trial was also terminated prematurely, without results. These failures necessitated a third attempt for 2016, and a subsequent fourth iteration in 2018. Those details follow.

MATERIALS AND METHODS

In early February of 2016, approximately 1000 12-inch terminal cuttings were collected from an established stand of eastern redcedar (*Juniperus virginiana* L.) at the Howe Conservation Education Area in rural Riley County, KS (39.329 N, 96.683 W). (Figure 2.1) This procedure was repeated in February of 2018, in the same location.

Figure 2.1 - Taking cuttings from parent trees at the Howe Conservation Education Area in rural Riley County, KS.

No more than 50 cuttings were taken from any individual tree, to reduce genotype influence on rooting response. These mature trees were approximately 30-50 years old, based on site history and size. For both 2016 and 2018, only male trees were selected, based on the presence of male pollen cones visible on branches.

Cuttings were collected over the course of approximately two hours in late morning, and were placed into mesh bags as they were collected. Ice (in the form of snow) was collected on site and packed with the bags to reduce desiccation of the cuttings. These cuttings were transported to the nearby greenhouse at Kansas State University, in Manhattan, KS. All cuttings were pooled, to limit the impact of parent tree influence on treatment variation, and graded based on size and needle fullness to assure consistency.

Figure 2.2 – A subset of cuttings being graded and wounded, ready for treatment.

Cuttings were re-cut at the base, to a standard 10-inch length, and the lower 3 inches of needles were stripped by hand to wound the stem and stimulate a wound response to initiate callus development. (Figure 2.2) Treatments were then applied to the cuttings, detailed in Table 2.1 and Table 2.2, in 2016 and 2018, respectively.

2016

For the 2016 treatments, commercially available sources of root-promoting compounds containing IBA (indole-3-butryic acid) and NAA (1-napthaleneacetic acid), and vitamin B1 (thiamine) were obtained and prepared according to procedures established by Boyer et al. (2013). These included Hortus Salts (Phytotronics, Inc., Earth City, MO), Dip‘N Grow (Dip‘N Grow, Inc., Clackamas, OR), and liquid Hormex (Maia Products, Inc., Westlake Village, CA). 70% isopropyl alcohol was used as the control, as the base solvent for the liquid formulation was also alcohol, as is common for most IBA/NAA formulations (Blythe, et al. 2007). The basal end of cuttings were dipped to a depth of two inches, in groups of 10, into the treatment solution for five-second duration and were immediately stuck into the prepared media. Each treatment was applied to 50 cuttings (Table 2.1). Treatment concentrations of IBA and NAA were selected to be within the range of successful concentrations reported in the literature, and on undiluted product concentration and one simple 1:1 dilution.

Table 2.1 - Summary of Treatments (2016)

IBA, NAA, B1 concentrations in parts per million (ppm)				
Treatment name	**n**	**IBA (indole-3-butyric acid)**	**NAA (1-napthaleneacetic acid)**	**Vitamin B1 (thiamine)**
Control	50	0	0	0
Hormex	50	130	2400	2500
Dip'N Grow 1:1	50	5000	2500	0
Dip'N Grow Conc.	50	10000	5000	0
Hortus Salts	50	10000	0	0

After treatments were applied, cuttings were immediately inserted into "D40 Deepots" (Stuewe & Sons, Inc., Tangent, OR) containing a rooting substrate mixture of two parts perlite and one part peat, by volume. Deepots were labeled according to treatment and randomized in treatment groups (rows) of 10 within a 50-container rack. These racks were placed on a bench with Redi-Heat heating mats (Phytotronics, Inc., Earth City, MO) calibrated to maintain in-container temperature at 70 F (Figure 2.3).

A fine-mist system (CoolNet Pro Foggers, Netafim USA, Fresno, CA) was placed 24 inches above the trays, set to provide mist for 10 seconds every 10 minutes. Slight adjustments were made to shorten or lengthen the mist interval based on weather. On very sunny days the mist interval was adjusted to 8 minutes, and rainy or overcast days were adjusted to an interval of 15 minutes. Greenhouse temperatures were set to 50-degree F nighttime and 75-degree daytime, and cuttings were exposed to a natural photoperiod with no supplemental lighting.

Every two weeks, a solution of Captan 50WP (48.9% N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide, Bonide Products Inc, Oriskany, NY) was applied, at a rate of 0.32 ounces per gallon, to limit foliar fungus problems over the approximately 20-week period of rooting.

In July of 2016, cuttings were lifted from containers and data was taken. Data collected included presence of callus (Figure 2.4) and number of roots (Figure 2.5). Cuttings with sufficient roots (at least 2-3 roots at least 1-inch in length) were transplanted into larger containers and labeled, to be grown out for field trial planting at a later date (Chapter 3).

2018

Additional treatments in 2018 were undertaken, based on the preliminarily significant findings of the 2016 treatments, indicating that there may be an effect of the ratio of NAA concentration versus IBA concentration on rooting success and number of roots developed. These treatments were made from chemical-grade IBA and NAA concentrations as opposed to the commercially-available formulations.

For the 2018 treatments, bioreagent grade indole-3-butryic acid (I5386) and 1-napthaleneacetic acid (N0640) was obtained from Sigma-Aldrich (St. Louis, MO) and prepared according to standard protocol (Boyer et al. 2013), using 70% isopropyl alcohol as a solvent. A 70% isopropyl alcohol control was also incorporated.

Cuttings were then subjected to one of ten treatments, detailed in Table 2.2 below. The basal end of cuttings were dipped to a depth of 2 inches, in groups of 10, into the treatment solution for five-second duration before being stuck into the prepared media identical to the 2016 rooting substrate. Each treatment was applied to 50 cuttings (Table 2.2). For truncated treatment names in Table 2.2, numerals indicate concentration of auxin components, “I” indicates presence of IBA, while “N” indicates presence of NAA.

Table 2.2 – Summary of Treatments (2018)

		IBA and NAA concentrations in parts per million (ppm)	
Treatment name	**n**	**IBA (indole-3-butyric acid)**	**NAA (1-napthaleneacetic acid)**
Control	50	0	0
25 I	50	2500	0
25 N	50	0	2500
25 IN	50	2500	2500
50 I	50	5000	0
50 N	50	0	5000
50 IN	50	5000	5000
100 I	50	10000	0
100 N	50	0	10000
100 IN	50	10000	10000

Immediately after treatments were applied, cuttings were inserted into 15-inch square by 5-inch deep AFLAT5 trays (Stuewe & Sons, Inc., Tangent, OR) containing a rooting substrate mixture of two parts perlite and one part peat, by volume. Rows of cuttings, with five cuttings per row, were labeled according to treatment and randomized within the tray. Ten trays were prepared, and were placed on a bench with Redi-Heat heating mats (Phytotronics, Inc., Earth City, MO) calibrated to keep media temperature at 70 F. (Figure 2.3)

A fine-mist system (CoolNet Pro Foggers, Netafim USA, Fresno, CA) was placed 24 inches above the trays, set to provide mist for 10 seconds every 10 minutes. Slight adjustments were made to shorten or lengthen the mist interval based on weather. On very sunny days the mist interval was adjusted to 8 minutes, and rainy or overcast days were adjusted to an interval of 15 minutes. Greenhouse temperatures were set to 50-degree F nighttime and 75-degree daytime, and cuttings were exposed to a natural photoperiod with no supplemental lighting.

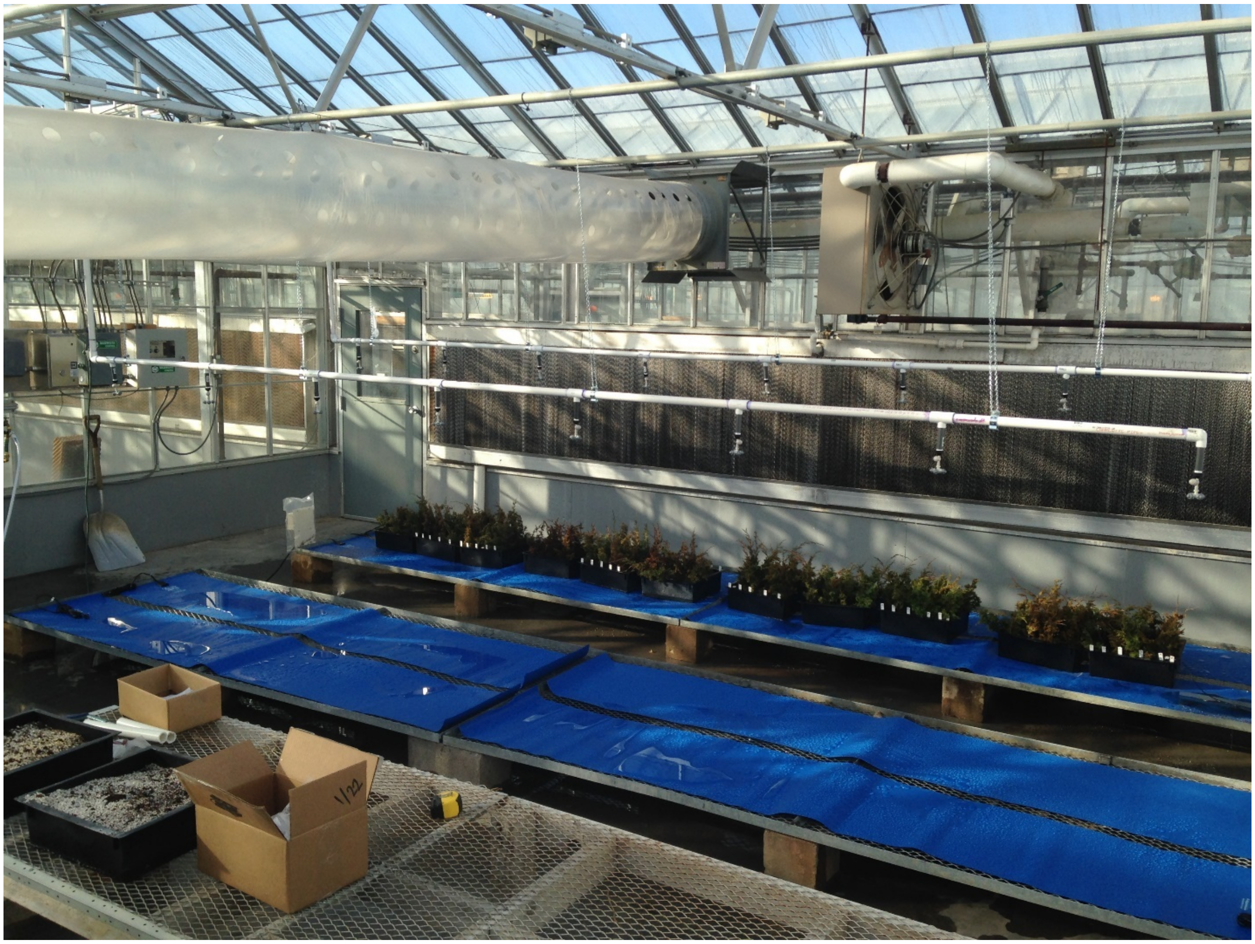


Figure 2.3 - Heat mats and mist system in greenhouse propagation area.

Every two weeks, a solution of Captan 50WP (48.9% N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide, Bonide Products Inc., Oriskany, NY) was applied, at a rate of 0.32 ounces per gallon, to limit foliar fungus problems over the approximately 20-week period of rooting.

In July 2018, cuttings were lifted from containers and data was collected. Data included presence of callus (Figure 2.4) and number of roots (Figure 2.5). Cuttings with sufficient roots (at least 2-3 roots at least 1-inch in length) were transplanted into larger containers and labeled, to be grown out for field trial planting at a later date (Chapter 3).

Figure 2.4 - Cuttings with varying amounts of callus presence, but no roots. Cutting at far right is dead, with no callus tissue present.

All statistical tests were performed using RStudio Cloud, at http://rstudio.cloud, and graphical results were created with Microsoft's Excel software .

Figure 2.5 - Root proliferation typical of successfully rooted cutting. Roots on this cutting would have been recorded as approximately 18-20.

RESULTS

2016

Callus Formation

Callus production was similar for all treatments (Figure 2.6). Between 22% and 28% of cuttings developed callus, regardless of treatment (Table 2.4). A chi-square test of independence was performed and showed no significant difference between treatments, X^2 (4, N = 250) = .74, p = .95. No treatment inhibited callus formation, as compared to the control. Callus production is typically considered a wound response, whereas presence of hormone induces pre-existing callus cells to differentiate into root cells, no difference in callus formation was expected. Therefore, it was expected that callus formation was uniform and unrelated to treatments.

Rooting Response

In all auxin treatments, roots were detected in 14-20% of samples (Figure 2.6, Table 2.5). No correlation between auxin concentration and rooting percentage was detected. When compared to the control with a Fisher's exact test there was a significant (p = .01) difference detected between all auxin treatments and the control, but no differences between the treatments themselves (Table 2.6).

Figure 2.6 - Chart of Callus and Rooting Response Results (2016) [p = 0.01]

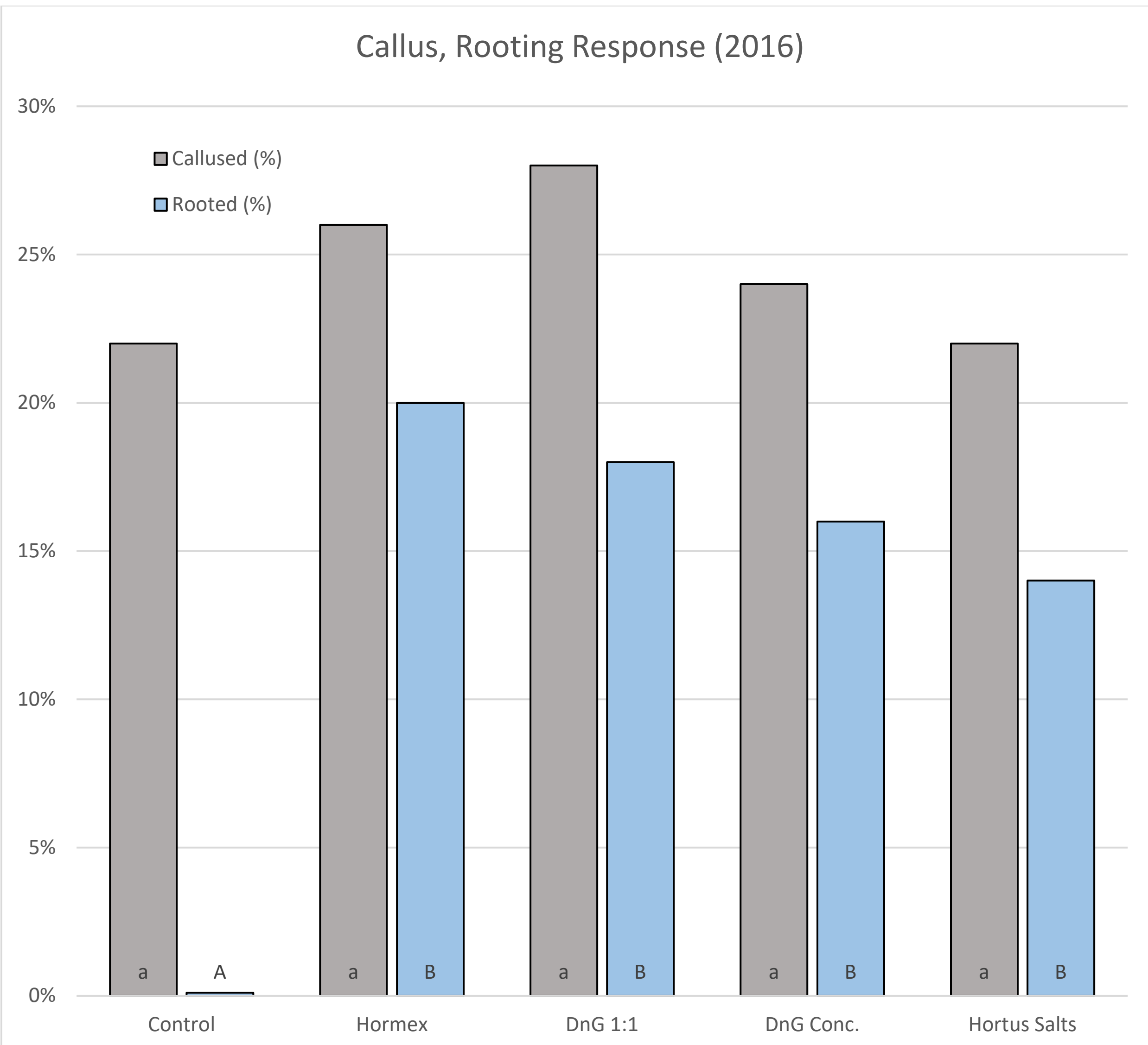


Table 2.3 - Summary of Callus Results (2016)

Treatment name	IBA (ppm)	NAA (ppm)	B1 (ppm)	n	Callus (#)	Callus
Control	0	0	0	50	11 a	22%
Hormex	130	2400	2500	50	13 a	26%
Dip'N Grow 1:1	5000	2500	0	50	14 a	28%
Dip'N Grow Conc.	10000	5000	0	50	12 a	24%
Hortus Salts	10000	0	0	50	11 a	22%

Table 2.4 - Summary of Rooting Results by Rooting Response (2016)

Treatment name	IBA (ppm)	NAA (ppm)	B1 (ppm)	n	Rooted (#)	Rooted
Control	0	0	0	50	0 A	0%
Hormex	130	2400	2500	50	10 B	20%
Dip'N Grow 1:1	5000	2500	0	50	9 B	18%
Dip'N Grow Conc.	10000	5000	0	50	8 B	16%
Hortus Salts	10000	0	0	50	7 B	14%

Table 2.5 - *p*-values for Pairwise Comparisons for Rooting Results by Rooting Response (2016)

	Control	Dip'N Grow Conc.	Dip'N Grow 1:1	Hormex
Dip'N Grow Conc.	.014	--	1	1
Dip'N Grow 1:1	.012	1	--	1
Hormex	.012	1	1	--
Hortus Salts	.012	1	1	1

Root Number

A simple count of roots per rooted cutting was taken, and recorded as an integer, regardless of root length, for all visible roots longer than 1 mm. Roots arising from the stem and significantly branched roots were counted. A one-way ANOVA was conducted (RStudio Cloud, Boston, Mass.) comparing effects of the treatments on the average number of roots per cutting.

There was a significant effect on average root numbers at the $p < .05$ level for the treatments [F(4, 245) = 6.71, $p = .008$). Post hoc comparisons using the Tukey HSD test indicated that the mean number of roots produced by the Dip'N Grow Concentrate (M = 1.06, SD = 3.27) treatments was significantly different than the control (M = 0, SD = 0). The Dip'N Grow Concentrate treatment produced significantly ($p = .04$) more roots per cutting than the control.

Figure 2.7 - Chart of Rooting Results by Roots per Cutting (2016) [$p = 0.05$]

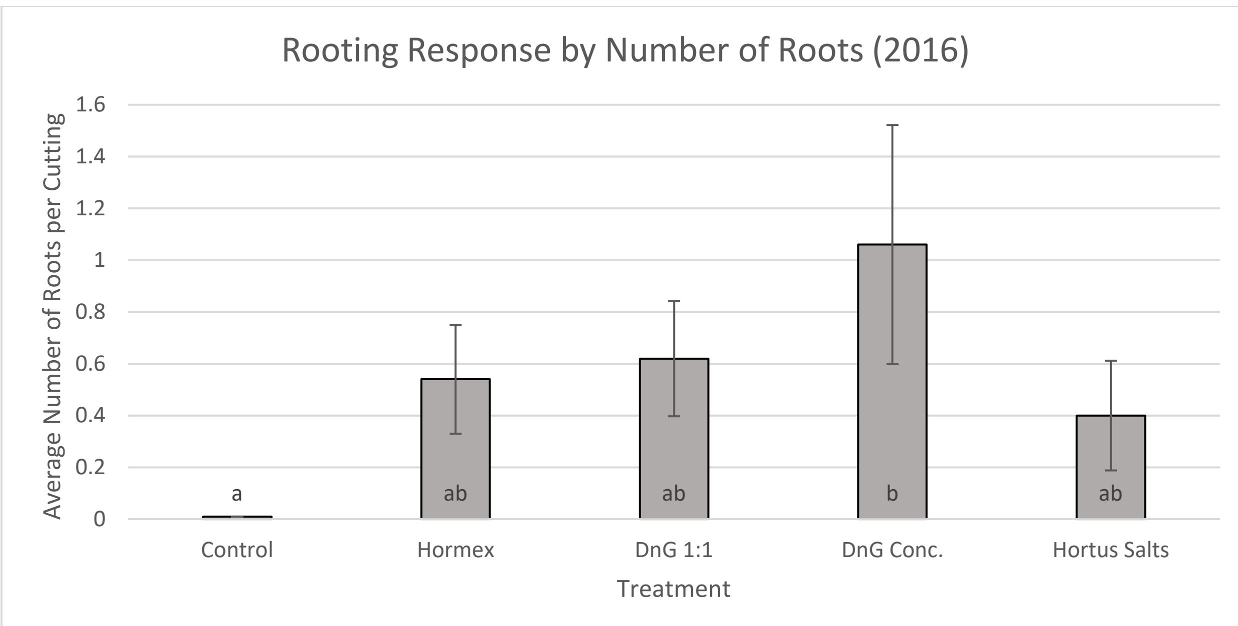


Table 2.6 - Summary of Rooting Results by Roots per Cutting (2016)

Treatment name	IBA (ppm)	NAA (ppm)	B1 (ppm)	n	Roots/ cutting	Roots/ success
Control	0	0	0	50	0.00 a	0.00
Hormex	130	2400	2500	50	0.54 ab	2.70
Dip'N Grow 1:1	5000	2500	0	50	0.62 ab	3.44
Dip'N Grow Conc.	10000	5000	0	50	1.06 b	6.63
Hortus Salts	10000	0	0	50	0.40 ab	2.86

The Dip'N Grow concentrate produced the highest average number of roots per cutting (Table 2.8), with 1.06 roots averaged across all 50 samples. This was higher than 1:1 diluted Dip'N Grow (0.62 roots per cutting), Hormex (0.54 roots per cutting), and Hortus Salts (0.40 roots per cutting), but only significantly (p = .04) different from the control.

Additionally, the Dip'N Grow concentrate produced the highest average number of roots per successfully rooted cutting, at 6.63, even though a slightly smaller number of cuttings (16%) rooted than compared to other treatments. While Hormex had the highest overall percentage of rooted cuttings, at 20%, few roots were produced per rooted cutting: just 2.70 on each successfully rooted sample, fewer than any of the other treatments.

2018

Callus Formation

A chi-square test of independence was performed and showed that a significant difference existed between treatments, X^2 (9, N = 500) = 110.62, $p < .001$. A post-hoc Fisher's exact pairwise test (Table 2.11) showed that the 50 N and 50 IN treatments resulted in significantly less callus formation than the 25 I, 25 N, 25 IN, 50 I, and 100 I treatments. However, these 50 N and 50 IN treatments were not significantly different from the control, 100 N or 100 IN treatments. The 100 N and 100 IN treatments resulted in significantly less callus formation than every other treatment except 50 N and 50 IN (Figure 2.8).

Similar to the findings in 2016, concentration of IBA and NAA did not have a significant impact on callus formation for all treatments, except those containing 5000-10000 ppm of NAA (Table 2.7). At that concentration of NAA, the simple effect of callus formation was significantly ($p < .001$) reduced when compared to the control.

Rooting Response

Unlike the 2016 treatments which did not demonstrate much variation between treatments, the 2018 treatments resulted in clear differences (Figure 2.9).

A chi-square test of independence was performed and showed that a significant difference existed between treatments, X^2 (9, N = 500) = 74.1, $p < .001$. A post-hoc Fisher's exact pairwise test (Table 2.10) showed that the 25 N and 25 IN treatments resulted in significantly more rooted cuttings than the control, 25 I, 100 N, and 100 IN treatments.

Figure 2.8 - Chart of Callus and Rooting Response Results (2018) [$p < 0.001$]

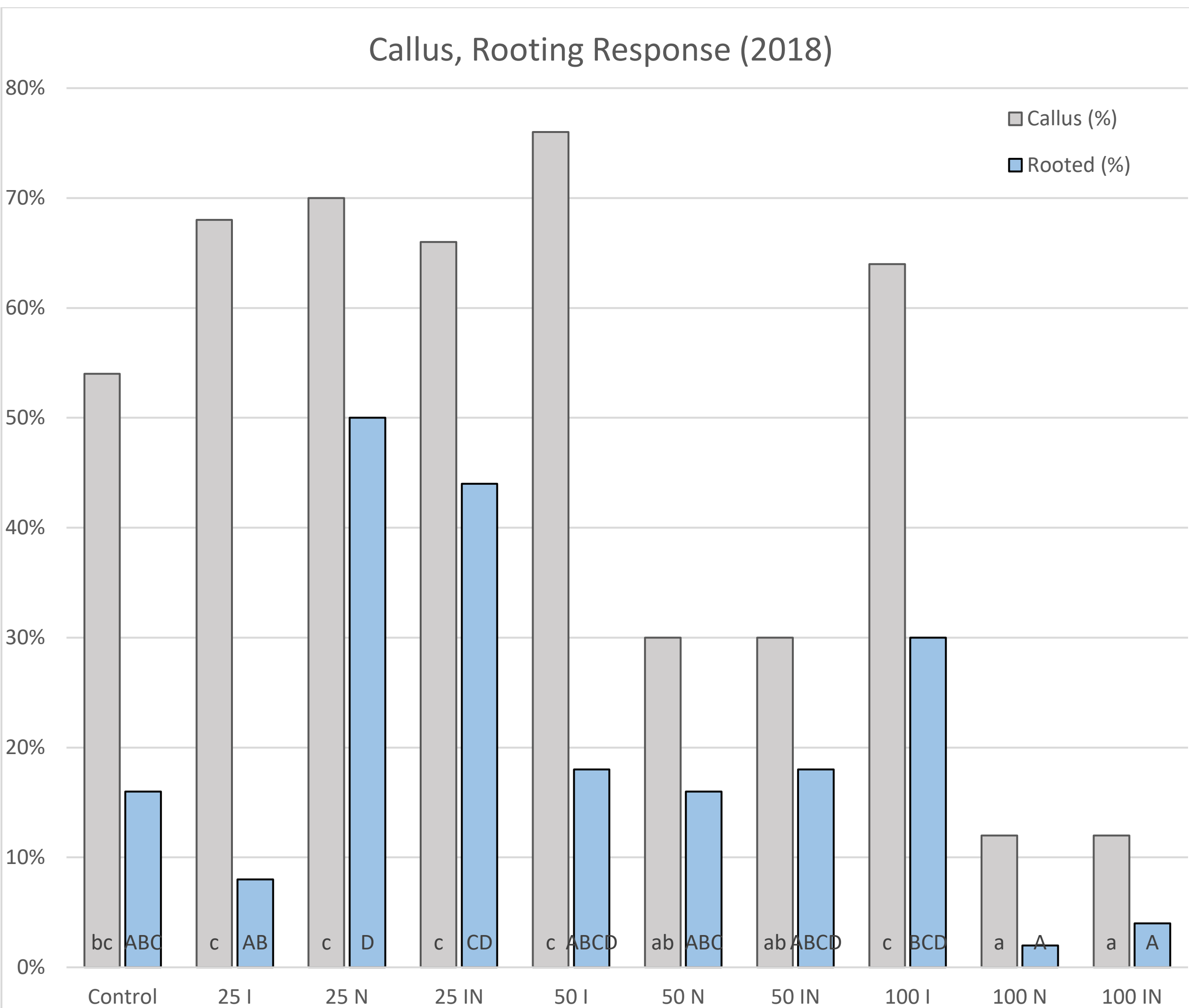


Table 2.7 - Summary of Callus Results (2018)

Treatment name	IBA (ppm)	NAA (ppm)	n	Callus (#)	Callus (%)
Control	0	0	50	27 bc	54%
25 I	2500	0	50	34 c	68%
25 N	0	2500	50	35 c	70%
25 IN	2500	2500	50	33 c	66%
50 I	5000	0	50	38 c	76%
50 N	0	5000	50	15 ab	30%
50 IN	5000	5000	50	15 ab	30%
100 I	10000	0	50	32 c	64%
100 N	0	10000	50	6 a	12%
100 IN	10000	10000	50	6 a	12%

Table 2.8 - *p*-values for Pairwise Comparisons for Callus Formation (2018)

	Control	25 I	25 N	25 IN	50 I	50 N	50 IN	100 I	100 N
25 I	0.317	--	--	--	--	--	--	--	--
25 N	0.223	1.000	--	--	--	--	--	--	--
25 IN	0.419	1.000	0.937	--	--	--	--	--	--
50 I	0.064	0.631	0.794	0.501	--	--	--	--	--
50 N	0.047	0.001	<0.001	0.001	<0.001	--	--	--	--
50 IN	0.047	0.001	<0.001	0.001	<0.001	1.000	--	--	--
100 I	0.535	0.937	0.795	1.000	0.387	0.003	0.003	--	--
100 N	<0.001	<0.001	<0.001	<0.001	<0.001	0.074	0.074	<0.001	--
100 IN	<0.001	<0.001	<0.001	<0.001	<0.001	0.074	0.074	<0.001	1.000

Table 2.9 - Summary of Rooting Results by Rooting Response (2018)

Treatment name	IBA (ppm)	NAA (ppm)	n	Rooted (#)	Rooted (%)
Control	0	0	50	8 ABC	16%
25 I	2500	0	50	4 AB	8%
25 N	0	2500	50	25 D	50%
25 IN	2500	2500	50	22 CD	44%
50 I	5000	0	50	9 ABCD	18%
50 N	0	5000	50	8 ABC	16%
50 IN	5000	5000	50	9 ABCD	18%
100 I	10000	0	50	15 BCD	30%
100 N	0	10000	50	1 A	2%
100 IN	10000	10000	50	2 A	4%

Table 2.10 - *p*-values for Pairwise Comparisons for Rooting Response (2018)

	Control	25 I	25 N	25 IN	50 I	50 N	50 IN	100 I	100 N
25 I	0.440	--	--	--	--	--	--	--	--
25 N	0.003	<0.001	--	--	--	--	--	--	--
25 IN	0.013	<0.001	0.816	--	--	--	--	--	--
50 I	1.000	0.329	0.005	0.025	--	--	--	--	--
50 N	1.000	0.440	0.003	0.013	1.000	--	--	--	--
50 IN	1.000	0.329	0.005	0.025	1.000	1.000	--	--	--
100 I	0.246	0.025	0.113	0.329	0.329	0.246	0.329	--	--
100 N	0.056	0.440	<0.001	<0.001	0.034	0.056	0.034	0.001	--
100 IN	0.056	0.440	<0.001	<0.001	0.034	0.056	0.034	0.001	1.000

Root Number

A one-way ANOVA was conducted to compare the effects of the treatments on the average number of roots per cutting (Figure 2.9). There was a significant effect on average root numbers at the $p < .05$ level for the treatments [$F(9, 490) = 6.71$, $p < .001$]. Post hoc comparisons using the Tukey HSD test indicated that the mean number of roots produced by the 25 N (M = 4.62, SD = 7.35) and the 25 IN (M = 3.20, SD = 5.35) treatments were both significantly different than the control (M = 0.5, SD = 1.34). The 25 N treatment produced significantly ($p <$ .001) more roots per cutting than the control, and the 25 IN treatments also produced significantly ($p = .03$) more roots per cutting than the control (Table 2.11).

Figure 2.9 - Chart of Rooting Results by Roots per Cutting (2018)

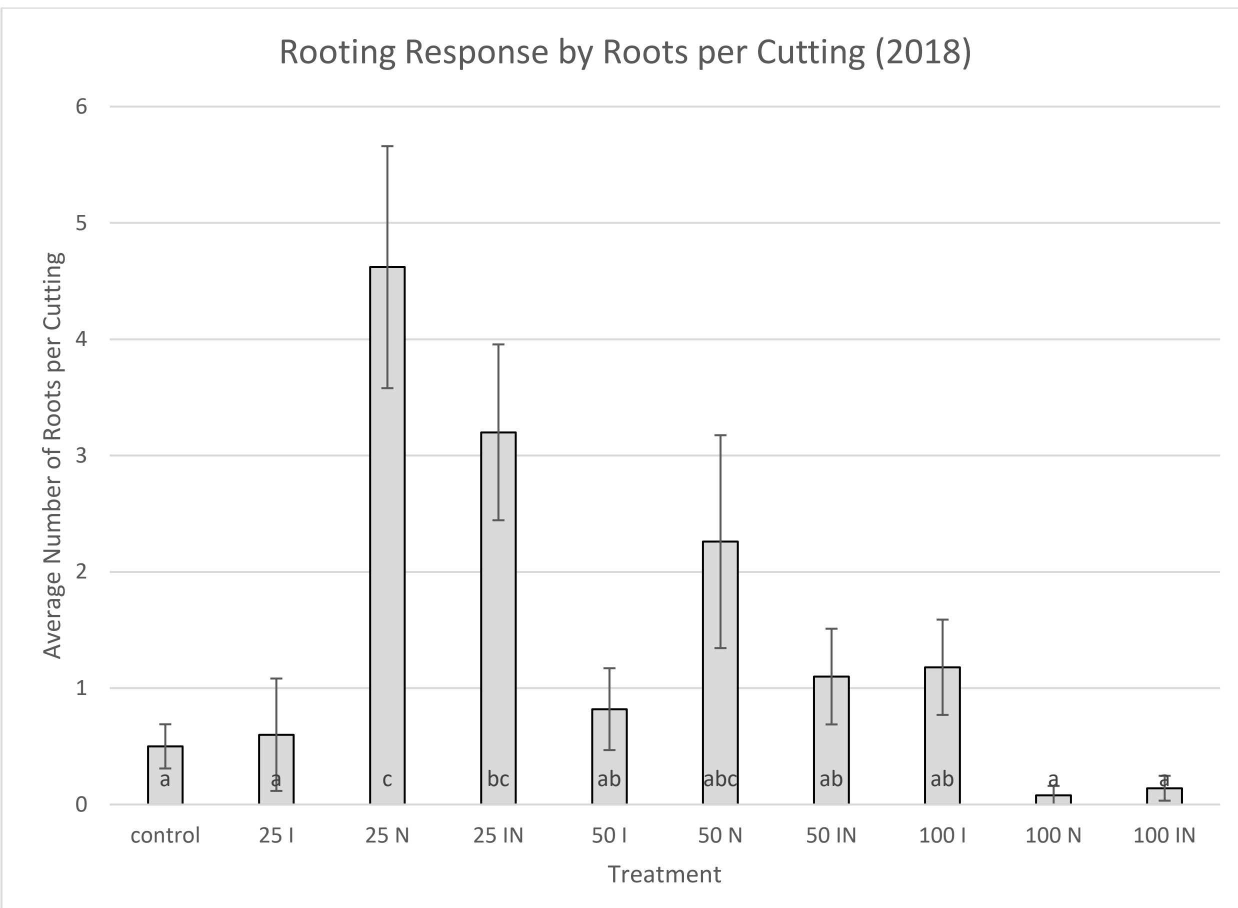


Table 2.11 - Summary of Rooting Results by Roots per Cutting (2018)

Treatment	IBA (ppm)	NAA (ppm)	n	Roots/cutting	Roots/success
Control	0	0	50	0.50 a	3.13
25 I	2500	0	50	0.60 a	7.50
25 N	0	2500	50	4.62 c	9.24
25 IN	2500	2500	50	3.20 bc	7.27
50 I	5000	0	50	0.82 ab	4.56
50 N	0	5000	50	2.26 abc	14.13
50 IN	5000	5000	50	1.10 ab	6.11
100 I	10000	0	50	1.18 ab	3.93
100 N	0	10000	50	0.08 a	4.00
100 IN	10000	10000	50	0.12 a	3.00

Taken as a whole, the treatments that resulted in the highest number of successful rooting responses and the highest number of roots were 25 N and 25 IN, which were significantly different (higher) than the control and several other treatments, in both evaluations.

DISCUSSION

The long period needed to develop callus and then induce differentiation into roots (16 to 20 weeks) may account for the relatively low percentage (8-20%) for most treatments, with only three treatments rooting at 30% or better in the combined 2016 and 2018 treatments. These rates are similar to those reported by Gil-Albert & Boix, 1978 (0-20%) and Bogdany, 1954 (40-75%), but lower than Zorg, 1953 (53%), Box & Beach, 1968 (82%), Henry, 1992 (87%), and Loach, 1988 (91%) reported using comparable methods. It is probable that other factors beyond auxin concentration play significant roles in rooting success; these could include geographical provenance, parent tree health, time of year, and time of day when cuttings are taken, suggested by Henry (1992), Duer (1981), and Ragonezi (2010).

The 50% rooting rate in the 2018 25 N treatment is low when compared to the standards expected in the plant propagation industry, reported by Wagner (1967). The long period needed to produce roots represents another limiting factor in the potential for commercialization of this process. A more comprehensive understanding of the timeframe for developing callus and adventitious roots may provide an opportunity to decrease the time needed and increase efficiency. Some other species of *Juniperus* responded to a post-callus re-treatment with auxin (Whalley 1965) which may be feasible with *Juniperus virginiana.*

Additionally, the low percentage of rooted cuttings may be linked to the age of the stock plants, which are older than the "ideal" age of 2-4 years recommended by Edson (1996) and Henry (1992). The greenhouse space used for this study did not have the ability to provide partial shade for the cuttings, potentially prematurely depleting the cuttings of stored reserves as they were exposed to bright sunlight. Shading may improve results, as is reported by Banko (1981).

As Kentelky (2011) indicates, concentration of auxin does not seem to affect the percentage of eastern redcedar cuttings that develop callus. Increasing concentrations of IBA and NAA are not linked directly to rooting success or number of roots developed, but instead there may be a "sweet spot" of NAA concentration between no response and phytotoxicity that can be identified. This effect is especially noticeable when comparing the different concentrations of NAA, independently of IBA concentration. Further study of this variable is warranted.

CONCLUSIONS

It is possible to induce adventitious rooting on eastern redcedar. However, the ability to produce roots at the levels reported in the literature may be lower for trees from the Great Plains provenance, as compared to the largely eastern North American sources obtained for these previous studies. Factors that may influence this could include stock plant age, genotype-driven predisposition for rooting response, and abiotic stresses from soil factors and water status.

Controlling for these outside factors, it may be possible to increase the percentage of rooted cuttings to economically viable levels (60-80%), as reported by Wagner (1967).

Field establishment of rooted cuttings is an important factor in assessing the successful rooting of these cuttings beyond initiation of adventitious roots alone. Unlike the ornamental nursery trade, conservation-grade plant material is smaller and younger, and is not transplanted into a highly-managed landscape. These conservation-grade seedlings are usually one or two years of age, 18-24” in height, and possess a root system in balanced proportion to top growth.

Comparing seedlings to cuttings, adventitious roots tend not to be as vigorous as seedling roots, making rooted cuttings potentially unsuitable for the additional stress that conservation-grade plant material often receives in the harsh conditions in which they are established; minimum levels of weed control and supplemental water are provided. Acceptable rates of survival for conservation plant seedlings are 70-90%, assuming acceptable levels of pre-plant and post-plant maintenance are provided.

Further study is warranted to examine the role that IBA vs NAA plays in induction of rooting in *Juniperus virginiana*, but it would appear that NAA concentration may be more closely linked to root response than IBA when it comes to developing multiple adventitious roots in this species.

Additionally, the plagiotropism of plants arising from cuttings has not been adequately studied. If lateral growth tends to persist on these cuttings, they become structurally unsuitable for conservation plantings. Further long-term study of these questions is required before introduction of male cutting sourced plant material is feasible for conservation purposes such as windbreaks and shelterbelts on challenging sites. Chapter 3 details an initial assessment of these questions.

Chapter 3 - Field Trials and Assessment

BACKGROUND

Rooted cuttings of eastern redcedar have not been assessed beyond the production phase, leaving uncertainty on establishment success and form. The objectives of this project are twofold, regarding assessment of establishment and assessment of growth habit.

First, will rooted cuttings of eastern redcedar establish at a similar rate to traditionally-produced eastern redcedar conservation-grade seedings?

Second, will the growth habit (form) of these rooted cuttings be suitable or similar to the growth habit of traditionally-produced eastern redcedar conservation-grade seedlings used for windbreak purposes?

While establishment practices for conservation seedlings are commonly defined as those that occur in the first 1-3 years (Strine 2004), some sense of establishment suitability could be understood within the shorter scope of this project, which began in summer 2018. Survival over the first two growing seasons is a suitable metric for assessing the likelihood of longer-term establishment, but with the caveat that there may be longer-term differences, such as poor root anchoring against wind events (Gilman et al. 2013, Gilman et al. 2014), that cannot be assessed in this time frame.

Similarly, windbreak utility is driven by windbreak geometry, as the area protected by the windbreak is a function of the windbreak height (Brandle et al. 1990), it is not possible to fully assess growth form suitability for windbreaks of trees that will not grow to a functional windbreak height during the term of this study.

However, indications of upright growth (or lack of plagiotropism) may be taken as a suitable analog for acceptable growth form likely to occur in the future. Whether this upright growth will exhibit typical apical dominance (dominance of the main, central stem in plant growth over side stems) or have a profusion of co-dominant stems (phenomenon of multiple central stems without a main leader) may not be clear, however.

MATERIALS AND METHODS

Five rooted cuttings (undetermined sex) that survived the 2015 treatments, and 34 rooted cuttings (from male parent trees) from the 2016 treatments were transplanted into quart-size containers with a one part peat to one part perlite (by volume) medium, and held in a glass-glazed greenhouse to grow a more vigorous root system prior to field trials. Regular watering was provided, and cuttings were fertilized with a 20-20-20 water-soluble fertilizer (Jack's, JR Peters, Allentown, PA) at a 150 ppm N rate on a monthly basis during the growing season. Growing season greenhouse temperatures were held at 85 F daytime and 75 F nighttime, and winter temperatures were 50 F day and night (as cool as environmental system settings allowed).

In May 2018, a planting site was selected at the Manhattan (KS) USDA NRCS Plant Materials Center (PMC) where land and basic maintenance would be provided for the purposes of assessing the establishment and growth of eastern redcedar cuttings (Figure 3.11). According to USDA NRCS Web Soil Survey information, the soil type at this site is Stonehouse-Eudora complex, a combination of silt loam and loamy fine sand with 0-2 percent slope. The high drainage of this soil type necessitated supplemental irrigation, provided as needed by PMC staff during the growing season.

At this site, an electric fence was erected to exclude deer and other nuisance animals from interference (mechanical damage) with the cuttings and seedlings (Figure 3.10).

Figure 3.10 - Deer fence at the Manhattan PMC field trial site.

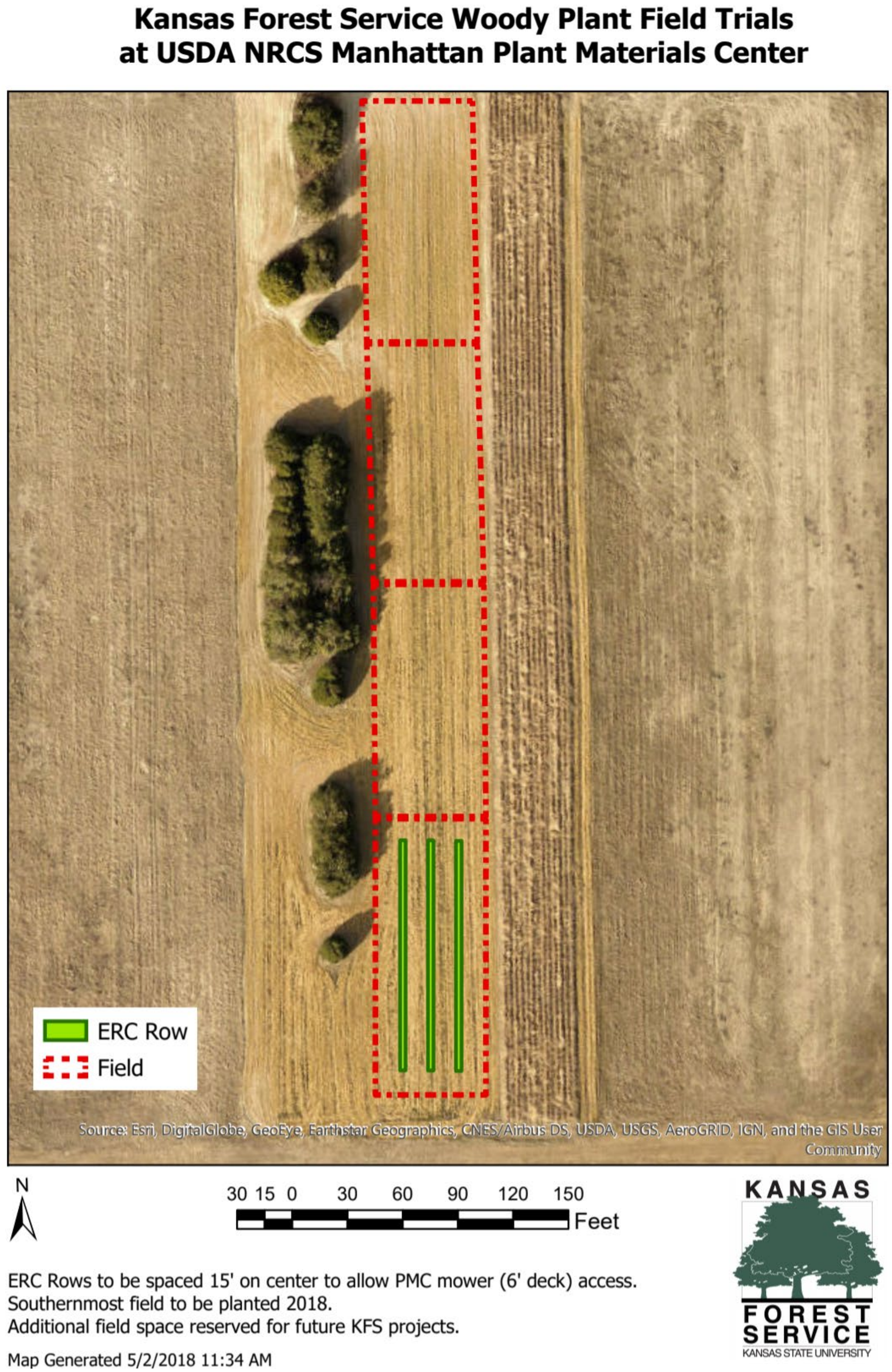


Figure 3.11 - Overview of field trial planting context at Manhattan USDA NRCS Plant Materials Center

After the erection of the electric fence, the planting site was prepared for planting by mowing and then disking strips in the existing warm-season grasses present on the site. The field site was planted with 26 cuttings from the 2016 propagation experiment (selected for consistency of size), and all 5 cuttings from the 2015 propagation experiment. Additionally, as a control, 26 seedlings were selected from a pool of 50 conservation-grade eastern redcedar containerized seedlings sourced from the Kansas Forest Service, selected based on consistency of size. No fertilizer or soil amendments were added to the site before or after planting.

The field was divided into three rows spaced approximately 15-feet apart, with in-row spacing of 6 feet between plants (Figure 3.11). Each transplant location was covered with a 4-foot square of black polypropylene weed barrier fabric from the Kansas Forest Service, part of standard practice for establishment of windbreaks in Kansas (Strine 2004, Armbrust 2017) (Figure 3.12 and Figure 3.13). These windbreaks are commonly planted for use around homesteads to shelter the landscape, field windbreaks to reduce wind erosion, and livestock windbreaks to provide winter shelter for animals. The Kansas Forest Service reported an average of 64,535 seedlings of eastern redcedar sold each year between 2016 and 2020 (Haller et al. 2020).

Figure 3.12 - Aerial photo of field trial planting site, showing row spacing.

Figure 3.13 - Planting rooted cuttings and eastern redcedar seedlings.

Figure 3.14 - Rooted cutting of eastern redcedar (foreground) and containerized eastern redcedar seedling (background), planted with polypropylene weed barrier fabric.

Row order was completely random as to whether a seedling or a cutting was planted on each plot. The five 2015 cuttings were planted last in order, to assist with future culling if they prove to be female (Figure 3.15).

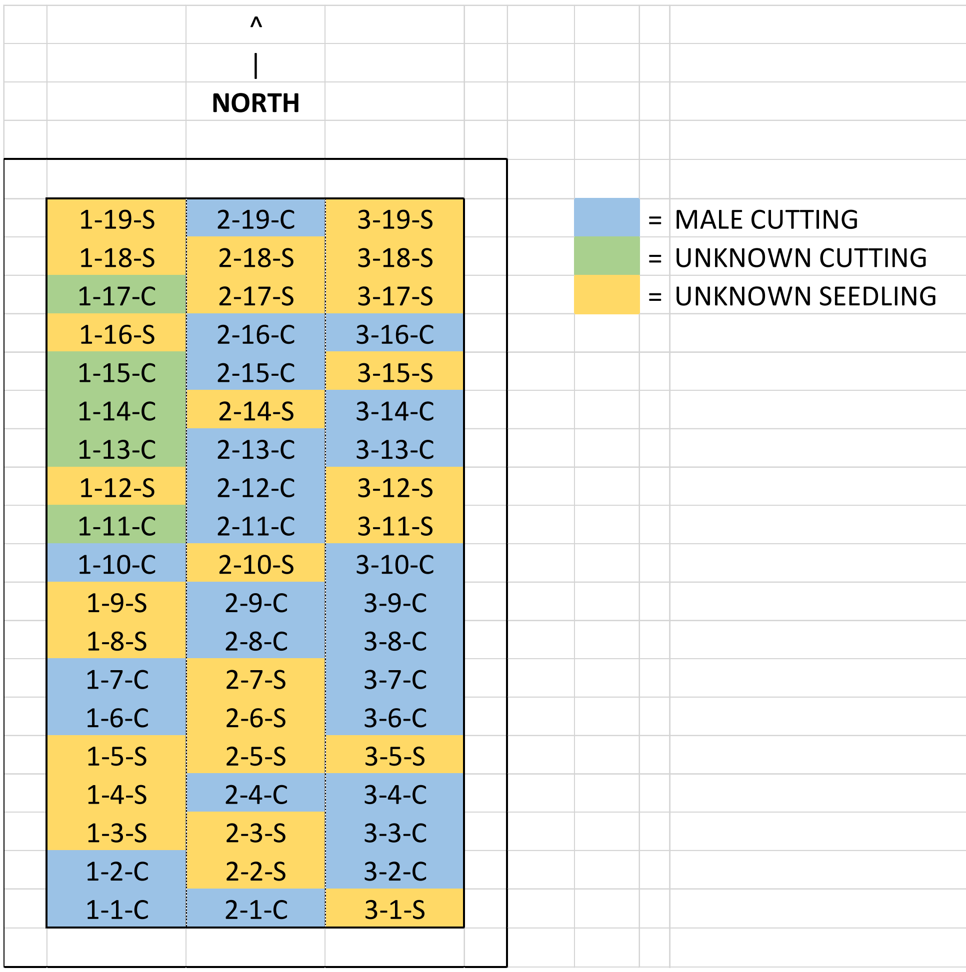


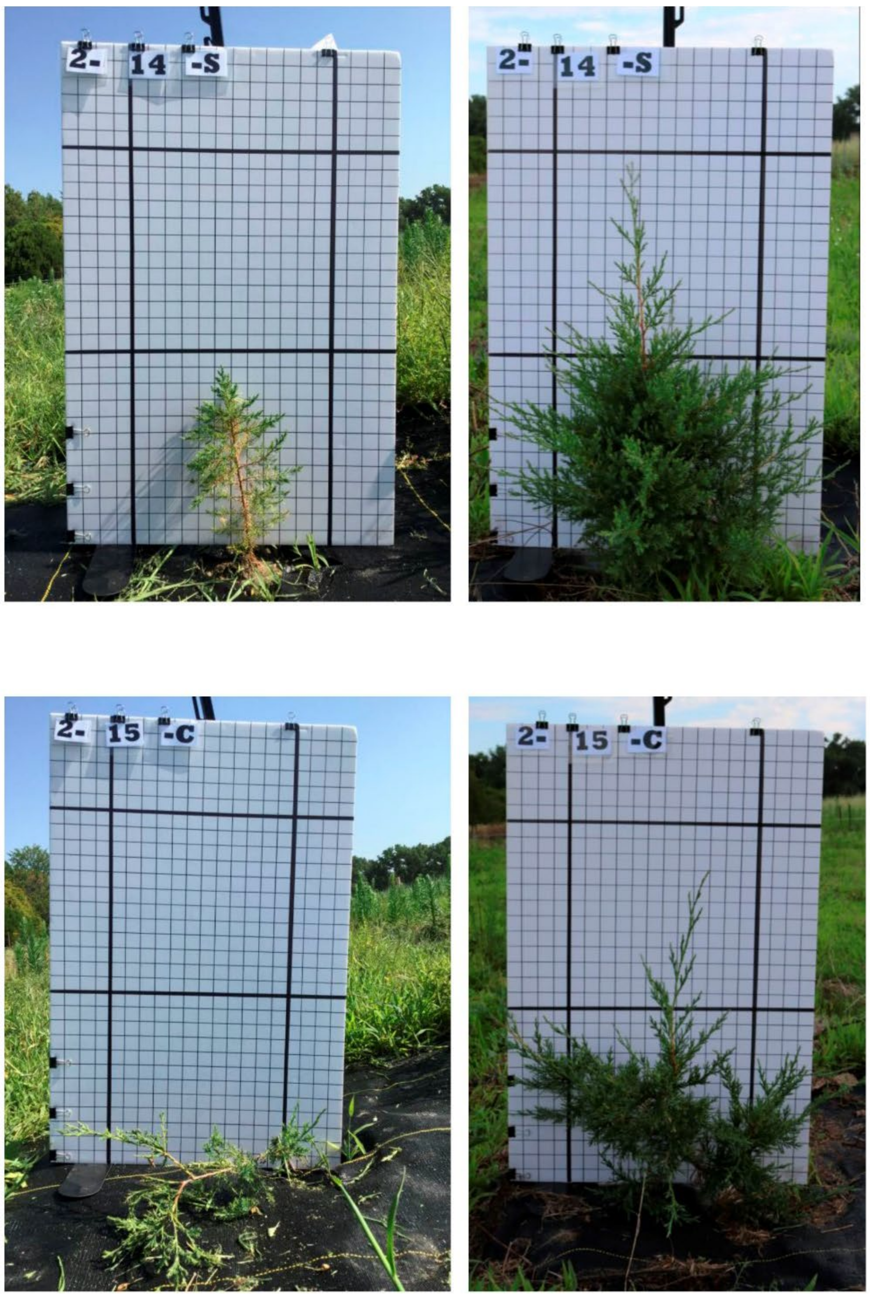
Figure 3.15 - Diagram of planting layout for field trial. First digit indicates row number, second digit is row order, and third letter indicates cutting (C) or seedling (S).

After planting (Figure 3.14), each seedling was watered in to settle the soil with well water on site. The site was mowed (between rows) several times during the growing season, by PMC staff. Weeds were removed by hand from the immediate rooting area of the seedling once per year, to simulate typical field establishment care practices.

An initial series of photos was taken in July 2018, two months after planting. A standard board was used, with a one-inch grid. All photos were taken from the south (facing north) to provide a baseline reference for future growth. In July 2019, one year later, a second series of photos was taken from the same aspect, with the same grid reference board (Figure 3.16). All photos are included in Appendix C.

July 2018

July 2019



July 2018

July 2019

Figure 3.16 - Reference grid board assessment for seedling (top) and cutting (bottom).

For assessment, three measurements were captured for each year. Survival was recorded as a 1 for alive and a 0 for dead. Height was measured to the nearest whole inch on the grid board. To measure deflection from vertical, a calculated deflection value was recorded based on the following protocol.

All photos were matched year-to-year for the same individual (as seen in Figure 3.16) and loaded as a pdf in Adobe Acrobat. Using the "measure" tool, the angle of the bold line on the grid board was recorded as a baseline. Then, the angle between the base of the stem and the uppermost tip of the primary growing point was recorded. (Figure 3.17) From these two measurements, a deflection from vertical (in degrees) was calculated according to the following formula:

$$Deflection = 90 - (Measured - Baseline)$$

A change in time was computed by subtracting the 2018 measurements from the 2019 measurements to assess growth and change in deflection angle, if any.

Figure 3.17 - Measuring baseline angle and angle of deflection with Adobe Acrobat measure tool. (Disregard distance readout.)

RESULTS

Between the planting date in May 2018 and the first collection of data in July 2018, only one eastern redcedar transplant died (1-6-C), a 2015 male cutting. All other transplants (seedlings and cuttings) appeared to be alive after two months.

Between July 2018 and July 2019, all transplants survived with the exception of 2-1-C, 3-6-C, and 3-18-S. This resulted in an establishment rate (survival rate) of 90.3% (28 / 31) for cuttings and 96% (25 / 26) for seedlings (Table 3.1). There was no significant difference between the seedling survival rate and the cutting survival rate according to an analysis by Fisher's Exact Test (p = .62).

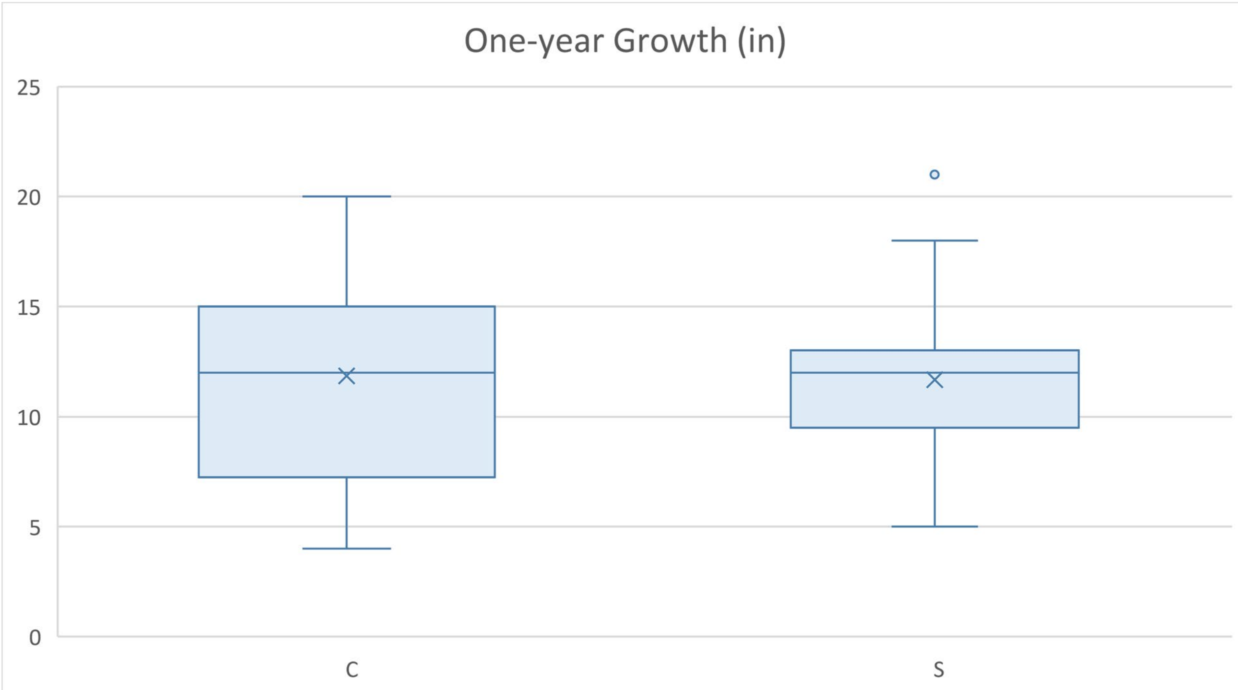
Table 3.1 - Survival differences between cuttings and seedling in field trial.

Status as of July 2019			
Type	**Alive**	**Dead**	**Total**
Seedling	24	1	25
Cutting	28	3	31

A one-way ANOVA was conducted to compare the effect of type (seedling or cutting) on growth rate for surviving individuals after one year (Figure 3.18). Average seedling growth rate was 11.68 inches, while the cuttings averaged 11.85 inches. There was not a significant difference in growth rate at the $p < .05$ level between the two groups [$F(1, 51) = .02$, $p = .88$].

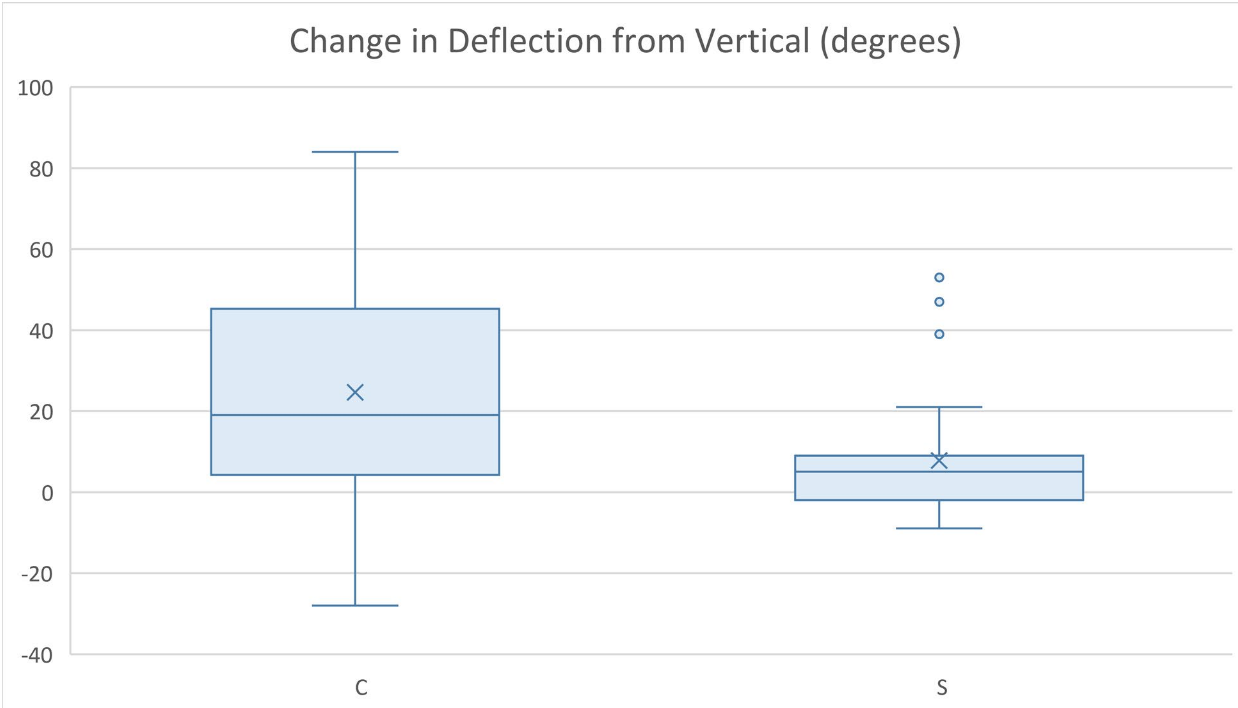
All statistical tests were performed using RStudio Cloud, at http://rstudio.cloud, and graphical results were created with Microsoft's Excel software .

Figure 3.18 - Comparison of growth rate between cuttings (C) and seedlings (S)



A one-way ANOVA was conducted to compare the effect of type (cutting or seedling) on change in deflection (Figure 3.19) for surviving individuals after one year. There was a significant difference in change in deflection at the $p < .05$ level between the two groups [$F(1, 51) = 7.12$, $p = .01$].

Figure 3.19 - Comparison of change in deflection between cuttings (C) and seedlings (S)



The majority (20 out of 26) of seedlings had an initial 2018 deflection from vertical of 10 degrees or less, and every surviving seedling (25 of 26) had a 2019 deflection of less than 20 degrees, leaving little room for change in deflection (Figure 3.21). Of the 26 surviving seedlings, 16 had a decrease in deflection (became more vertical) and 10 had an increase in deflection (became less vertical. Of the 10 seedlings that became less vertical, the change was minor: between 1 and 9 degrees.

Only two cuttings had an initial deflection of 10 degrees or less in 2018, and 9 had a deflection from vertical of greater than 60 degrees (Figure 3.20). One year later, 26 out of 28 surviving cuttings had a deflection of less than 40 degrees, with 23 of 28 having a deflection of 30 degrees or less. Additionally, 23 of 28 cuttings had a decrease in deflection (became more vertical) after one year.

In Figure 3.20 and 3.21, this change in deflection is visualized by individual lines representing individual cuttings (Figure 3.20) or seedlings (Figure 3.21). The steepness of slope in this line is directly related to the degree to which the individual cutting or seedling became more (or less) vertical over the course of one growing season. Dashed lines indicate individual plants that did not survive to the time of data collection in 2019. Mortality is indicated by a 90-degree deflection data point.

Most cuttings had a perceptible "lean" towards horizontal at the time of planting, accounting for the wide spread on the left-hand (2018) side of Figure 3.20. It is clear that many of these individuals became more upright by the narrowing of the spread on the right-hand (2019) side of this figure.

In contrast, the spread of the left-hand (2018) side of Figure 3.21 indicates that while some seedlings had a notable deflection from vertical at the time of planting, the very narrow spread on the right-hand (2019) side of Figure 3.21 shows that nearly every seedling was almost vertical after one growing season.

Figure 3.20 – Visualization of change in deflection for cuttings

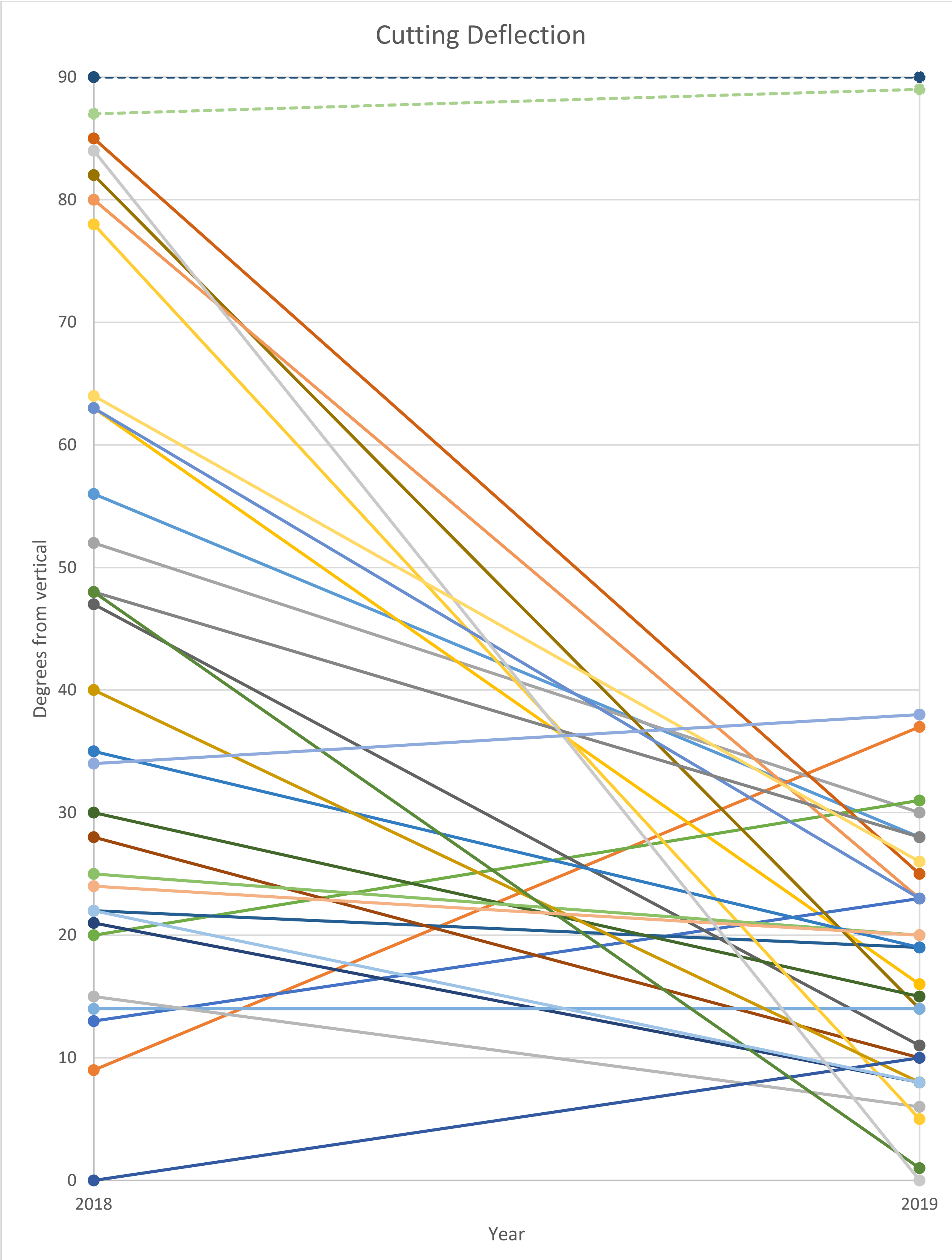
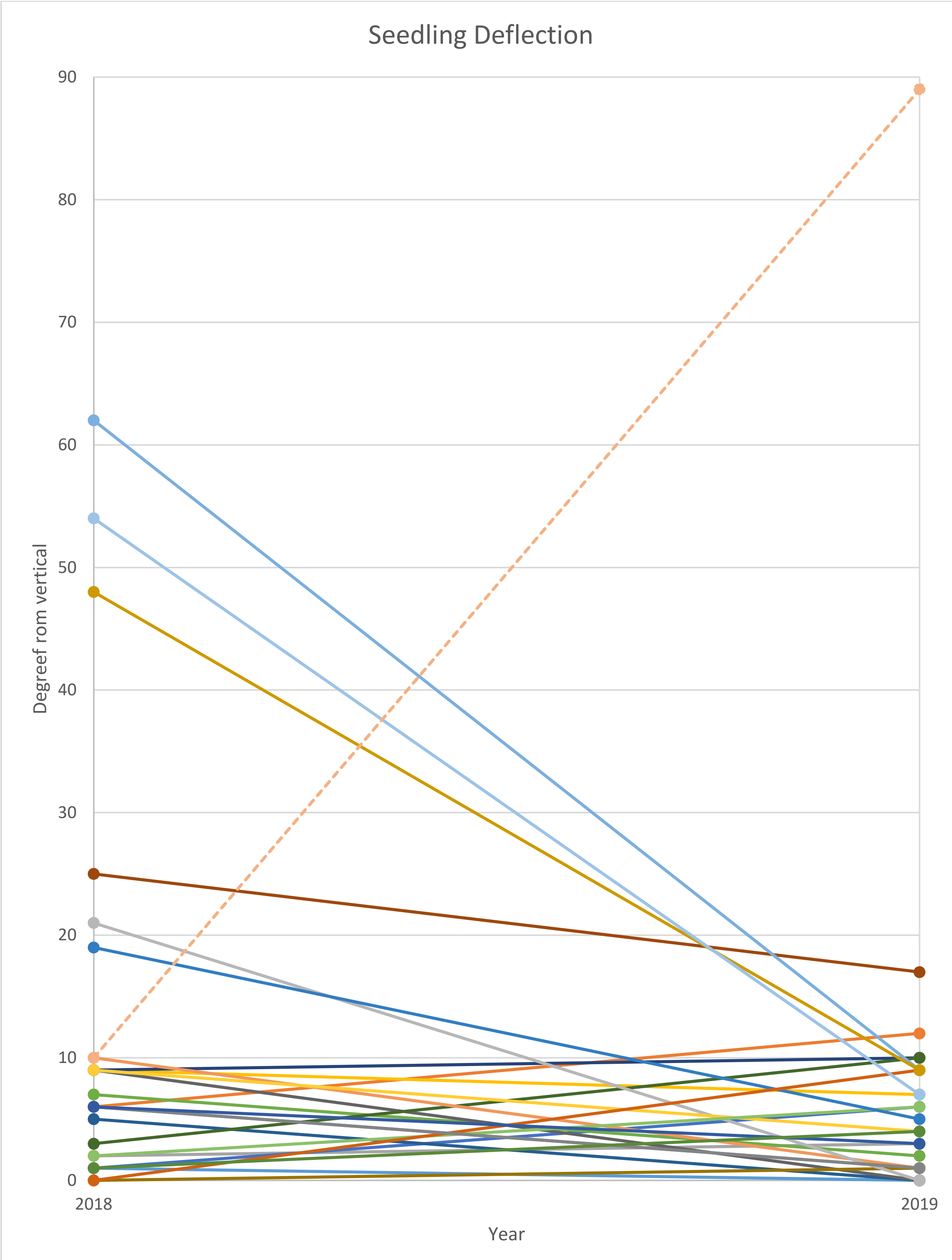


Figure 3.21 – Visualization of change in deflection for seedlings



DISCUSSION

Under standard conservation establishment practices and maintenance, there is no difference in survival rate or growth rate (measured by height) between similar-size seedlings and potted plants produced from cuttings. As noted by Henry (1992), this research to determine differences between seedling and cutting field establishment is previously undescribed, and novel.

Initial deflection from vertical of seedlings is very low, with any lean self-corrected in one growing season. For cuttings, there is often a pronounced lean or horizontal growth that seems to indicate horizontal plagiotropism, but for a majority of cuttings, vigorous vertical growth corrects this lean and the tree has a significantly reduced deflection from vertical in one growing season. This is similar to the findings of Edson (1996), where all "bent leaders" began to develop upright growth after one growing season, though Edson's study was on the closely related *Juniperus scopulorum* and not *J. virginiana.*

There is a persistent and pronounced tendency for cuttings to have a more "splayed" growth form than the tight apically-dominant cone found in seedlings, even though the "main leader" of the cuttings may have vertical growth present. This splayed or open growth could potentially have an impact on the future form and function of a windbreak comprised of cuttings.

Further study is warranted to assess differences, if any, that may exist between collecting cuttings in different months, from varying parts of the native range of *Juniperus virginiana*, from trees of various ages, and from collecting cuttings from various portions of the canopy. These factors could potentially impact rooting percentage, growth rate, and overall form.

CONCLUSIONS

The perceived barriers to use of eastern redcedar cuttings in conservation plantings may not be as significant as assumed. Survival and growth rate are similar to seedlings, given similar care in the field. In the short term, vertical growth form appears to be adequate for cuttings, but longer-term assessments are required to determine suitability for windbreak purposes, which commonly begin to have benefit as the windbreak reaches 10-15 feet tall at 10-20 years of age. The tallest seedling or cutting in this study was just under 3 feet tall after one growing season in the field, so the implications of the form portion of this study are somewhat limited by its timeframe.

No assessment of rooting strength (resistance to directional wind forces) could be assessed between the seedlings and the cuttings at the time of publication. It is conceivable that there may be differences in the anchoring function of the root systems derived from seed roots (seedlings) and adventitious roots (cuttings) in the long term.

In addition, there exists another factor that was not assessed in the scope of this study. For conservation purposes, the cost of seedlings to establish a windbreak is minimal, around \$1-2 per seedling. With increased production cost, time of production, and lower “success rate” at producing material that meets conservation grading standards, it is likely that the cost of cuttings would be several times higher than the cost of seedlings. An economic feasibility study would establish this difference, and a survey of landowners and public agencies (i.e., NRCS, conservation districts) that support cost-share on windbreak establishment would be able to better assess this dimension of cutting feasibility for windbreaks, beyond a propagation/physiological basis, as is contained in this study.

References

Armbrust, R. 2017. Weed Control Options in Tree Plantings. Kansas State Research and Extension. L848. 12 p.

Banko, T.J. 1981. Propagation of upright junipers. Proc. Intl. Plant Propagators Soc. 31:658–665.

Blythe, E.K., J.L Sibley, K.M. Tilt, J.M. Ruter. 2007. Methods of auxin application in cutting propagation: a review of 70 years of scientific discovery and commercial practice. J. Environ. Hortic. 25, 166–185.

Bogdany, J. 1954. The rooting of *Juniperus virginiana* 'Canaerti' and *Juniperus virginiana* 'Keteleeri' from cuttings. Proc. Intl. Plant Propagators Soc. 4:45–47.

Boyer, C.R., J.J Griffin, B.M Morales, E.K. Blythe. 2013. Use of Root-Promoting Products for Vegetative Propagation of Nursery Crops. Kansas State Research and Extension. MF3105. 4 pp.

Box, B.H., L.C. Beech. 1968. Vegetative propagation trials of eastern redcedar and Arizona cypress in the greenhouse. Tree Planters' Notes. 19(3):1–2.

Brandle, J. R., S. Finch. 1991. How windbreaks work. University of Nebraska Extension. EC 9 1-1763-B

Chong, C., B. Hamersma. 1995. Automobile radiator antifreeze and windshield washer fluid as iba carriers for rooting woody cuttings. HortScience. 30(2):363–365.

Chong, D. 2003. Influence of bottom heat and mulch on rooting of evergreen cuttings. Proc. Intl. Plant Propagators Soc. 53:496–500.

Cunningham, R.A., R.M. King. 2000. Juniper seed sources in the Great Plains. Gen. Tech Rep. RMRS-GTR-51. USDA Forest Service. 19 p.

Duer, R.A. 1981. Cutting propagation of *Juniperus scopulorum* cultivars. Proc. Intl. Plant Propagators Soc. 31:141–144.

Edson, J.L., D.L. Wenny, R.K. Dumroese, A. Leege-Brusven. 1996. Mass propagation of rocky mountain juniper from shoot tip cuttings. Tree Planter's Notes. 47(3):94–99.

Frampton, L.J, J.F. Hodges. 1989. Nursery Rooting of Cuttings from Seedlings of Slash and Loblolly Pine, Southern Journal of Applied Forestry, 13(3):127–132.

Gil-Albert, F., E. Boix. 1978. Effect of treatment with IBA on rooting of ornamental conifers. Acta Horticulturae. 79:63–77.

Gill, J.G.S. 1983. Comparisons of Production Costs and Genetic Benefits of Transplants and Rooted Cuttings of *Picea sitchensis*, Forestry: An Intl. J. of Forest Res., 56(1):61–73.

Gilman, E.F., J. Miesbauer, C. Harchick, R.C. Beeson. 2013. Impact of tree size and container volume at planting, mulch, and irrigation on *Acer rubrum* L. growth and anchorage. Arbor. & Urban For, 39:173–181.

Gilman, E.F., C. Harchick. 2014. Root system morphology influences lateral stability of *Swietenia mahagoni*. Arboriculture & Urban Forestry, 40(1):27–35.

Hartmann, H.T., D.E. Kester, F.T. Davies, R.L. Geneve. 2011. Plant propagation: Principles and practices. Prentice Hall. Upper Saddle River, N.J. 928 p.

Van Haverbeke, D.F., R.M King. 1990. Genetic variation in Great Plains *Juniperus*. Research Paper RM-292. USDA Forest Service. 8 p.

Haller, M., A. Yoder, E. Peterson. 2020. Conservation Tree Planting Program Annual Report. Kansas Forest Service. Manhattan, KS. 12 p.

Henry, P.H., F.A. Blazich, L.E. Hinesley. 1992. Nitrogen nutrition of containerized eastern redcedar. II. Influence of stock plant fertility on adventitious rooting of stem cuttings. J. Amer. Soc. Hort. Sci. 117(4):568–570.

Henry, P.H., F.A. Blazich, L.E. Hinesley. 1992. Vegetative propagation of eastern redcedar by stem cuttings. HortScience. 27(12):1272–1274

Hill, J.B. 1962. The propagation of *Juniperus chinensis* in greenhouse & mistbed. Proc. Intl. Plant Propagators Soc. 12:173–178.

Ikeuchi M., et al. 2017. Wounding triggers callus formation via dynamic hormonal and transcriptional changes. Plant Physiol. 175 1158–1174. 10.1104/pp.17.01035

Kentelky, E. 2011. The analysis and growth peculiarities of *Juniperus* species propagated by cuttings. UASVM Horticulture, 68(1):380–385.

Lawson, E. R. 1990. *Juniperus virginiana* L. Eastern redcedar. Silvics of North America. USDA Forest Service. 1:131-140.

Loach, K. 1988. Hormone applications and adventitious root formation in cuttings – a critical review. Acta Horticulturae. 227:126–133.

Ragonezi, C., K. Klimaszewska, M.R. Castro, M. Lima, P. de Oliveira, M.A. Zavattieri. 2010. Adventitious rooting of conifers: influence of physical and chemical factors. Trees 24: 975–992.

Strine, J. 2004. Windbreaks for Kansas. Kansas State Research and Extension. MF2120. 10 p.

Wagner, G. 1967. Speeding production of hard-to-root conifers. Proc. Intl. Plant Propagators Soc. 17:113.

Westervelt, D.D. 1959. The use of cutting-grafts for producing grafted junipers. (Call Number: LD2668 .T4 1959 W47) Masters Thesis. Kansas State University, Manhattan, KS. K-Rex.

Whalley, J. 1965. Propagation of difficult plants. Proc. Intl. Plant Propagators Soc. 15:338–339.

Zorg, P.G. 1953. The propagation of junipers from cuttings. Proc. Intl. Plant Propagators Soc. 3:81–85.

Appendix A - Explanation of Statistical Analysis Challenges

In statistical analysis, a process called a one-way analysis of variance, or "one-way ANOVA," is a common method to analyze significance of the differences between values for one independent variable. When two independent variables are desired to be analyzed, to assess variance and interaction between the variables, a factorial ANOVA can be used, but the data must be complete. In other words, if two independent variables are compared with a factorial ANOVA, then four groups would be compared. If data for only 3 of those groups exist, or were recorded, then a factorial ANOVA cannot be used to analyze this data in a valid fashion.

In an experimental design that features two treatment factors (IBA and NAA) and four concentrations (0, 2500, 5000, 10000 ppm) each, a complete factorial design would result in 16 groups, which could be analyzed by a factorial ANOVA in a conventional way. However, in a dataset that is missing 6 of those treatment combination groups, factorial ANOVA would not be applicable. The table below illustrates this dataset visually.

Table A.2 Summary of 2018 Treatments

	IBA 0	**IBA 2500**	**IBA 5000**	**IBA 10000**
NAA 0	DATA EXISTS	DATA EXISTS	DATA EXISTS	DATA EXISTS
NAA 2500	DATA EXISTS	DATA EXISTS	*NO DATA*	*NO DATA*
NAA 5000	DATA EXISTS	*NO DATA*	DATA EXISTS	*NO DATA*
NAA 10000	DATA EXISTS	*NO DATA*	*NO DATA*	DATA EXISTS

For this dataset, because of missing combinations, a factorial ANOVA would not be available. Simple linear analysis is possible for some of the data, such as concentration of IBA and concentration of NAA, but analysis that includes the IBA*NAA combinations at 2500, 5000 and 10000 would not be available. To analyze the entire dataset, instead of select subsets, other procedures must be explored.

One procedure that potentially can be used to analyze a dataset with two independent variables with missing data, making it an incomplete factorial, is a procedure within SAS called "GLIMMIX." According to SAS documentation[1], "The GLIMMIX procedure fits statistical models to data with correlations or nonconstant variability and where the response is not necessarily normally distributed. These models are known as generalized linear mixed models (GLMM)."

According to Dr. Pabodha Galgamuwa (personal correspondence) it is possible and reasonable to use a procedure such as GLIMMIX to analyze this dataset with "missing" groups, but caution must be used in the interpretation. For instance, some hypotheses cannot be tested as main effects, but instead tested as simple effects. This phenomenon is common in biological fields, and can be appropriately addressed through procedures such as GLIMMIX, as described by Kathleen Kiernan of SAS[2].

In the text "Analysis of Messy Data," authors George A. Milliken and Dallas E. Johnson (2009) caution the reader that, "Many statistical packages contain routines that calculate test statistics for experiments with missing treatment combinations, but it is shown in this chapter that the observed values of those test statistics often have little, if any, meaning."

However, this does not necessarily mean that no useful statistical meaning can be obtained from experiments with missing treatment combinations, only that such meaning must

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[1] https://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_glimmix_a0000001394.htm

[2] https://www.sas.com/content/dam/SAS/support/en/sas-global-forum-proceedings/2018/2179-2018.pdf

be carefully considered, keeping the limitations of the selected analysis tool in mind. For instance, using GLIMMIX on a dataset with missing groups would still produce multiple results, but some must be discarded as meaningless; according to Galgamuwa, “the software will automatically calculate Type I, Type II, Type III SS and corresponding test statistics by default. But, those interpretations are meaningless due to this missing treatment combinations issue. So it is important that you figure out which questions you can ask from the dataset and what question you can’t address. Then you can actually write that linear combination as a question in SAS to get the desired output. This by no means degrade the value of the analysis, figuring this out would strengthen the overall analysis and the interpretations more valid.”

So, even for a dataset that is not fully factorial, several linear analyses can be performed to determine simple effects of the independent variables. While meaningful analysis of the interaction of the two variables is precluded by the lack of some groups, the simple effects of each variable can still be analyzed and interpreted to have meaning. In the experimental example cited here, there is certainly enough data to generate meaningful interpretations of the simple effects of IBA at 0, 2500, 5000 and 1000; and also of NAA at 0, 2500, 5000, and 10000.

Clearly, the best scenario for a dataset problem of this sort is to take care to design a fully factorial experiment in the first place, to allow for factorial ANOVA instead of a less-powerful GLIMMIX procedure. This aligns with the suggestion of Milliken and Johnson, in reference to experiments where some treatment combinations are never observed, that, “These kind of experimental situations often occur in practice, mostly by chance but sometimes by design. When the experimenter does have control over the experiment, extreme care should be taken to ensure that all treatment combinations are observed.”

While taking this advice into consideration for future experimental design is important, there is still the question of how to deal with existing data. Rather than discarding data from an experiment that lacks certain observations, however, procedures such as GLIMMIX can glean some meaningful interpretations from those data, as long as care is taken to only consider the valid results of that procedure, and not simply take the entire product of the procedure at face value as results are generated by SAS.

Because of the limitations with using GLIMMIX to assess treatment interactions, and the lack of access to the Kansas State University Statistical Consulting Lab due to the global COVID-19 crisis, other statistical tests were utilized in this report. For the statistical analysis contained in this report, the best available statistical tests for the data were selected to evaluate simple effects, as opposed to analysis of factorial effects, which sufficed for understanding treatment effects. For contingency tables with binary data, Chi squared tests were used, but if any cells contained a value of less than 5, Fisher's Exact test was used. If a Chi-squared test determined that there was a significant difference, Fisher's Exact test was used post hoc to determine significance. For data that was non-binary (such as root numbers) a one-way ANOVA was used for analysis, with post hoc Tukey's HSD tests to determine the source of significance between treatments.

All statistical tests were performed using RStudio Cloud, at http://rstudio.cloud, and graphical results were created with Microsoft's Excel software.

Appendix B - Field Trial Assessment Photos

The following photos were taken one year apart. The first photo in each set is at time of planting, while the second photo shows growth and form one year later. The reference board used in each photo depicts the identification of the transplant by row, number, and a designation of C for cutting or S for seedling. This can be referenced to the map in Figure B.22. The reference board is made up of a grid of one-inch squares, with bold lines every 12 inches. All photos are taken from the south side, facing north.

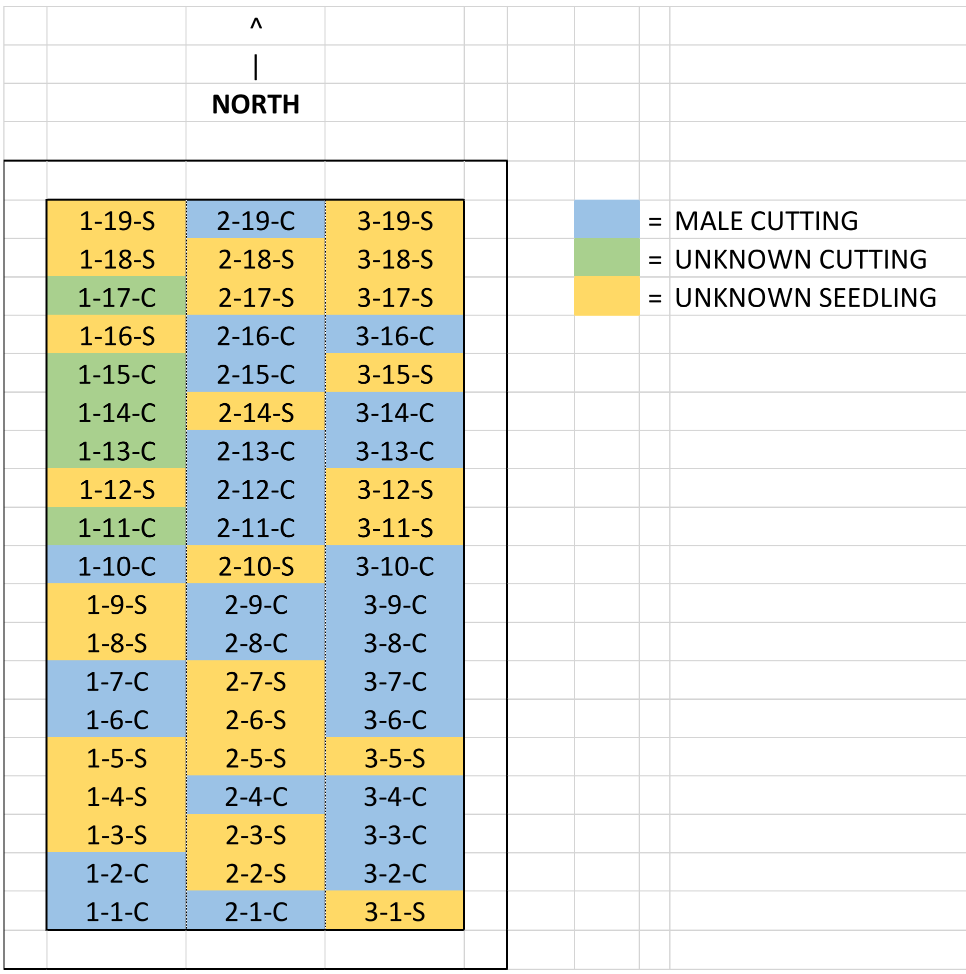

Figure B.22 - Diagram of planting layout for field trial. First digit indicates row number, second digit is row order, and third letter indicates cutting (C) or seedling (S).

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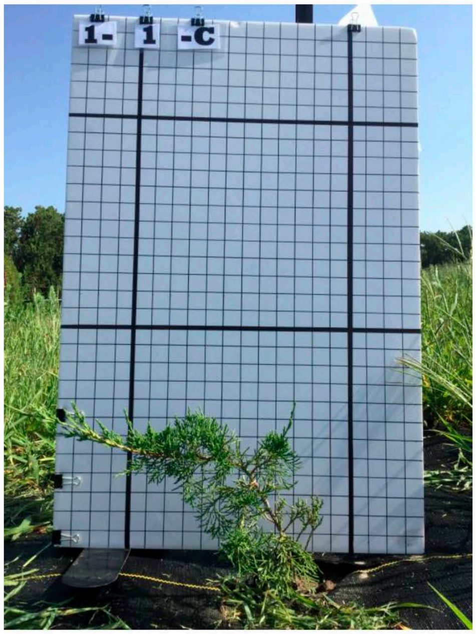

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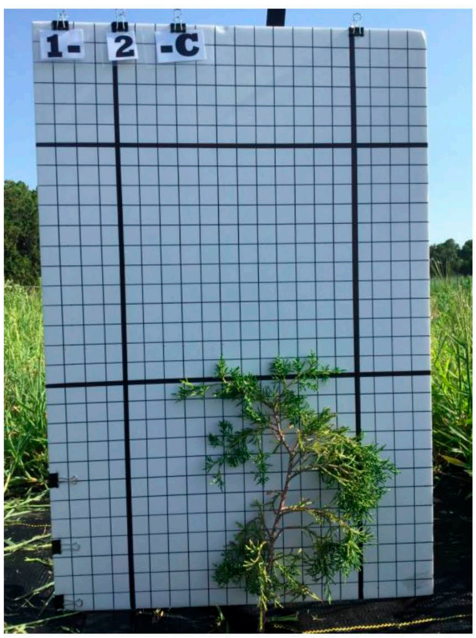

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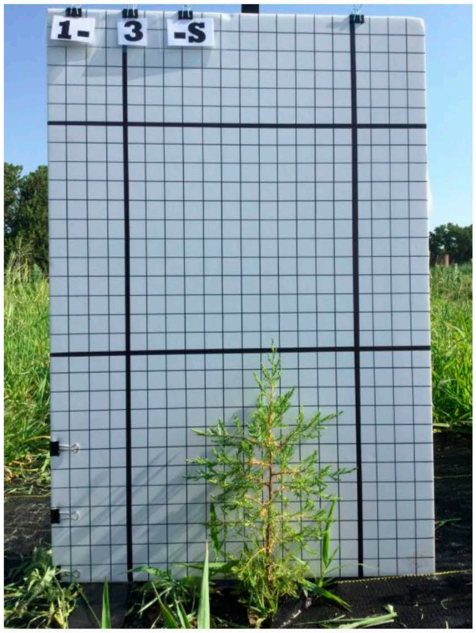

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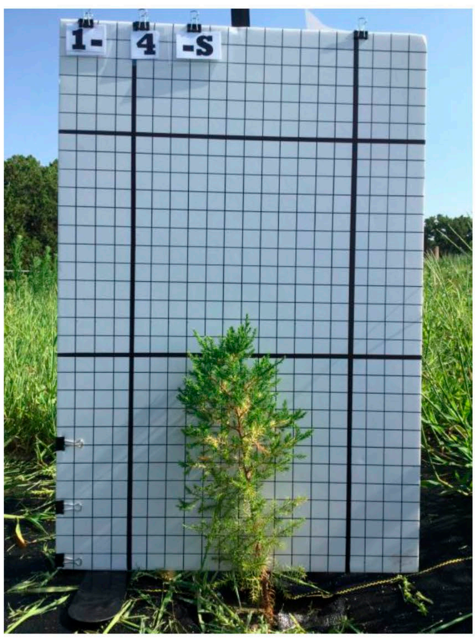

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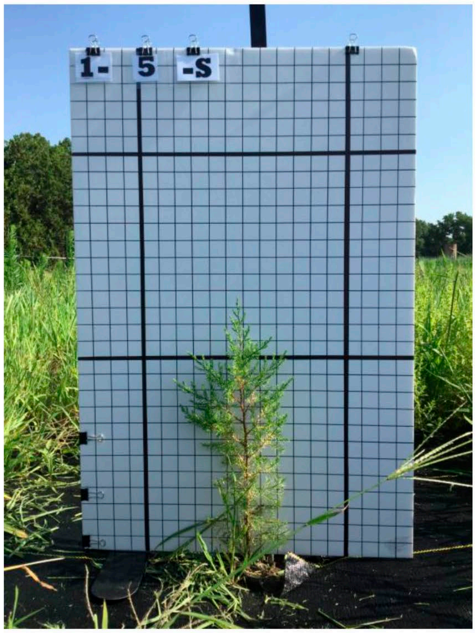

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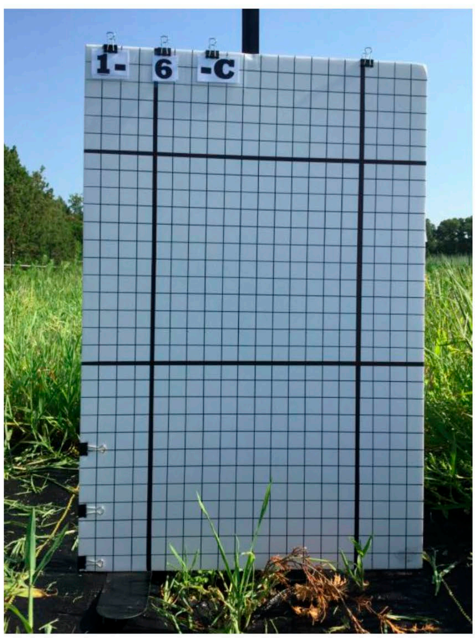

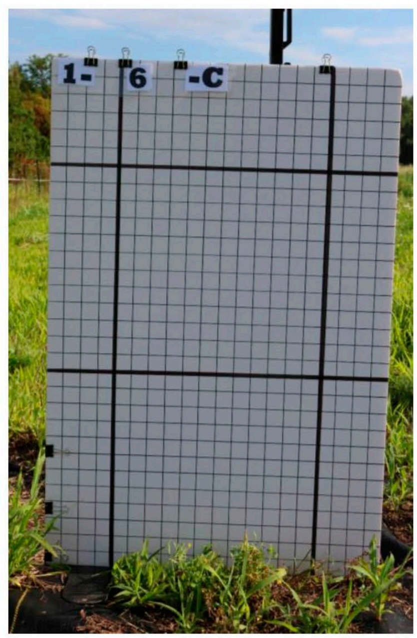

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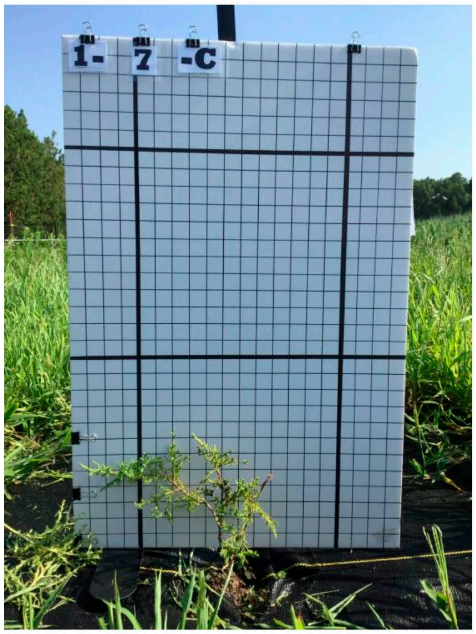

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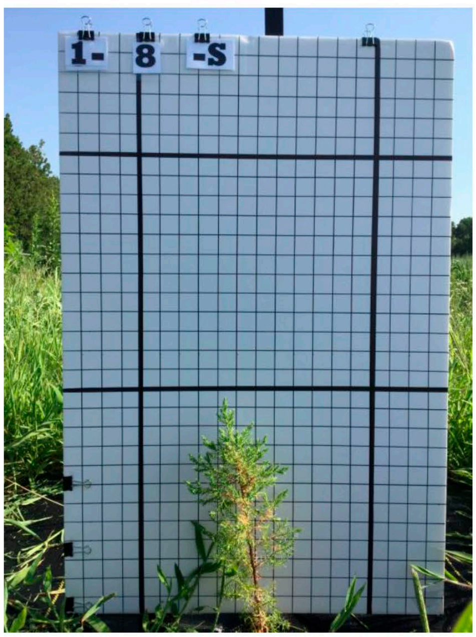

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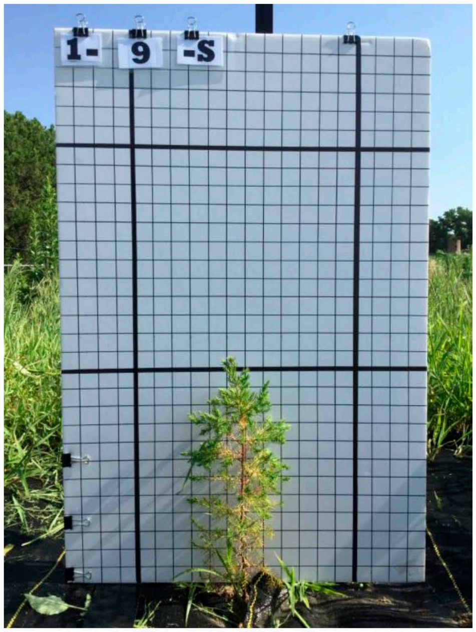

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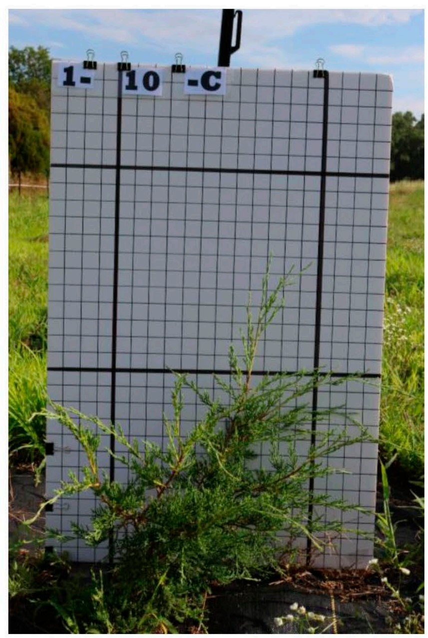

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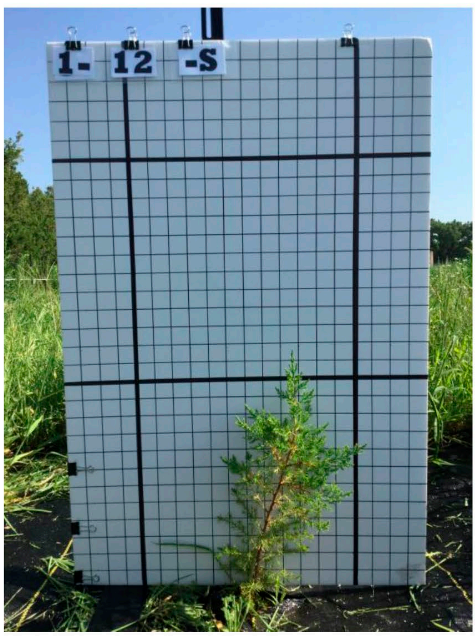

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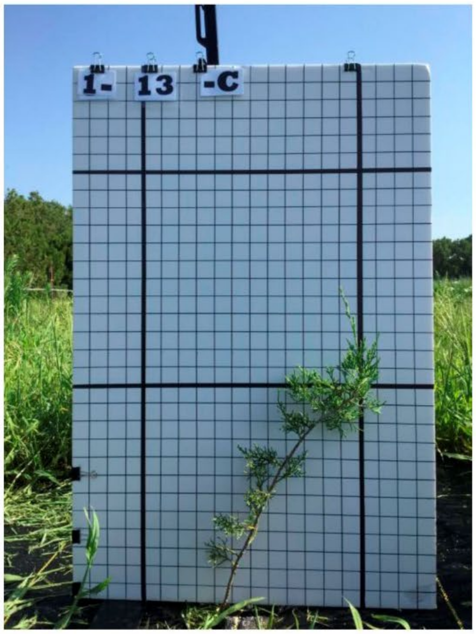

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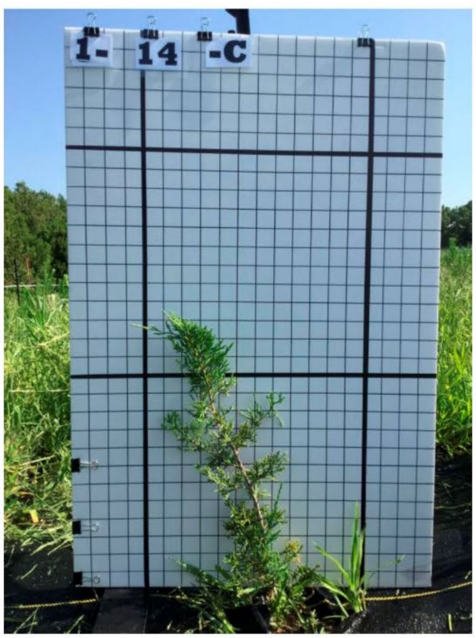

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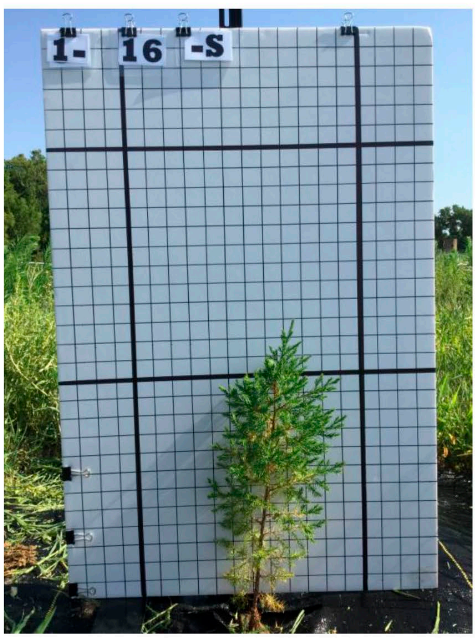

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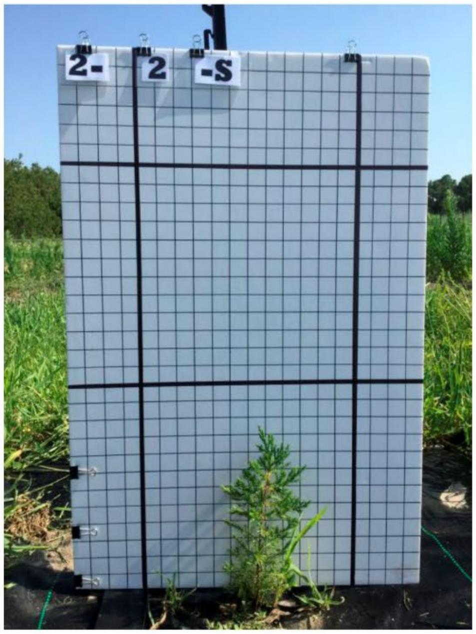

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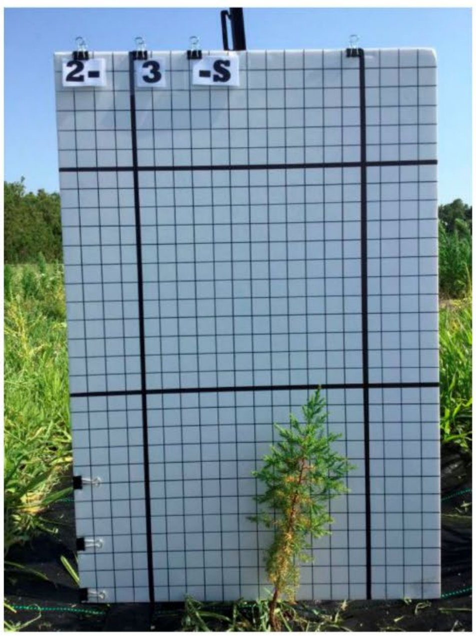

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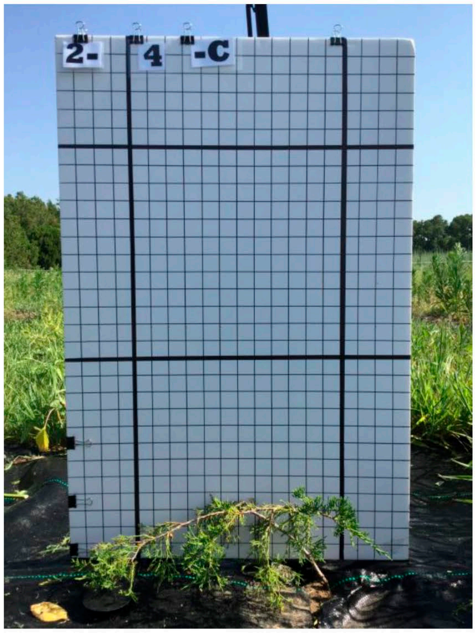

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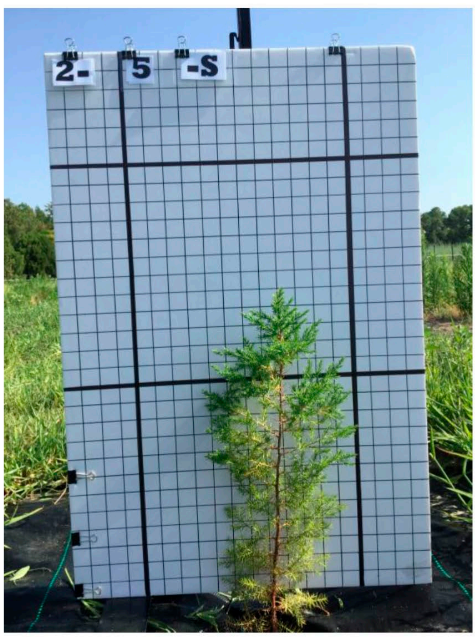

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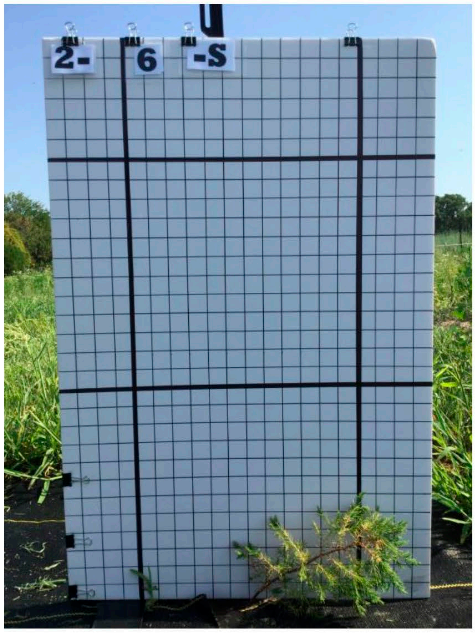

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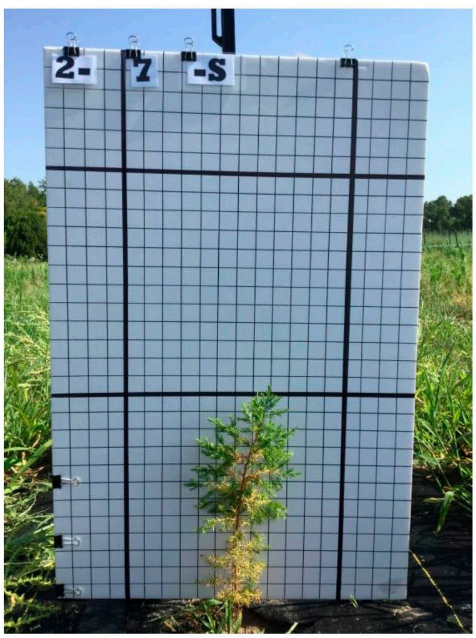

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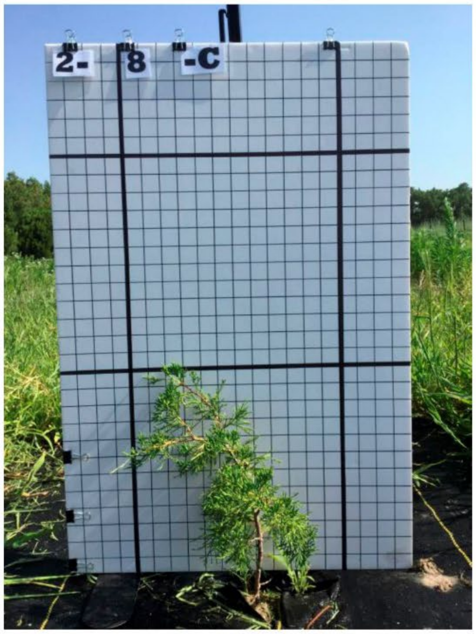

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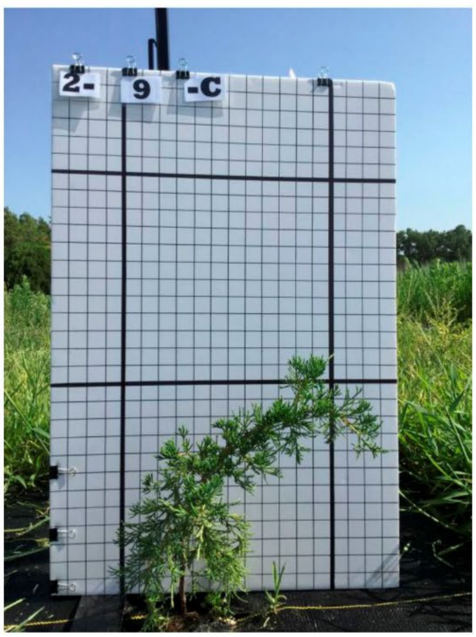

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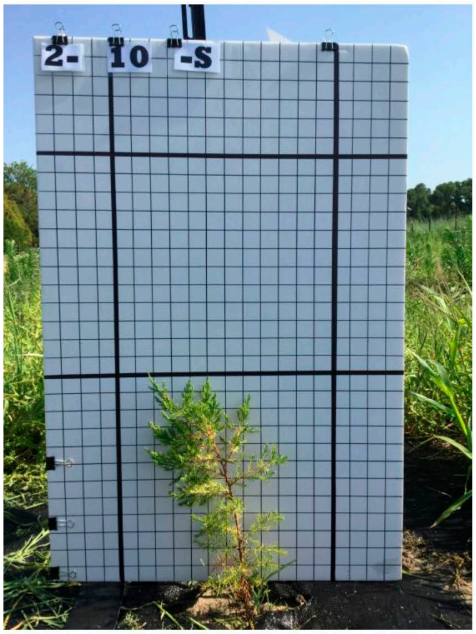

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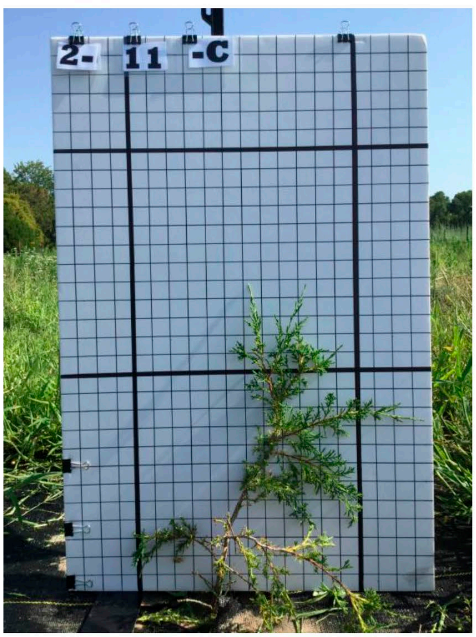

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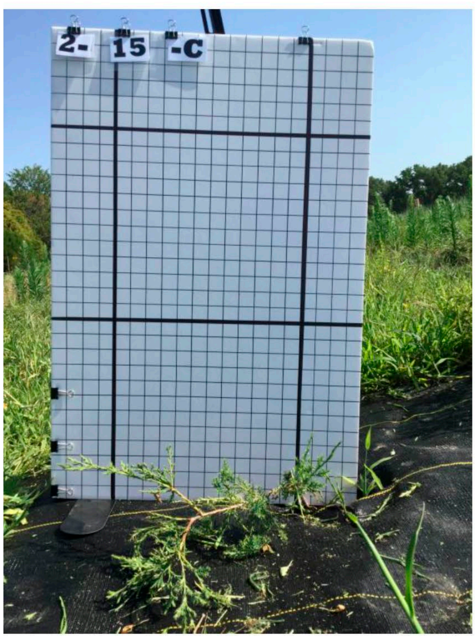

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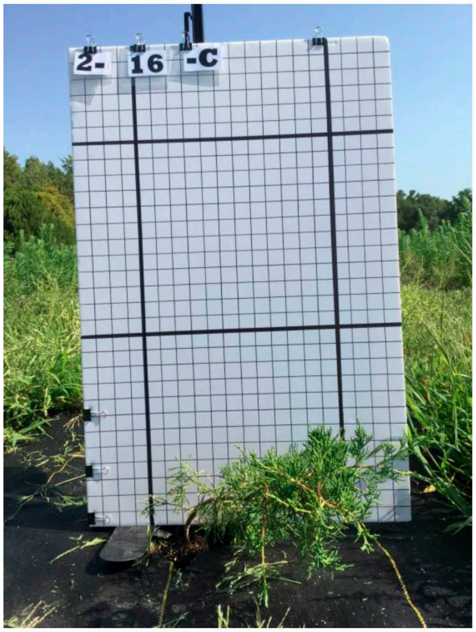

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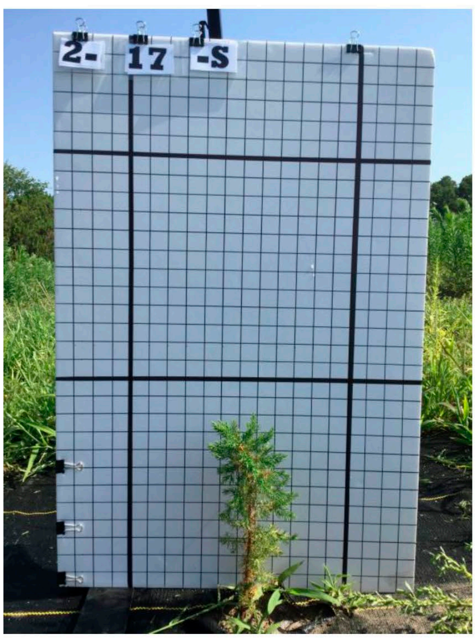

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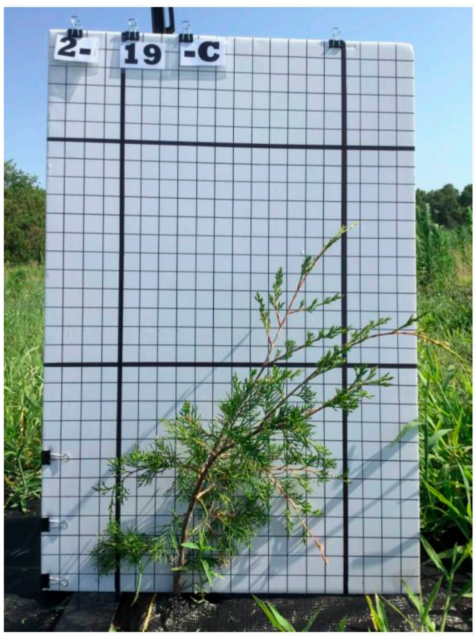

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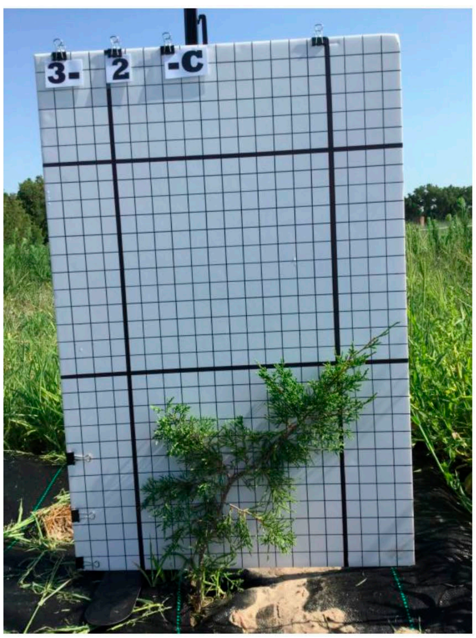

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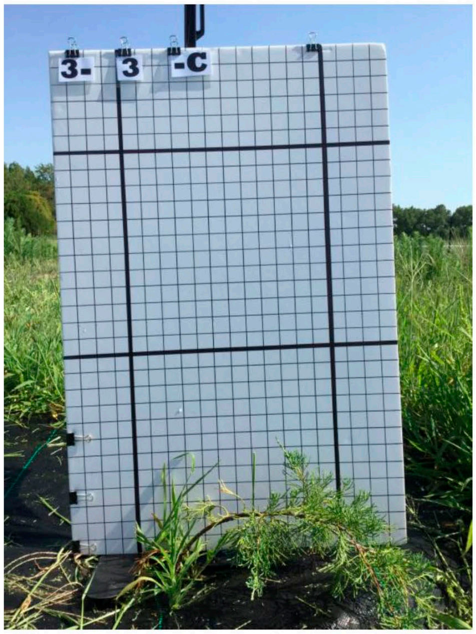

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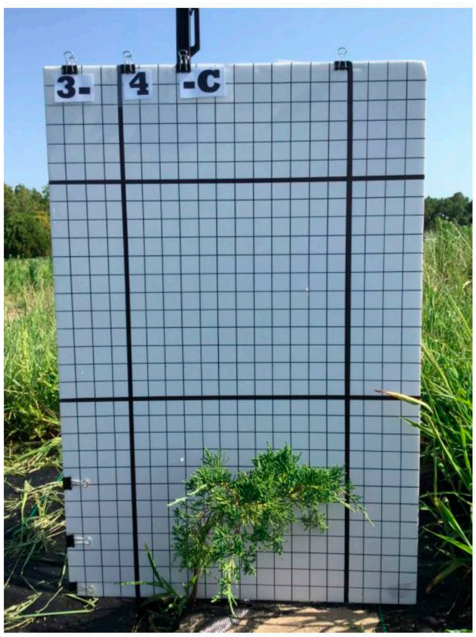

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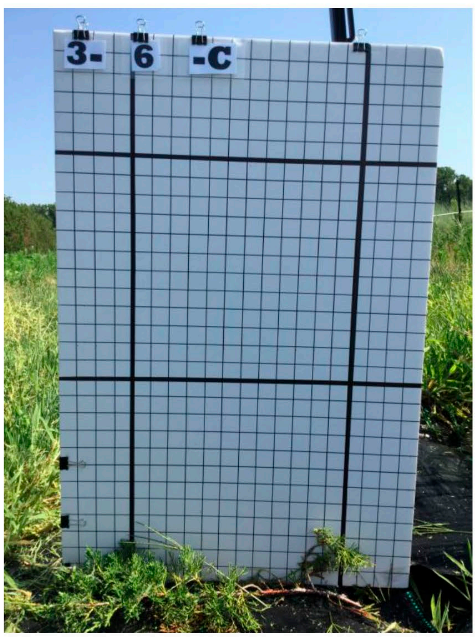

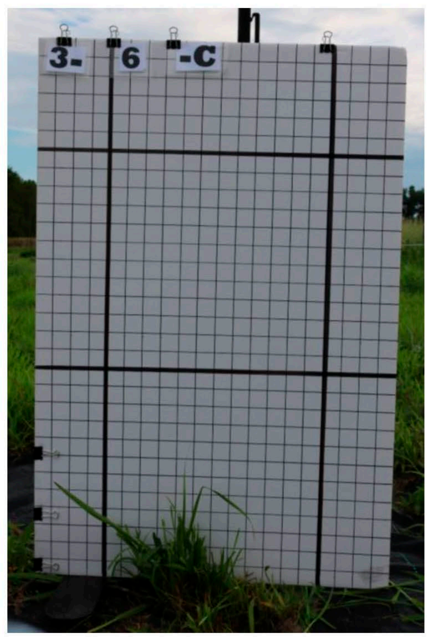

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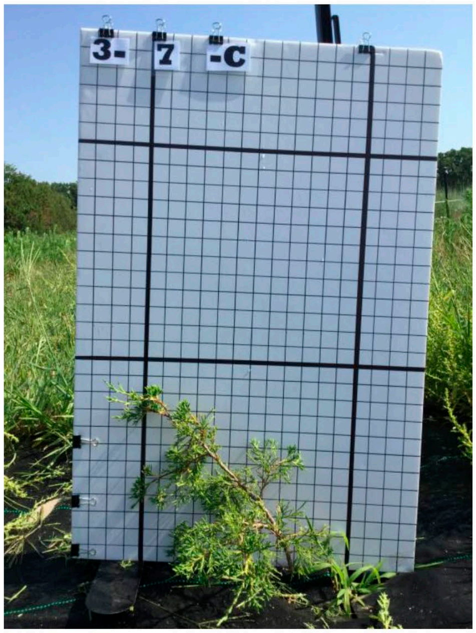

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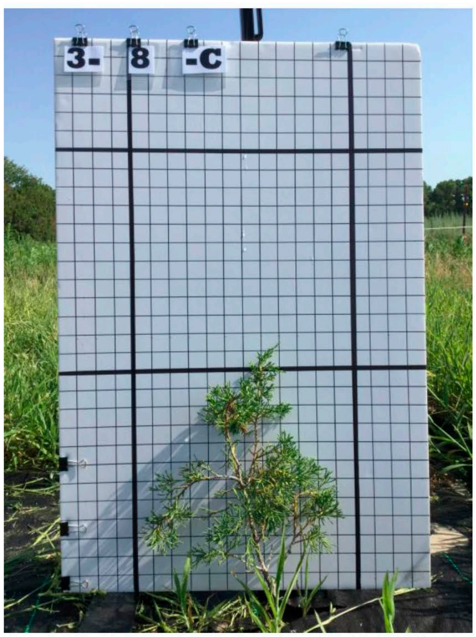

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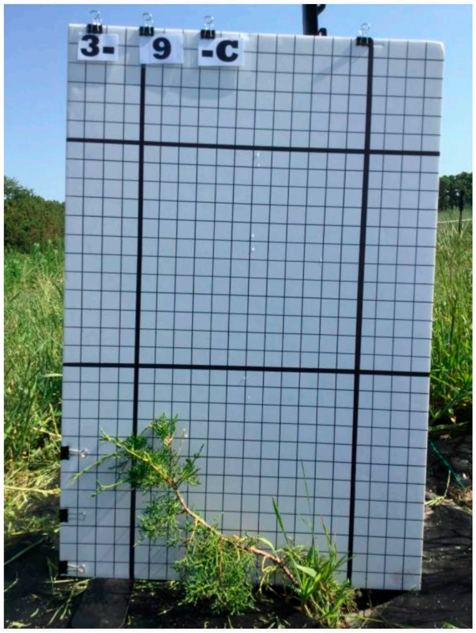

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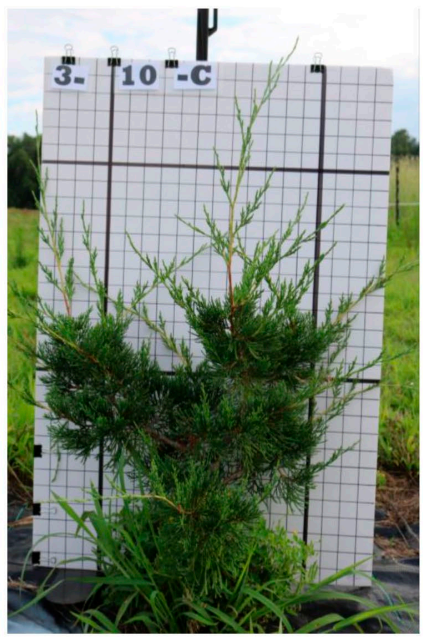

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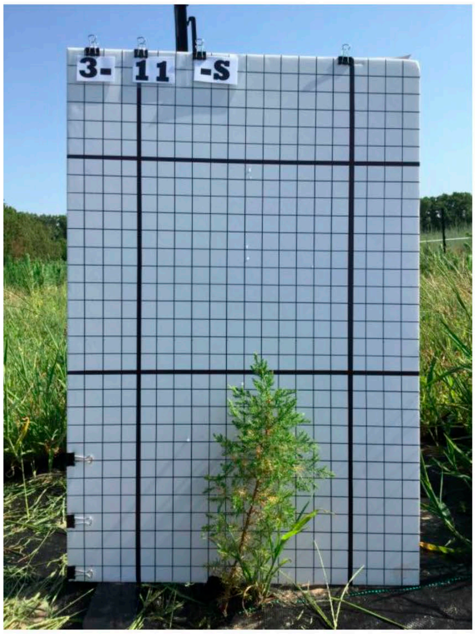

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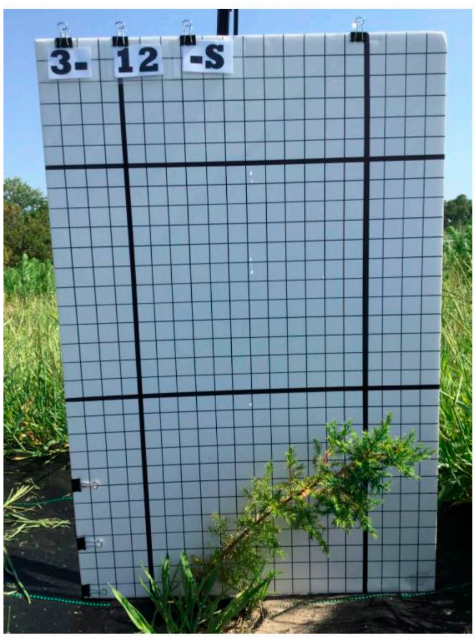

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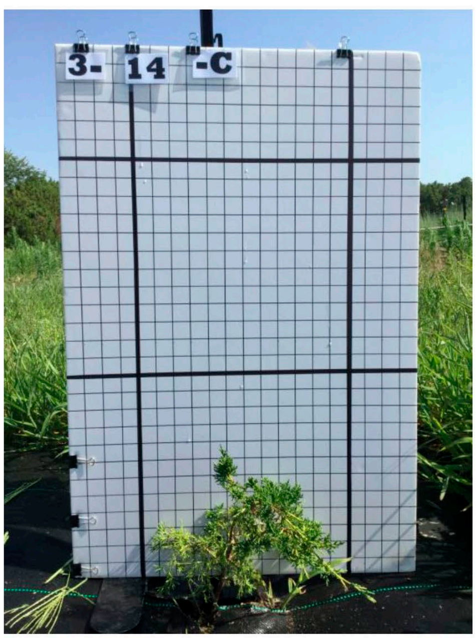

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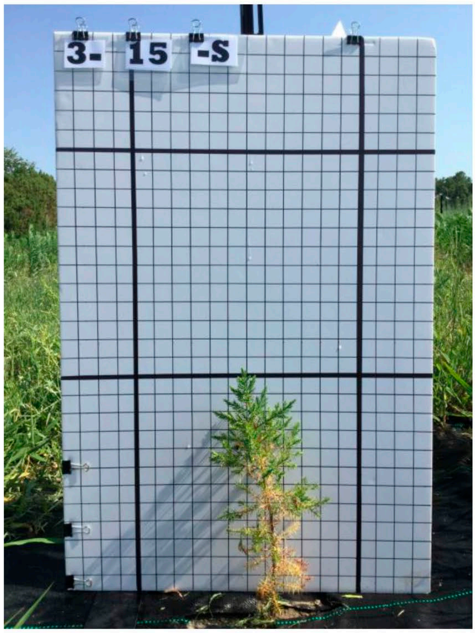

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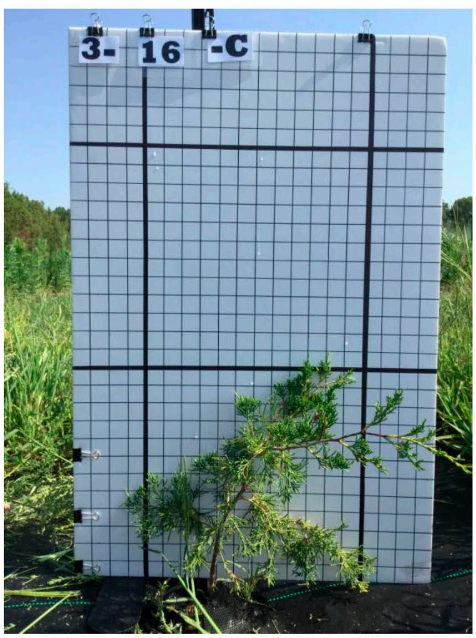

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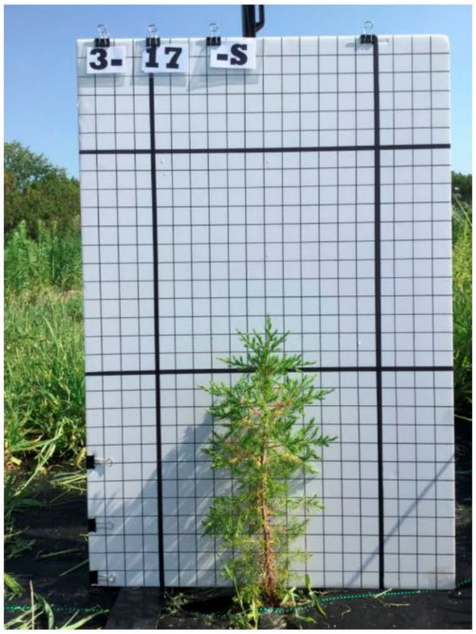

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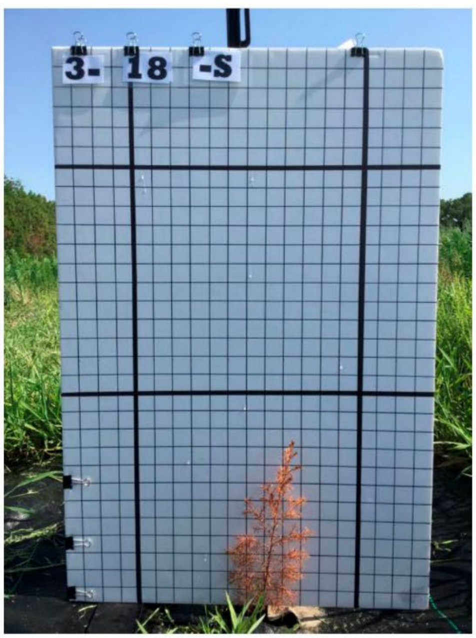

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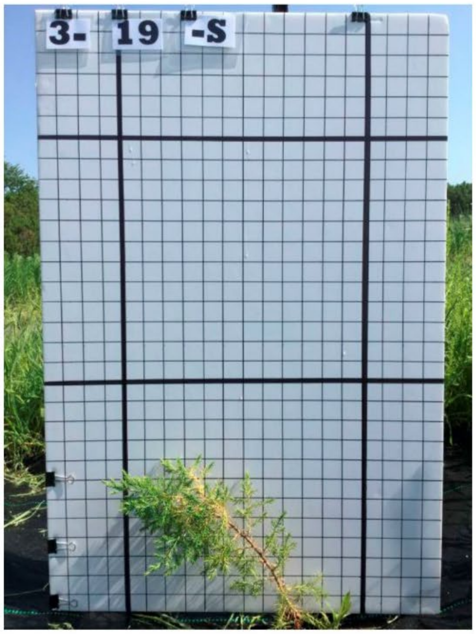

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