THESIS

STOCK PLANT MANAGEMENT OF LAVANDULA ANGUSTIFOLIA 'WEE ONE' USING PLANT

GROWTH REGULATORS AND PROPAGATION TECHNIQUES USED TO CREATE OPTIMAL

PROTOCOLS FOR SEVERAL PLANT SELECT® SPECIES

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ABSTRACT

STOCK PLANT MANAGEMENT OF LAVANDULA ANGUSTIFOLIA 'WEE ONE' USING PLANT

GROWTH REGULATORS and PROPAGATION TECHNIQUES USED TO CREATE OPTIMAL

PROTOCOLS FOR SEVERAL PLANT SELECT® SPECIES

Lavandula angustifolia 'Wee One' is a drought tolerant dwarf herbaceous perennial

being promoted by Plant Select®. The increased demand for this herbaceous perennial has

resulted in problems with stock plant management and propagation due to the relatively small

vegetative growth. The objective of this study was to determine the effects of plant growth

regulators applied as foliar sprays on the vegetative growth of *Lavandula angustifolia* 'Wee

One' propagation stock plants. Five chemical plant growth regulators were applied at the

optimal recommended rates : 1)Ethephon (2-Chloroethyl) phosphonic acid [500 mg·L^{-1} (ppm)]

(Verve, Nufarm Americas, Inc., Alsip,IL). 2) Kinetin, Gibberellic Acid, Indole-3-butyric Acid [500

mg·L^{-1} (ppm)] (Gravity, Winfield Solutions, LLC, St.Paul, MN), 3) N-(phenylmethyl)-IH-purine 6-

amine, Gibberellins A4A7 [100 mg·L^{-1} (ppm)] (Fascination, Valent USA Corp., Fresno, CS), 4)N-

(phenylmethyl)-1H-purine-6-amine [400 mg·L^{-1} (ppm)] (Configure, Fine Agrochemicals Limited,

Worcester, U.K.), 5) Gibberellin A3 [100 mg·L^{-1} (ppm)] (ProGibb T&O, Valent USA Corp., Fresno,

CS). Fifteen replications of *Lavandula angustifolia* 'Wee One' were evaluated once for four

months for plant height, width, number of cuttings, and fresh & dry weight of the cuttings. This

study was replicated twice, the first experiment was performed from March 2020 to July 2020

and the second experiment was performed from August 2020 to December 2020. *Lavandula*

angustifolia 'Wee One' stock plants that were treated with ProGibb T&O at 100 mg·L^{-1} (ppm) resulted in larger stock plants with more cuttings produced.

A secondary rooting study was conducted at the same time of each experiment. Cuttings were taken at the same time of day and stuck in trays of 26-strip Jiffy® Preforma media and placed under mist with bottom heat at a temperature of 23.9°C. Number of visible roots and rooting percentages were then recorded every week for four weeks. Rooting of *Lavandula angustifolia* 'Wee One' resulted in no observed differences between plant growth regulator treatments and the control.

In conclusion, the use of plant growth regulators resulted in increases of propagation material for Lavender stock plants. Foliar applications of ProGibb T&O at 100 mg·L^{-1} (ppm) caused an increase in growth of vegetative material and increased the number of cuttings produced from each stock plant with no decreases in the rooting percentage of those cuttings.

Epilobium canum subsp. *garrettii* 'PWWG01S', *Osteospermum* species, and *Pterocephalus depressus* are three herbaceous perennials being promoted by Plant Select®. The increased demand for these perennials has resulted in problems with current propagation protocols and production of rooted cuttings. The objective of the propagation techniques study was to determine the optimal combination of rooting hormone, root zone heating temperature, and hormone application methods that would result in higher rooting percentages of cuttings in four weeks. The first experiment focused on two concentrations of Dip N Grow rooting hormone applied to a cutting and placed on two different root zone heating temperatures. Three replications of this experiment occurred from July 2019 to September 2019. The second experiment focused on two rooting hormones (Dip N Grow and Hortus IBA)

applied at a single concentration with two different application methods of quick dip (30 seconds) or immersion (3 minutes). Two replications of this experiment occurred from February 2020 to March 2020.

After these two experiments, recommendations for propagation protocols can be written. *Pterocephalus depressus* prefer quick dip application of 30 seconds with either rooting hormone at 500 mg·L^{-1} (ppm) at a 23.9°C root zone heating temperature. *Osteospermum* species prefer an immersion application of 3 minutes with Dip N Grow at 500 mg·L^{-1} (ppm) at a 20°C root zone heating temperature. *Epilobium canum* subsp. *garrettii* 'PWWG01S' prefer a quick dip application of 30 seconds with either hormone at 500 mg·L^{-1} (ppm) with a root zone heating temperature of 20°C. All these recommended propagation techniques resulted in faster rooting and higher rooting percentage when compared to the untreated controls.

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CHAPTER 1: Importance to Industry

The horticulture industry has become an important part of the economy in the United

States. According to the United States Department of Agriculture 2019 Census, horticultural

operations recorded a revenue of $13.8 billion dollars in 2019. Potted herbaceous perennials

were responsible for $900 million dollars in 2019 (Perdue & Hamer, 2020).

The horticulture industry has seen a rapid increase in popularity due to the current

pandemic occurring in the United States. The increase was relatively due to the large numbers

of homeowners working from home and commercial businesses that could start landscaping

projects without disrupting employees. The attractiveness to herbaceous ornamental

perennials is the season after season dependability compared to annuals that must be replaced

each year.

Ornamental herbaceous perennials are in high demand as landscape plants in the

western United States. Hardy herbaceous perennials are wanted by homeowners and

commercial businesses due their relatively low maintenance which has showed continuous

growth each season. Plant Select® is a collaborative program between Colorado State

University, Denver Botanic Gardens and professional horticulturalists to provide plants

designed to thrive in the high plains and mountainous regions of the western states. Each year,

a committee of Plant Select® members, help create literature on plants that will be released

into their program yearly. These plants range from herbaceous perennials to woody shrubs that

can be utilized in every part of the landscape (*Plant Select*, 2021).

With the higher demands for ornamental perennials, growers of Plant Select® have seen

an increase in problems associated with propagation and stock plant management. Four plants

of interest from the Plant Select® program are *Lavandula angustifolia* 'Wee One',
Osteospermum species, *Epilobium canum subsp. garretti* 'PWWG01S', and *Pterocephalus depressus*. Research is lacking for these four species of specific propagation protocols for the
horticulture industry. While most of these species can be produced from seed, selected clones
have been chosen by Plant Select® due to desirable plant characteristics. To maintain these
desired characteristics, these perennials must be propagated vegetatively.

Due to the needs for specific propagation protocols for certain Plant Select®
introductions, two experiments were designed to help Plant Select® growers with propagation
of the four selected perennials. *Lavandula angustifolia* 'Wee One' was researched for stock
plant management using multiple plant growth regulators. *Osteospermum* species, *Epilobium canum subsp. garretti* 'PWWG01S', and *Pterocephalus depressus* was researched for finding the
optimal propagation techniques to root cuttings in 4 weeks or less.

UNIT 1: PLANT GROWTH REGULATOR STUDY

CHAPTER 2: Literature Review

2.1 Background on *Lavandula angustifolia* 'Wee One'

Lavandula angustifolia(Mill.) is a herbaceous perennial that is in the Lamiaceae family. The Lamiaceae family is native to the Mediterranean region and has many species that have medical uses. *Lavandula angustifolia*, English Lavender, is a widely cultivated perennial due to the attractive blooms and aromatic scents. English lavender is a narrow leafed variety that grows 31 – 92 cm in height and seems to be the most cold hardy variety of lavender species sold (Davis & McCoy, 2020).

Lavandula angustifolia 'Wee One' is a selected dwarf clone of lavender being promoted by Plant Select®. This cultivar of lavender has a compact growth habit maturing to about 25.4 cm with attractive lavender-blue flowers. It is being promoted for xeric landscaping and small spaces(*Plant Select*, 2021).

Propagation of lavender by seed is a slow process. The germination rate for seed propagation of lavender is very low and sporadic. Due to poor germination and the selection of a specific clones, lavender is primarily propagated by vegetative cuttings or layering. Cuttings are harvested from stock plants that are either grown in a greenhouse or in an open field (American Horticultural Society & Toogood, 1999).

Lavender cuttings should be taken from early to mid-summer for softwood cuttings. Softwood cuttings of 6-8 cm are taken then striped of the bottom foliage. Cuttings can take 4-8 weeks to root (Davis & McCoy, 2020). Fungicide applications are important to apply during rooting as lavender is highly subject to vascular wilts (Davis & McCoy, 2020).

2.2 Vegetative Propagation with Plant Growth Regulators

Softwood cuttings are the primary source for herbaceous perennial production. Softwood cuttings are taken from actively growing vegetative tissues. These tissues are formed early in the season before flowering or the formation of hardwood. Softwood cuttings are more difficult tissues to deal with in a production setting due to the high rate of desiccation but are the most rapid to root. Other types of cuttings can take longer to root, longer to harvest those cuttings and occurs later in the growing season (Nau, 1996).

2.3 Perennial Stock Plant Management

The time of season for taking cuttings is important when harvesting from plants outdoors, these conditions can be easily manipulated in a greenhouse setting with stock plants. Management of these stock plants indoors is important to keep plant health at an optimal range to have economically feasible production levels. Fertilization and pest management are two important variables to managing stock plant health and keeping new vegetative growth (Stanley, n.d.; Twardowski et al., 2012).

Pest management of lavender stock plants is important as lavender is suspectable to vascular wilts and stem rot (Davis & McCoy, 2020). Keeping soil moisture levels at the optimal range helps to prevent diseases from occurring. Continuous observation of stock plants also allows growers to know if additional pesticide treatments may be required. Maintaining fertilization to keep up with nutritional demands of stock plants is important for keeping plant material healthy. Fertilization is helpful for maintaining a continuous flush of new growth. This new flush of growth is considered softwood. Softwood cuttings are known to root faster and easier than older adult plant material (Nau, 1996). Not all plant material falls under this rule but

for lavender, the soft new vegetative growth is required when doing softwood cutting

propagation (American Horticultural Society & Toogood, 1999).

Several management tools used indoor or outdoor for keeping stock plants in this

softwood state is pruning and/or application of plant growth regulators. Pruning of lavender

would occur early in the season and after blooming to induce a flush of new growth (Davis &

McCoy, 2020). The problem with pruning treatments is that ideally only two flushes of

softwood vegetative growth would occur in a season before the vegetative material forms

semi-woody branches at the base of the lavender cuttings. An additional method that is gaining

popularity in the horticulture industry for stock plant management is the use of plant growth

regulators.

2.4 Plant Growth Regulators

Plant growth regulators are widely available for horticultural use. Multiple forms of

active ingredients can be used for a variety of effects on various plant species. The three most

common active ingredients are forms of gibberellins, cytokinin and ethylene (Davies, 2010).

These three plant growth regulators can be natural hormones that are extracted from plant

tissues or synthetically made compounds. Five different plant growth regulators were tested in

this stock plant management study. Each plant growth regulator has one of the three active

ingredients or a combination of them.

2.4.1 Gibberellins

Gibberellins (GA) is synthesized in young shoot tissues and developing seeds. Most GA is

biosynthesized in the chloroplasts of plant cells (Davies, 2010). Gibberellins are broken down

into multiple forms of GA based on chemical structure characteristics. Many of these

gibberellins are found in plants but only a selected few are readily available for use in the horticulture industry.

Gibberellic acid (GA_3) is the most widely available compound used. GA_3 is the pre-cursor of GA_1 that is used by plants. GA_1 causes elongation of stems by promoting cell division and elongation (Davies, 2010).

Bluebird Nursery in Nebraska has been using gibberellic acid for years to break dormancy or to obtain an early batch of cuttings on two herbaceous perennials. *Scabiosa* and *Heuchera* were noted for having little new growth and moving into dormancy in the greenhouse. GA applications were researched to see if dormancy could be reversed and if new growth could be encouraged. Gibberellic acid treatments were shown to encourage a flush of new growth after only one treatment. With two treatments of GA, these herbaceous perennials had a large increase in the total number of cuttings taken from a stock plant (Ackerman & Hamernik, 1994). Another research group reported that gibberellins negatively affect the formation of roots when applied to certain species. Their findings showed that GA caused roots to be shorter in length and thinner (Fonouni-Farde et al., 2019).

2.4.2 Cytokinin

Cytokinin occurs in root tips and developing seeds. Movement of cytokinin occurs in the xylem of a plant. Cytokinin causes high levels of cell division to occur when present in a cell (Davies, 2010). The effects can be even more pronounced when in the presence of auxin. Along with the induction of cell division, plant cells can be encouraged to grow lateral buds (Davies, 2010). Benzyladenine (BA) is a synthetic form of cytokinin that is readily available for use as a plant growth regulator (CFNP TAP *Report for 6-Bnzyladenine*, 2004).

Research completed at Green Leaf Enterprises, showed that applications of BA increased the number of lateral offshoots in many perennial species (Martin & Singletary, 1999). This company also noted with increased concentrations of BA there was no significant increase in lateral offshoots. This finding could be beneficial for growers since low concentrations can be used and have the same results as a higher concentration (Martin & Singletary, 1999). Auburn University reported similar results with *Hosta* species treated with BA. Offsets of *Hosta* increased when treated with BA and increased further when treated with more than one application of BA. Plants were not affected when these offsets were removed and retreated with BA (Garner et al., 1996)

2.4.3 Ethephon

Ethylene is produced in most plant tissues as a response to stress. Ethylene is commonly found in the gas form in plants and moves through cells by diffusion across membranes (Davies, 2010). Ethylene is often used in the horticulture industry since it can result in many wanted effects such as fruit ripening, flower/leaf senescence and shoot/root growth and differentiation (Davies, 2010). Ethephon is the liquid form of ethylene that is sold as a plant growth regulator. Ethephon is readily absorbed by plants. Once ethephon is absorbed, ethylene is released and used by plant cells (Lopez & Walters, 2017).

Ethephon use is the greenhouse varies depending on desired effect. Ethephon has been applied on a few herbaceous perennials at Michigan State University to research the potential use for stock plant management. On *Coreopsis verticillata* and *Veronica longifolia*, ethephon treatments increased lateral branching and number of vegetative cuttings. One perennial, *Dianthus caryophyllus* which had ethephon treatments, reported increased lateral branching

but marginal leaf necrosis occurred (Glady et al., 2007). Further studies from Michigan State showed that ethephon would be a valuable plant growth regulator for several herbaceous perennials for height control in the greenhouse (Hayashi et al., n.d.).

2.5 Study Objectives

The objectives of the stock plant management study for *Lavandula angustifolia* 'Wee One' was to determine if plant growth regulator treatment(s) result in more vegetative propagation material of high propagation quality. The rooting study objective was to determine if the plant growth regulator treatment(s) had any effects on the rooting percentage of these cuttings. The final objective was to develop a stock plant management protocol for growers to improve their propagation.

CHAPTER 3: Methods and Materials

3.1 Perennial Stock Plant Management with Plant Growth Regulators

This study was conducted at Colorado State University Horticulture Center which is located at 1707 Centre Avenue, Fort Collins, CO. The first experiment was conducted starting in February 2020 and continued until July 2020. The second experiment was conducted starting in July 2020 and continued until December 2020.

This research was conducted to examine the stock plant management of *Lavandula angustifolia* 'Wee One' from the Plant Select® program. Plants of uniform size (72 plug tray) were purchased from a local greenhouse (Gulley Greenhouse, Fort Collins, CO) for the first experiment. Plants of uniform size were rooted and grown by CSU for the second experiment. A total of 90 plants per variety were selected, so that five replicates of three plants (15 total)

were placed in a randomized complete design and placed throughout the greenhouse bench for each of the five treatments and control group.

Figure 3.1.1 Stock plant bench before automatic irrigation installation located at the CSU Horticulture Center at 1707 Centre Avenue, Fort Collins, CO.

The plants were transplanted from the 72 count plug into black #1 (2.84L) containers. All containers were disinfected with an anti-fungal, anti-bacterial and anti-algae solution for ten minutes prior to use to prevent contamination from previous experiments. The media used in this study for the stock plants was Pindstrup Orange, which is a peat moss substrate composed of blonde peat moss and a starting charge of fertilizer.

Groups of fifteen plants were randomly selected for a specific plant growth regulator treatment. Five chemical plant growth regulators were applied at the optimal recommended rates 1)Ethephon (2-Chloroethyl) phosphonic acid [500 mg·L^{-1} (ppm)] (Verve, Nufarm Americas,

Inc., Alsip,IL), 2) Kinetin, Gibberellic Acid, Indole-3-butyric Acid [500 mg·L^{-1} (ppm)] (Gravity,

Winfield Solutions, LLC, St.Paul, MN), 3) N-(phenylmethyl)-IH-purine 6-amine, Gibberellins

A4A7 [100 mg·L^{-1} (ppm)] (Fascination, Valent USA Corp., Fresno, CS), 4)N-(phenylmethyl)-1H-

purine-6-amine [400 mg·L^{-1} (ppm)] (Configure, Fine Agrochemicals Limited, Worcester, U.K.), 5)

Gibberellin A3 [100 mg·L^{-1} (ppm)] (ProGibb T&O, Valent USA Corp., Fresno, CS). A control group

of fifteen plants was left untreated in both experiments. The treatments were applied using a

Patriot 350 (13.25 liter) CO_2 sprayer starting two weeks before the first data collection date and

then monthly throughout the duration of both replications.

Figure 3.1.2 Patriot 350 (13.25 liter) CO_2 sprayer.

The first experiment treatments were applied on February 28, 2020, March 27, 2020,

April 24, 2020, and May 22, 2020. The second experiment treatments were applied on July 31,

2020, August 28, 2020, September 25, 2020, and October 23, 2020. The harvest of cuttings

were taken monthly, approximately two weeks after the PGR treatment applications on a

monthly basis. These treatments were based on the recommendations on the product label and on interviews from Colorado greenhouse growers.

The lavender stock plants were placed on a single rolling greenhouse bench with dimensions of 1.54 m by 12.19 m. The five groups of three plants for each treatment were randomly assigned a location on the greenhouse bench selected by a random number generation in Microsoft Excel, resulting in a complete randomized design. Groups of three containers were space 30 cm apart on the bench. The plants were individually numbered using a number of 1 to 90. Data was collected separately for each plant.

The greenhouse used for the stock plant portion of the study was run by a Wadsworth control system. The greenhouse, number 118, was heated by natural gas and forced air heater. The greenhouse was cooled passively by automatic ridge vents and automatic pulled shade cloths and actively by a pad and fan system. The Wadsworth system had preset daytime temperatures that were maintained between 22.7-23.9°C. The temperatures at night were maintained between 16.8-18.9°C. No supplemental lightning was used during experiments.

During initial establishment period, plants were watered by hand when over 75% of those plants had visually dry soil with a 14-4-14 fertilizer at 200 parts per million (ppm) nitrogen for every watering. Fertilizer was constantly injected using a Dosatron® model D14MZ2. Once a majority of all plants had roots striking the sides of the #1 containers, drip irrigation was installed to each pot. Each #1 container had one emitter placed above the media . The fertilizer regimen was switched to a 20-10-20 fertilizer at 200 ppm nitrogen continual feed. Using 4 liters per hour emitters, irrigation was done twice a week for 30 minutes, for a total of 4 liters of fertilized water per week per plant.

3.2 Cutting Protocol

3.2.1 Step by Step Protocol for Lavandula angustifolia 'Wee One'

1. Take all ideal vegetative cuttings from the stock plant.

    - Ideal cuttings should be at least 2 centimeters in length or longer

    - Cuttings should also be of a healthy stem caliber of 0.6 to 0.9 mm

2. Do not remove more than a 1/3 of growth. Do not remove any woody cuttings.

3. Clean stock plant of any dead stems and leaves.
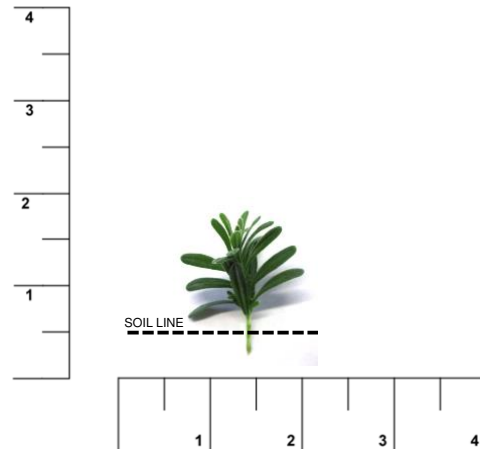
Figure 3.2.1 Photograph of *Lavandula angustifolia* 'Wee One' cutting protocol provided by Gulley Greenhouse, Fort Collins, CO.

3.3 Data Collection

Initial measurements of height and width were taken before the first application of PGR treatments for all 90 plants. Parameters measured monthly were plant height, width, number of cuttings taken, total fresh weight of cuttings, and total dry weight of cuttings. Plants were measured in centimeters at the highest point from the base of the plant and at two perpendicular widths. These three measurements of height and two widths were used to create

a growth index used for analysis. Photographs were taken at each sampling date to help document the differences between the treatment groups, before cuttings were removed from the individual plants.
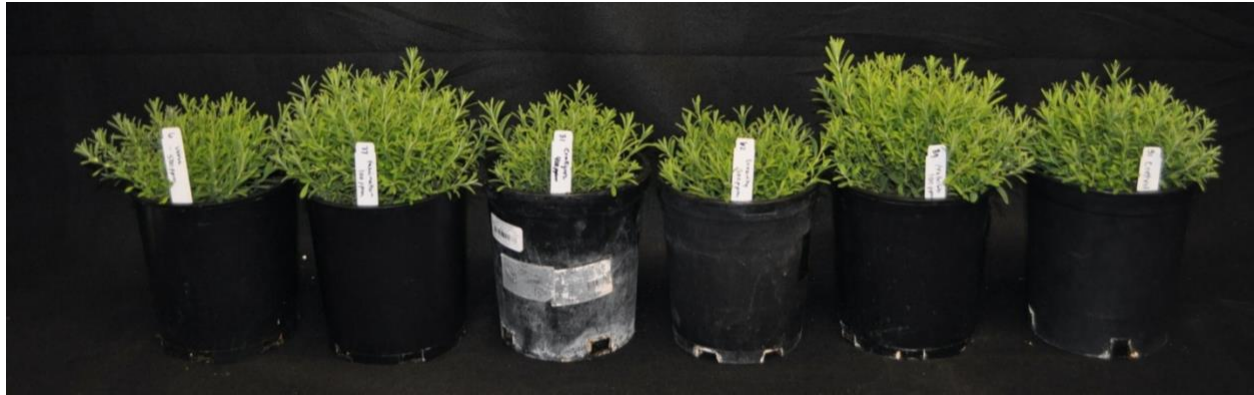
Figure 3.3.1 Photograph of *Lavandula angustifolia* 'Wee One' stock plants for Experiment #1 showing the difference between treatments of Verve, Fascination, Configure, Gravity, ProGibb, and Control.

Cuttings from each individual stock plant were counted, placed in a paper bag and weighed to determine the fresh weight. After fresh weights were taken, cuttings were then placed in a drying oven at 70° C for a minimum of 48 hours. After the cuttings were completely dried, the bags were weighed again to obtain the dry weights. After the final harvest of cuttings, stock plants were allowed to grow for four weeks before taking final top growth collection and root ratings. No pruning was completed on the stock plants between rounds of cuttings.

Cuttings were collected for the first experiment on March 14, 2020, April 11, 2020, May 8, 2020, and June 8, 2020. A selection of cuttings were also taken from the extra treated stock plants and stuck on the same dates as cutting collections. These extra cuttings were used during the rooting study. Final data of top growth and root ratings was collected on July 8, 2020 for the first replication.

Cuttings were collected for the second experiment on August 14, 2020, September 11, 2020, October 9, 2020, and November 16, 2020. A selection of cuttings were also taken from the extra stock plants and stuck on the same dates as cutting collections. These extra cuttings were used during the rooting study. Final data of top growth and root ratings was collected on December 4, 2020.

One month after the last cutting harvest for each experiment, all fifteen stock plants from each treatment had all the vegetative growth removed, dried, and weighed. This was done to simulate the average growth of the plant between harvest events. The root balls were removed from the pots and based on a determined rating scale of zero to five (zero being no roots and five being vigorous fibrous root system), given a visual rating. A visual reference was photographed and displayed as root ratings were taken for the individual plants for consistency.

Figure 3.3.2 Photograph of *Lavandula angustifolia* 'Wee One' rooting of the stock plant for Experiment #2 on the control.

3.4 Rooting Study

Five stock plants from each treatment were randomly selected at the beginning of each replication and grown under the same conditions for a rooting study. The only variables of the rooting experiment were the plant growth regulator treatments and the control. Cuttings were harvested from each treatment two weeks after application of the plant growth regulators on March 14, 2020, April 11, 2020, May 8, 2020, and June 8, 2020 for the first experiment. For the second experiment, cuttings were harvested on August 14, 2020, September 11, 2020, October 9, 2020, and November 16, 2020. Cuttings were taken at the same time of day, in the morning, and stuck in trays of 26-strip filled with Jiffy Performa plugs, and placed under mist with bottom heat at a temperature of 23.9°C. The media in a Jiffy Performa plug is a blend of coco coir and peat moss with a small amount of binder. Rooting data of number of roots and rooting percentage was then collected every week for four weeks.

The rooting study had five stock plants from each treatment saved and the treatments were continued with the five plant growth regulator treatments being applied monthly and an untreated control and with cuttings taken two weeks after the applications. Control stock plants were left untreated for both experiments. Cuttings were taken from all five stock plants in each treatments. Ten randomly selected cuttings were chosen and then dipped for 30 seconds in 500 parts per million IBA solution (Dip-N-Grow) and stuck in 26-strip plug trays. The plug trays were placed on heating mats that maintained a soil temperature of 23.9°C. The mist times on the bench were adjusted weekly; week one was 10 seconds every 15 minutes, week two was 10 seconds every 30 minutes, week three was 20 seconds every 60 minutes, and week

four was 20 seconds every 60 minutes. This schedule was active for the entire 24 hour period each day and controlled by a Nova 1626 ET six zone misting control system.

3.5 Data Analysis

Data analysis was done using R version 4.0.4. Response variables include: average number of cuttings per plant per treatment, dry and fresh weight of cuttings, final dry weight, root ratings of stock plants and growth index. Terms included in the model were predictor variables matching to the plant growth regulator treatments and the control (6 levels). Pairwise comparisons and least squares means were calculated using eemeans package for each response variable. Significant differences were noted using α =0.05 and 95% confidence intervals.

Response variables for the rooting study include: average rooting percentage per plant per treatment and average number of roots per plant per treatment. Average number of roots were analyzed using a one-way Anova model. Pairwise comparisons and least square means were calculated using the emmeans package for each response variable. Significant differences were noted using α =0.05 and 95% confidence intervals. Rooting percentages were calculated in Microsoft Excel.

CHAPTER 4: Results and Discussion

4.1 Growth Index

A single parameter for size was calculated to represent overall plant growth by averaging the measured height and two widths of each plant. Statistical analysis of growth index was completed for each time point beginning with initial measurements and occurring

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before each data collection period. Subsequent analyses contain all treatments averaged over the five time points.

Analysis of variance of *Lavandula angustifolia* 'Wee One' Experiment #1 revealed no significant effect of treatment for the average growth index and all pairwise comparisons showed no significant difference at the significant level of 0.05 (Figure 4.1.1). The smallest plants were the control and the largest plants were treated with ProGibb 400 ppm (Table 4.1.1 and Figure 4.1.2).

```
## Response: GI
##            Sum Sq Df F value Pr(>F)
## Treatment 11.585  5   1.642 0.1647
## Residuals 76.196 54
```

Figure 4.1.1 *Lavandula angustifolia* 'Wee One' Experiment #1 one-way ANOVA table for size index with Treatment as the predictor.

Table 4.1.1 *Lavandula angustifolia* 'Wee One' Experiment #1 size index ((height + width 1 + width2)/3) and 95% confidence intervals for each PGR treatment.

```
##  Treatment    emmean    SE df lower.CL upper.CL .group
##  Control        8.07 0.376 54     7.31     8.82  a
##  Gravity        8.13 0.376 54     7.38     8.89  a
##  Configure      8.37 0.376 54     7.62     9.13  a
##  Verve          8.46 0.376 54     7.71     9.22  a
##  Fascination    9.03 0.376 54     8.27     9.78  a
##  ProGibb        9.25 0.376 54     8.49    10.00  a
```

Figure 4.1.2 *Lavandula angustifolia* 'Wee One' Experiment #1 bar graph of average size index per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance of *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a significant effect of treatment for the average size index and some of the pairwise comparisons showed significant difference at the significant level of 0.05 (Figure 4.1.3 and Table 4.1.2). The smallest plants were treated with Verve at 100 ppm and the largest plants were treated with ProGibb T&O 400 ppm (Table 4.1.2 and Figure 4.1.4). Experiment #2 showed significance in the growth index between treatments. While experiment #2 had significant differences, the data followed the same trend as experiment #1. The application of plant growth regulators has an effect on the overall growth of a stock plant when compared to a control. In both experiments, stock plants treated with ProGibb T&O had the largest growth index. The active ingredient in ProGibb T&O is gibberellic acid. Gibberellic acid causes cell elongation and increases in lateral

branching (Davies, 2010). Similar results were seen in stock plants treated with gibberellic acid by the Bluebird Nursery that showed an increase in the number of cuttings taken (Ackerman & Hamernik, 1994). An increase in the number of cuttings taken correlates to an increase in the size and lateral branching of a stock plant.

```
## Response: GI
##            Sum Sq Df F value     Pr(>F)
## Plant      22.500  5   8.503 5.477e-06 ***
## Residuals  28.578 54
```

Figure 4.1.3 *Lavandula angustifolia* 'Wee One' Experiment #2 one-way ANOVA table for size index with Treatment as the predictor.

Table 4.1.2 *Lavandula angustifolia* 'Wee One' Experiment #2 size index ((height+width 1+ width2)/3) and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Plant        emmean   SE df lower.CL upper.CL .group
##  Verve          4.35 0.23 54     3.89     4.81  a
##  Control        4.99 0.23 54     4.53     5.45  ab
##  Gravity        5.08 0.23 54     4.62     5.54  ab
##  Configure      5.36 0.23 54     4.90     5.82   b
##  Fascination    5.68 0.23 54     5.22     6.14   bc
##  ProGibb        6.33 0.23 54     5.87     6.79    c
```
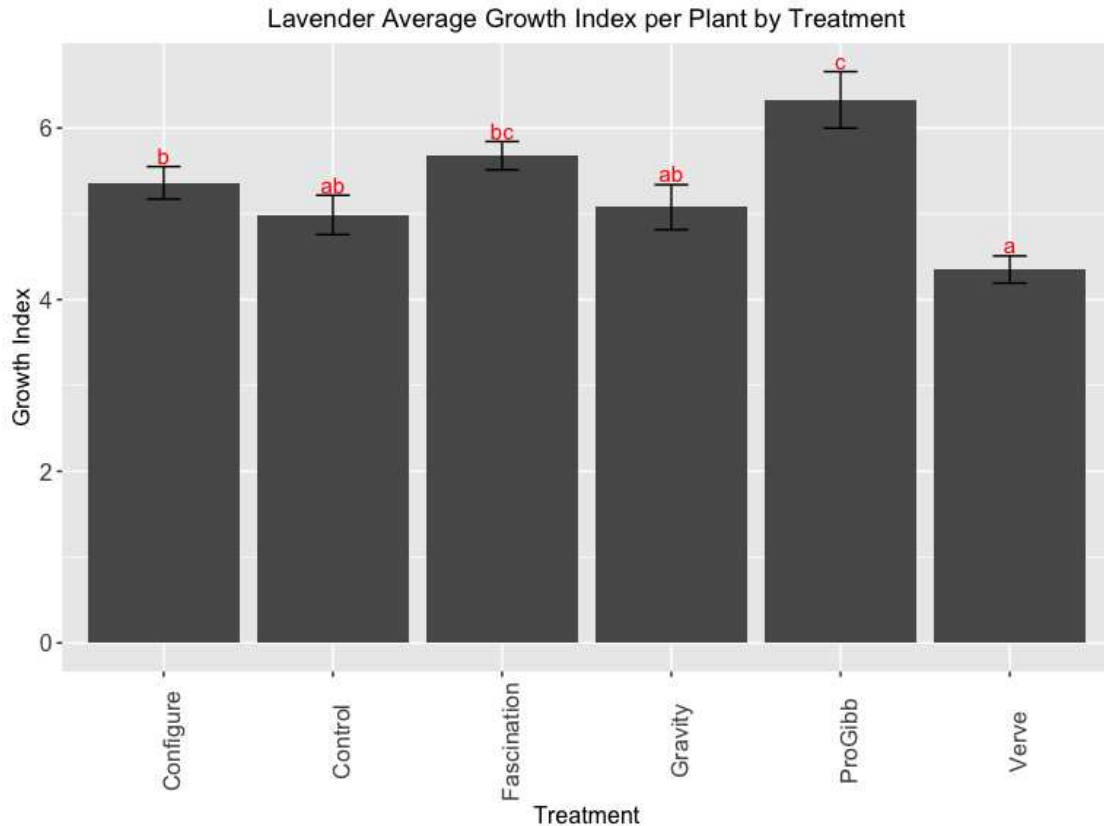
Figure 4.1.4 *Lavandula angustifolia* 'Wee One' Experiment #2 bar graph of average size index per treatment. Standard error bars indicate a 95% confidence interval for the mean.

4.2 Average Number of Cuttings Per Plant

The average number of harvested cuttings was totaled and averaged over the four harvest dates for analysis. Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed a significant effect of treatment for the average number of cuttings and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.2.1 and Table 4.2.1). The plants treated with Configure had the smallest number of cuttings and plants treated with Gravity had the highest number of cuttings (Table 4.2.1 and Figure 4.2.2).

21

```
## Response: Cut
##           Sum Sq Df F value  Pr(>F)
## Treatment 2390.8  5  2.8705 0.02273 *
## Residuals 8995.0 54
```

Figure 4.2.1 *Lavandula angustifolia* 'Wee One' experiment #1 one-way ANOVA table for average number of cuttings with Treatment as predictor.

Table 4.2.1 *Lavandula angustifolia* 'Wee One' Experiment #1 average number of cuttings and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
## Treatment   emmean   SE df lower.CL upper.CL .group
## Configure    44.1 4.08 54     36.0     52.3  a
## Fascination  45.3 4.08 54     37.1     53.5  ab
## ProGibb      48.6 4.08 54     40.4     56.8  ab
## Control      51.0 4.08 54     42.9     59.2  ab
## Verve        57.4 4.08 54     49.2     65.6  ab
## Gravity      61.7 4.08 54     53.5     69.9   b
```
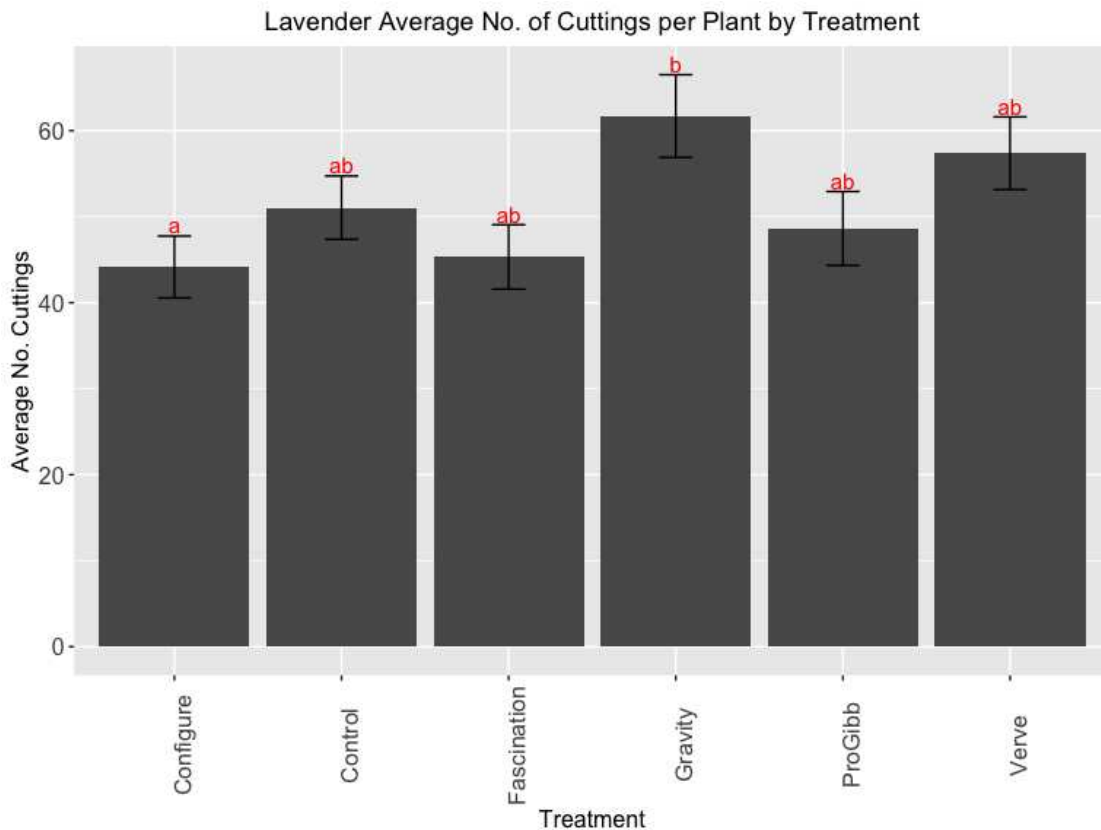

Figure 4.2.2 *Lavandula angustifolia* 'Wee One' Experiment #1 bar graph of average number of cuttings per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a

significant effect of treatment for the average number of cuttings and the pairwise comparisons

showed a significant difference at the significant level of 0.05 (Figure 4.2.3 and Table 4.2.2). The

plants treated with Verve had the smallest number of cuttings and plants treated with ProGibb

had the highest number of cuttings (Table 4.2.2 and Figure 4.2.4). In experiment #1 and #2, the

significant effects in treatment shows that PGR has an effect on the growth of vegetative

material after treatment. In both experiments, stock plants treated with a gibberellic acid plant

growth regulator had the highest number of cuttings. Stock plants treated with gibberellic acid

by the Bluebird Nursery showed an increase in the number of cuttings taken from a stock plant

(Ackerman & Hamernik, 1994). The results of increased number of cuttings strongly correlates

with each plant growth regulator ingredient of either GA, BA, or ethephon that causes an

increase in lateral branching and cell elongation (Davies, 2010).

```
## Response: Cut
##             Sum Sq Df F value    Pr(>F)
## Treatment 458.31   5  12.316 5.494e-08 ***
## Residuals 401.91 54
```
Figure 4.2.3 *Lavandula angustifolia* 'Wee One' experiment #2 one-way ANOVA table for average number of cuttings with Treatment as predictor.

Table 4.2.2 *Lavandula angustifolia* 'Wee One' Experiment #2 average number of cuttings and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
## Treatment     emmean    SE df lower.CL upper.CL .group
## Verve           9.45 0.863 54     7.72     11.2  a
## Gravity        11.47 0.863 54     9.75     13.2  ab
## Control        11.53 0.863 54     9.80     13.3  ab
## Configure      13.10 0.863 54    11.37     14.8  b
## Fascination    14.65 0.863 54    12.92     16.4   bc
## ProGibb        18.10 0.863 54    16.37     19.8    c
```
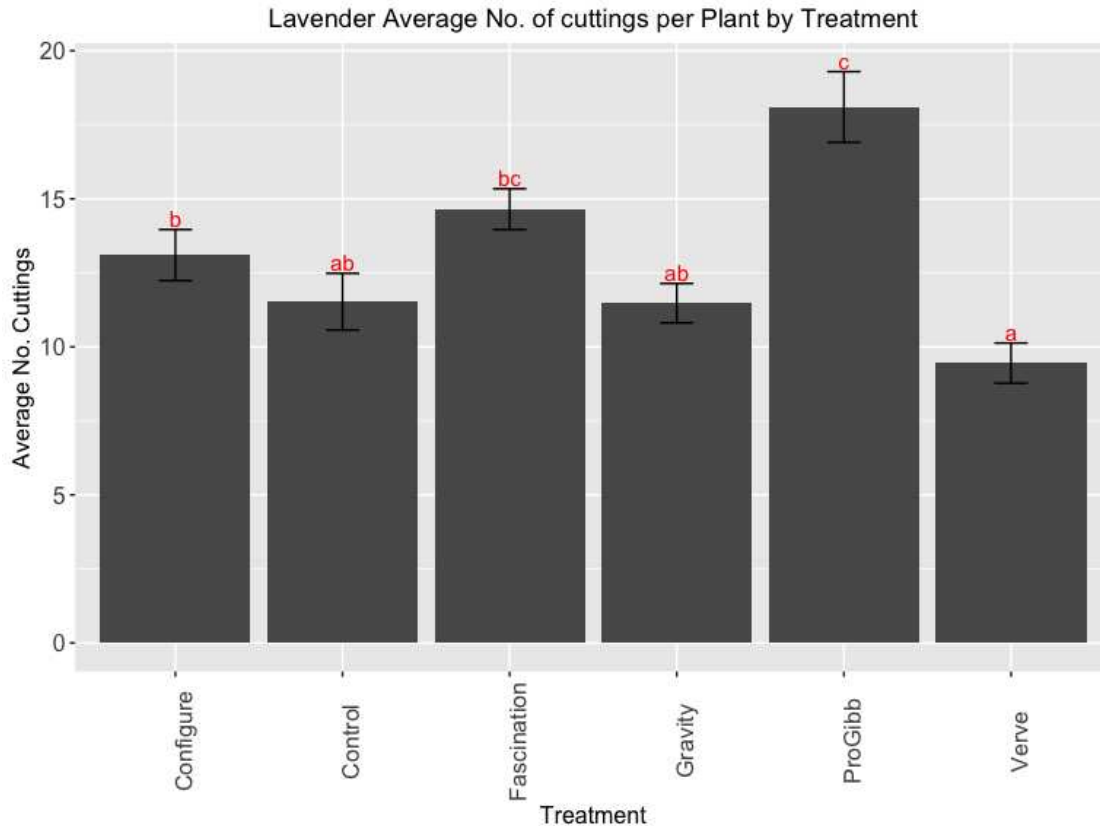
Figure 4.2.4 *Lavandula angustifolia* 'Wee One' Experiment #2 bar graph of average number of cuttings per treatment. Standard error bars indicate a 95% confidence interval for the mean.

4.3 Fresh Weight Per Cutting

Average fresh weight per cutting was calculated by dividing the total fresh weight of cuttings by the total number of cuttings harvested for each plant averaged over the four harvest dates. Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed a significant effect of treatment for the average fresh weight of cuttings and all pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.3.1 and Table 4.3.1). The plants treated with Verve had the smallest fresh weight of cuttings and plants treated with ProGibb T&O had the largest fresh weight of cuttings (Table 4.3.1 and Figure 4.3.2)

24

```
## Response: AvgFW
##              Sum Sq Df F value    Pr(>F)
## Treatment 0.0053965  5  9.2453 2.107e-06 ***
## Residuals 0.0063039 54
```

Figure 4.3.1 *Lavandula angustifolia* 'Wee One' experiment #1 one-way ANOVA table for average cutting fresh weight of top growth with Treatment as predictor.

Table 4.3.1 *Lavandula angustifolia* 'Wee One' Experiment #1 average cutting fresh weight and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment    emmean       SE df lower.CL upper.CL .group
##  Verve        0.0652 0.00342 54   0.0583   0.0720  a
##  Gravity      0.0660 0.00342 54   0.0592   0.0729  ab
##  Fascination  0.0749 0.00342 54   0.0680   0.0817  abc
##  Configure    0.0796 0.00342 54   0.0727   0.0864   bcd
##  Control      0.0804 0.00342 54   0.0736   0.0873    cd
##  ProGibb      0.0931 0.00342 54   0.0862   0.0999     d
```
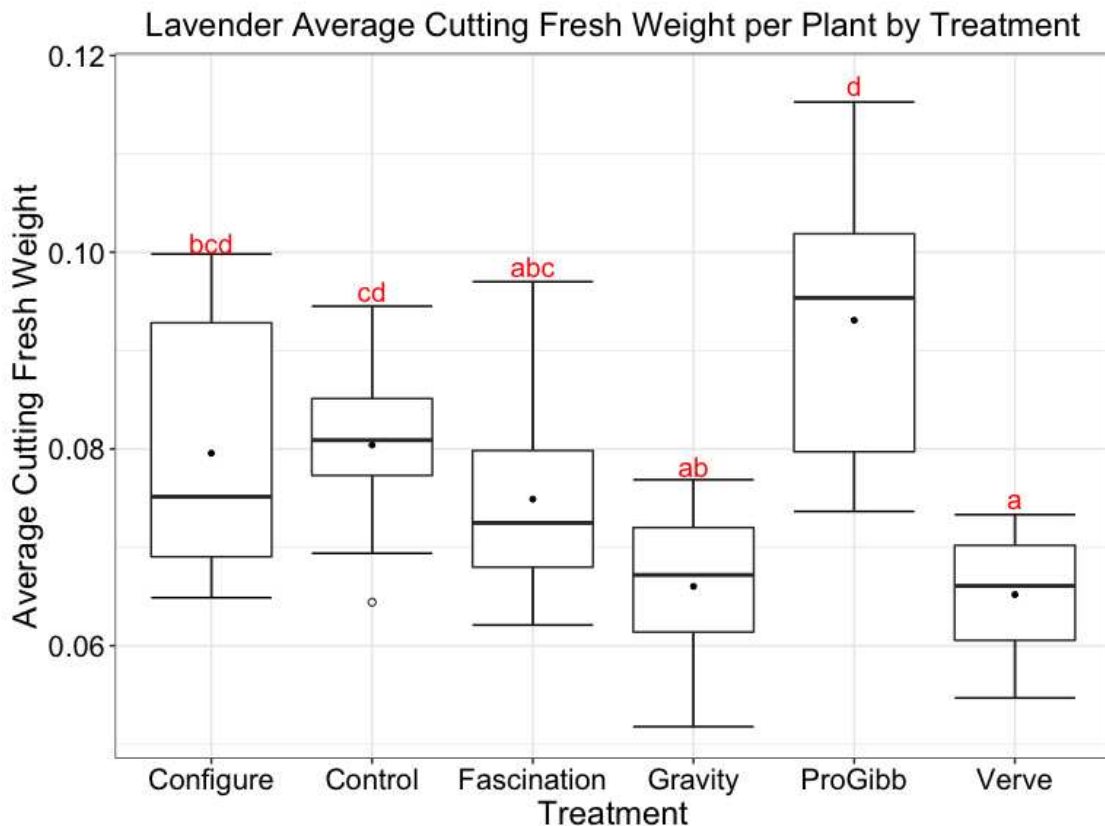

Figure 4.3.2 *Lavandula angustifolia* 'Wee One' Experiment #1 boxplot of average cutting fresh weight per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a significant effect of treatment for the average fresh weight of cuttings and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.3.3 and Table 4.3.2). The plants treated with Verve had the smallest fresh weight of cuttings and plants treated with Configure had the largest fresh weight of cuttings (Table 4.3.2 and Figure 4.3.4). The significant differences in experiment #1 and #2 shows an effect of PGR on the fresh weight of cuttings compared to the untreated control cuttings. Cuttings treated with plant growth regulators causes cell elongation and cell differentiation (Davies, 2010). This cell elongation increases the length of cuttings on the stock plant, therefore increasing the calculated fresh weight after cuttings are harvested.

```
## Response: AvgFW
##              Sum Sq Df F value  Pr(>F)
## Treatment 0.0012844  5  3.1964 0.01339 *
## Residuals 0.0043396 54
```

Figure 4.3.3 *Lavandula angustifolia* 'Wee One' experiment #2 one-way ANOVA table for average cutting fresh weight with Treatment as predictor.

Table 4.3.2 *Lavandula angustifolia* 'Wee One' Experiment #2 average cutting fresh weight and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment   emmean      SE df lower.CL upper.CL .group
##  Verve       0.0389 0.00283 54   0.0333   0.0446  a
##  Fascination 0.0455 0.00283 54   0.0398   0.0512  ab
##  Gravity     0.0500 0.00283 54   0.0443   0.0557  ab
##  ProGibb     0.0501 0.00283 54   0.0444   0.0558  ab
##  Control     0.0506 0.00283 54   0.0449   0.0563  ab
##  Configure   0.0530 0.00283 54   0.0473   0.0587   b
```

Figure 4.3.4 *Lavandula angustifolia* 'Wee One' Experiment #2 boxplot of average cutting fresh weight per treatment. Standard error bars indicate a 95% confidence interval for the mean.

4.4 Dry Weight Per Cutting

Average dry weight per cutting were calculated by dividing the total dry weight of cuttings by the total number of cuttings harvested for each plant during each harvest date and averaged over the four harvest dates.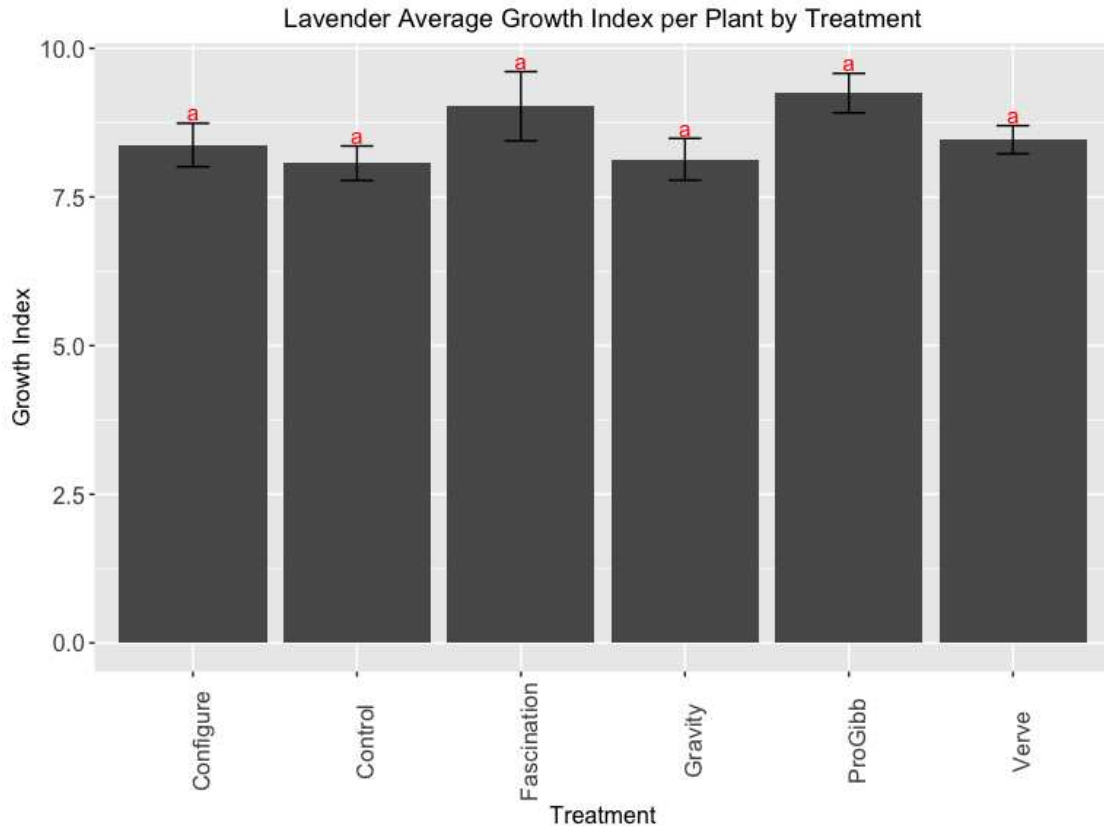 Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed a significant effect of treatment for the average dry weight of cuttings and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.4.1 and Table 4.4.1). The plants treated with Gravity had the smallest dry weight of cuttings and plants treated with ProGibb T&O had the largest dry weight of cuttings (Table 4.4.1 and Figure 4.4.2)

```
## Response: AvgDW
##               Sum Sq Df F value   Pr(>F)
## Treatment 3.9580e-05  5  4.3208 0.002226 **
## Residuals 9.8934e-05 54
```

Figure 4.4.1 *Lavandula angustifolia* 'Wee One' experiment #1 one-way ANOVA table for average cutting dry weight with Treatment as predictor.

Table 4.4.1 *Lavandula angustifolia* 'Wee One' Experiment #1 average cutting dry weight and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment    emmean       SE df lower.CL upper.CL .group
##  Gravity      0.0103 0.000428 54  0.00941   0.0111  a
##  Verve        0.0109 0.000428 54  0.01007   0.0118  a
##  Fascination  0.0110 0.000428 54  0.01016   0.0119  a
##  Control      0.0116 0.000428 54  0.01078   0.0125  ab
##  Configure    0.0118 0.000428 54  0.01094   0.0127  ab
##  ProGibb      0.0128 0.000428 54  0.01199   0.0137   b
```
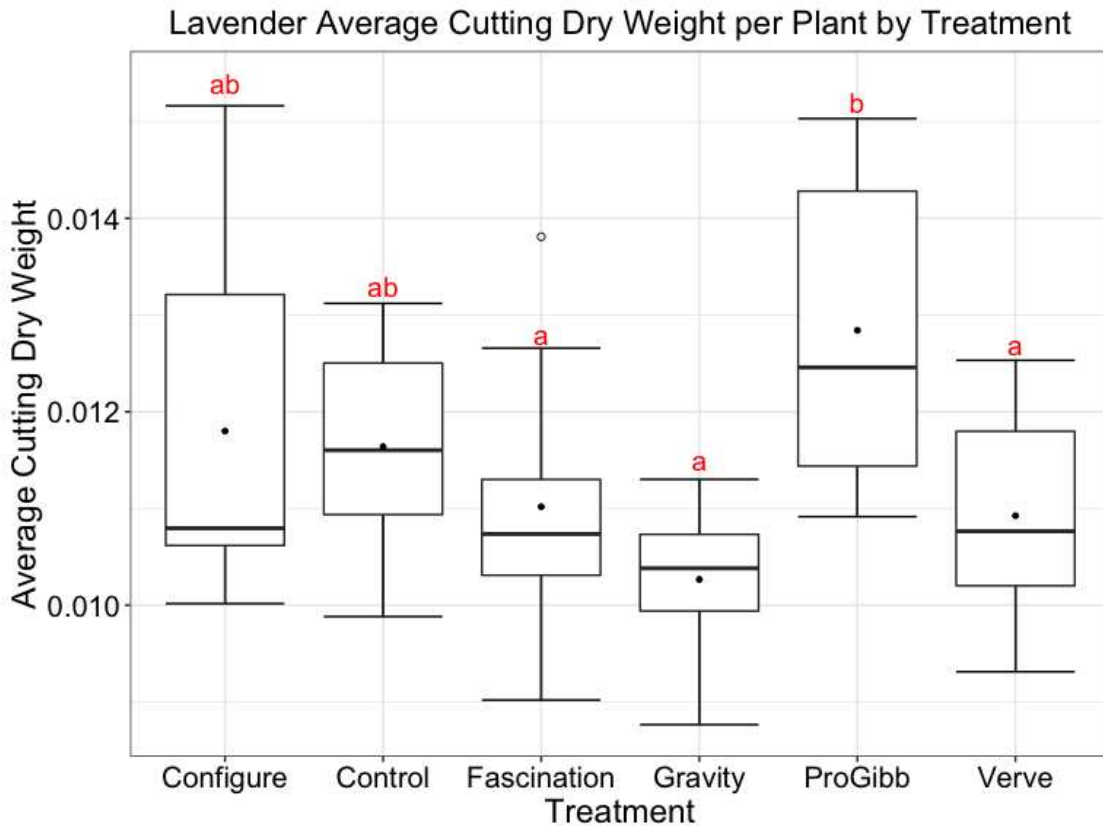

Figure 4.4.2 *Lavandula angustifolia* 'Wee One' Experiment #1 boxplot of average cutting dry weight per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed no

significant effect of treatment for the average dry weight of cuttings and the pairwise

comparisons showed little significant difference at the significant level of 0.05 (Figure 4.4.1 and

Table 4.4.1). The plants treated with Fascination had the smallest dry weight of cuttings and

plants treated with Configure had the largest dry weight of cuttings (Table 4.4.1 and Figure

4.4.2). Dry weights of cuttings correlates with the fresh weight of cuttings and follows the same

trends in both experiments. Dry weights of cuttings shows the amount of water lost from those

cuttings.

```
## Response: AvgDW
##               Sum Sq Df F value  Pr(>F)
## Treatment 4.2909e-05  5  2.3573 0.05239 .
## Residuals 1.9658e-04 54
```

Figure 4.4.3 *Lavandula angustifolia* 'Wee One' experiment #2 one-way ANOVA table for average cutting dry weight with Treatment as predictor.

Table 4.4.2 *Lavandula angustifolia* 'Wee One' Experiment #2 average cutting dry weight and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
## Treatment     emmean       SE df lower.CL upper.CL .group
## Fascination 0.00859 0.000603 54  0.00738   0.0098  a
## Verve       0.00939 0.000603 54  0.00818   0.0106  ab
## ProGibb     0.00994 0.000603 54  0.00873   0.0112  ab
## Gravity     0.01041 0.000603 54  0.00920   0.0116  ab
## Control     0.01054 0.000603 54  0.00933   0.0117  ab
## Configure   0.01122 0.000603 54  0.01001   0.0124   b
```
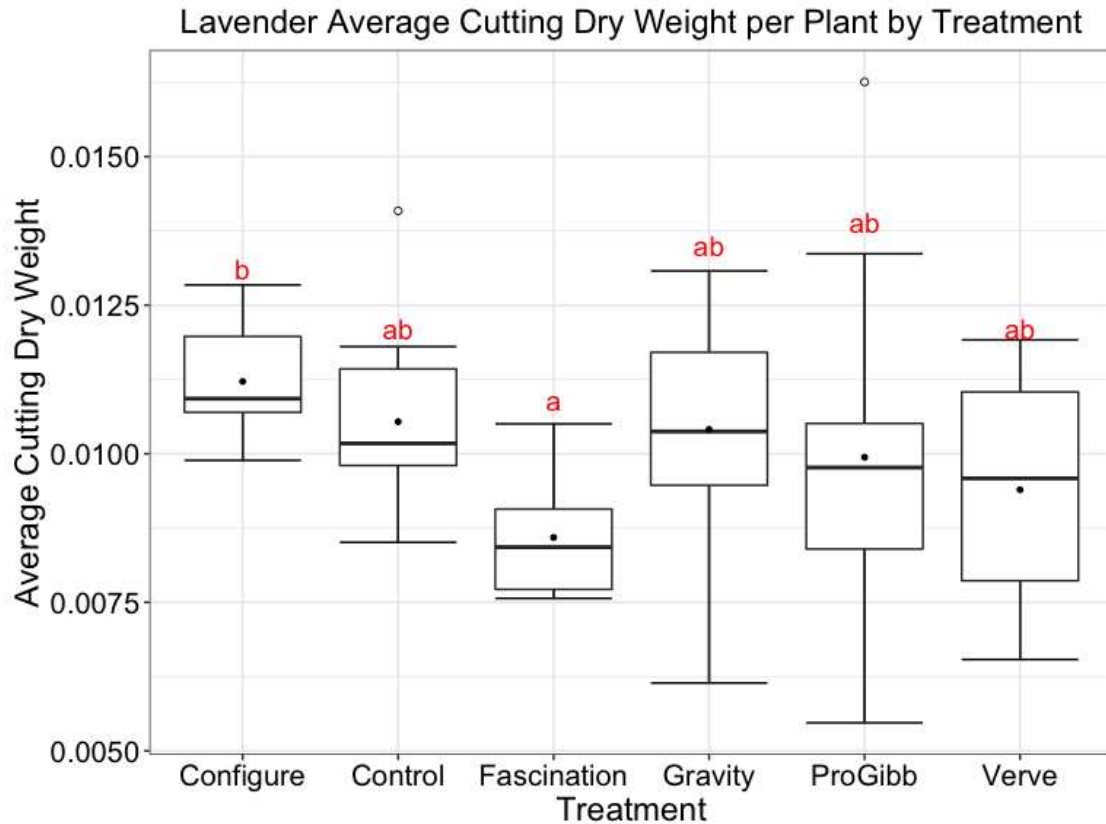
Figure 4.4.4 *Lavandula angustifolia* 'Wee One' Experiment #2 boxplot of average cutting dry weight per treatment. Standard error bars indicate a 95% confidence interval for the mean.

4.5 Final Dry Weight

Final dry weight of stock plants was determined by cutting off all top growth at the crown of the plant. The top growth was placed in a paper bag and weighed. The top growth was then dried at 70°C for at least 4 days in paper bags before weighting for a dry weight of the top growth. This was performed one month after the fourth and final harvest of cuttings. The month duration was meant to simulate the amount of growth the plants were putting on in-between cutting events.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed a significant effect of treatment for the average final dry weight of top growth and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.5.1 and

Table 4.5.1). The plants untreated had the smallest dry weight of top growth and plants treated

with ProGibb T&O had the largest dry weight of top growth (Table 4.5.1 and Figure 4.5.2)

```
## Response: DW
##             Sum Sq Df F value     Pr(>F)
## Treatment 115.12   5  8.3089 7.067e-06 ***
## Residuals 149.63 54
```

Figure 4.5.1 *Lavandula angustifolia* 'Wee One' experiment #1 one-way ANOVA table for final dry weight of top growth with Treatment as predictor.

Table 4.5.1 *Lavandula angustifolia* 'Wee One' Experiment #1 average final dry weight of top growth and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment    emmean      SE df lower.CL upper.CL .group
##  Control        4.29 0.526 54     3.24     5.35  a
##  Gravity        4.86 0.526 54     3.81     5.92  ab
##  Configure      4.97 0.526 54     3.92     6.03  ab
##  Verve          6.38 0.526 54     5.32     7.43  abc
##  Fascination    7.05 0.526 54     6.00     8.11   bc
##  ProGibb        8.24 0.526 54     7.18     9.29    c
```
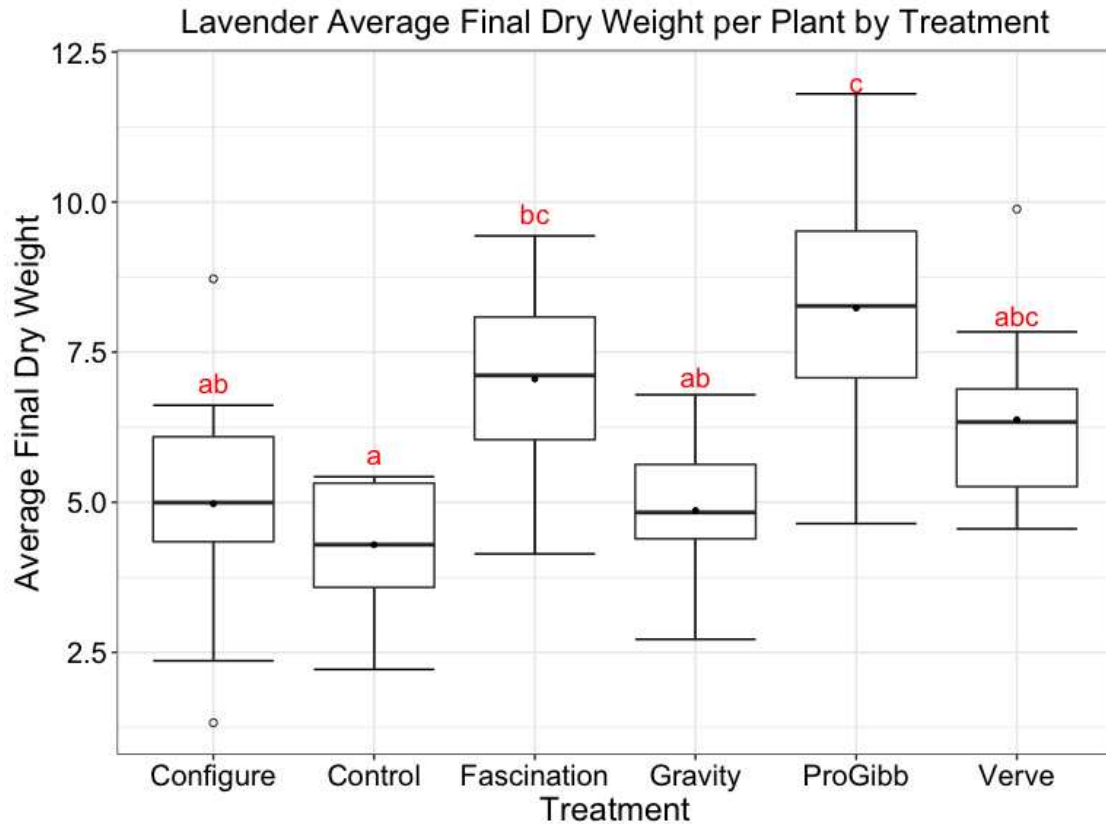
Figure 4.5.2 *Lavandula angustifolia* 'Wee One' Experiment #1 boxplot of average final dry weight of top growth per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a significant effect of treatment for the average final dry weight of top growth and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.5.3 and Table 4.5.2). The plants untreated had the smallest dry weight of top growth and plants treated with ProGibb T&O had the largest dry weight of top growth (Table 4.5.2 and Figure 4.5.4). These trends show that application of plant growth regulators has an effect on the overall growth of the stock plants. Gibberellic acid, benzyladenine, and ethephon have an effect on the lateral branching and cell elongation when applied to plants (Davies, 2010).

```
## Response: DW
##            Sum Sq Df F value  Pr(>F)
## Treatment 1.5909  5  4.8111 0.00104 **
## Residuals 3.5714 54
```

Figure 4.5.3 *Lavandula angustifolia* 'Wee One' Experiment #2 one-way ANOVA table for final dry weight of top growth with Treatment as predictor.

Table 4.5.2 *Lavandula angustifolia* 'Wee One' Experiment #2 average final dry weight of top growth and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment    emmean     SE df lower.CL upper.CL .group
##  Control       0.487 0.0813 54    0.324    0.650 a
##  Gravity       0.540 0.0813 54    0.377    0.703 ab
##  Verve         0.589 0.0813 54    0.426    0.752 abc
##  Configure     0.756 0.0813 54    0.593    0.919 abc
##  Fascination   0.859 0.0813 54    0.696    1.022  bc
##  ProGibb       0.919 0.0813 54    0.756    1.082   c
```
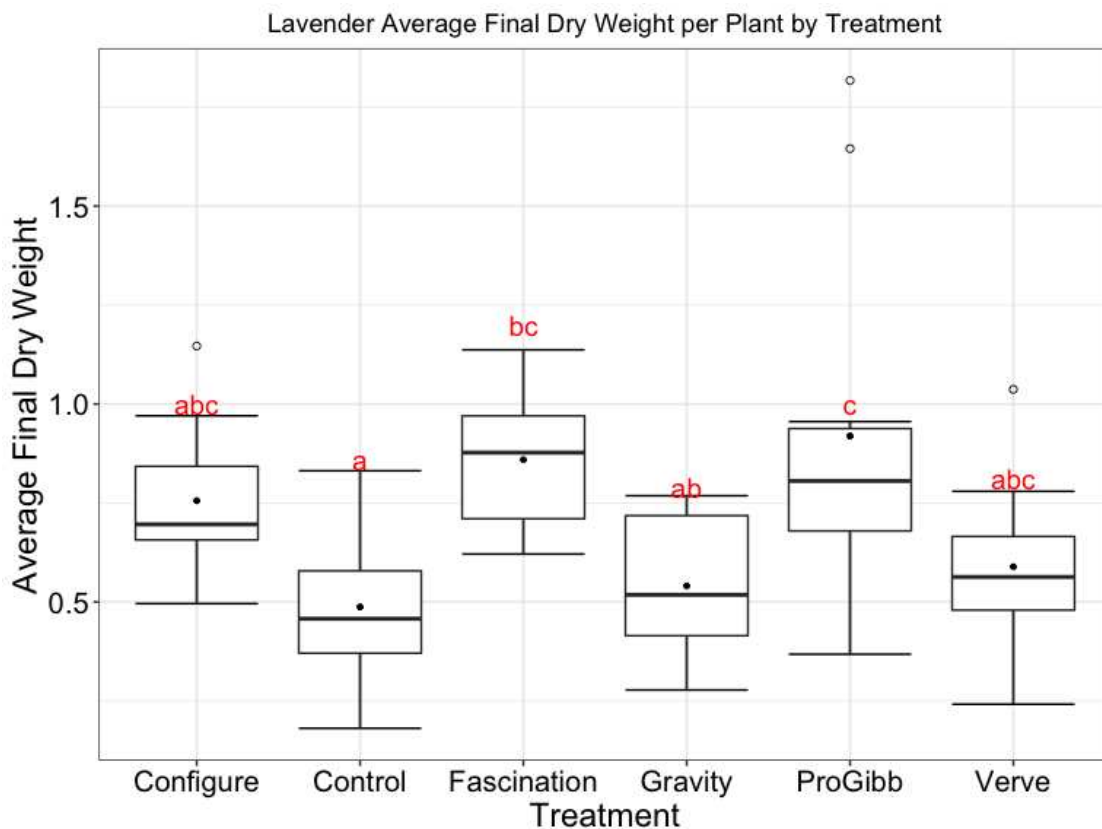

Figure 4.5.4 *Lavandula angustifolia* 'Wee One' Experiment #2 boxplot of average final dry weight of top growth per treatment. Standard error bars indicate a 95% confidence interval for the mean.

4.6 Root Ratings

Root rating were conducted at the end of the experiment after the top growth was harvested for the final dry weight. These ratings were calculated using a scale of 1-5 with 1 being lightly rooted to 5 being fully rooted throughout the container.
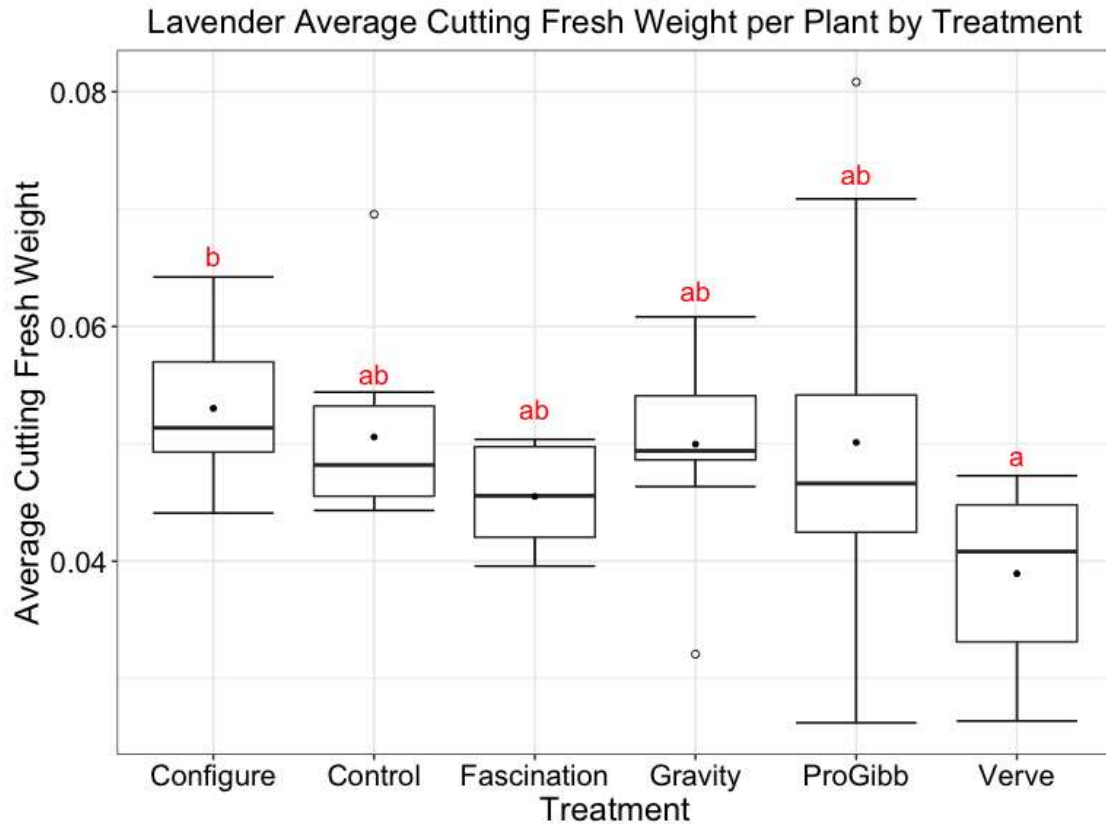
Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed no significant effect of treatment for the average root rating and the pairwise comparisons showed no significant difference at the significant level of 0.05 (Figure 4.6.1 and Table 4.6.1). The plants treated with Configure had the lowest root rating and plants untreated had the highest root rating (Table 4.6.1 and Figure 4.6.2).

```
## Response: RootRating
##            Sum Sq Df F value Pr(>F)
## Treatment   0.75  5   0.648 0.6642
## Residuals  12.50 54
```

Figure 4.6.1 *Lavandula angustifolia* 'Wee One' Experiment #1 one-way ANOVA table for root rating of stock plants with Treatment as predictor.

Table 4.6.1 *Lavandula angustifolia* 'Wee One' Experiment #1 root rating of stock plants and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##   Treatment    emmean    SE df lower.CL upper.CL .group
##   Configure       3.5 0.152 54     3.19     3.81  a
##   Fascination     3.8 0.152 54     3.49     4.11  a
##   Gravity         3.8 0.152 54     3.49     4.11  a
##   ProGibb         3.8 0.152 54     3.49     4.11  a
##   Verve           3.8 0.152 54     3.49     4.11  a
##   Control         3.8 0.152 54     3.49     4.11  a
```
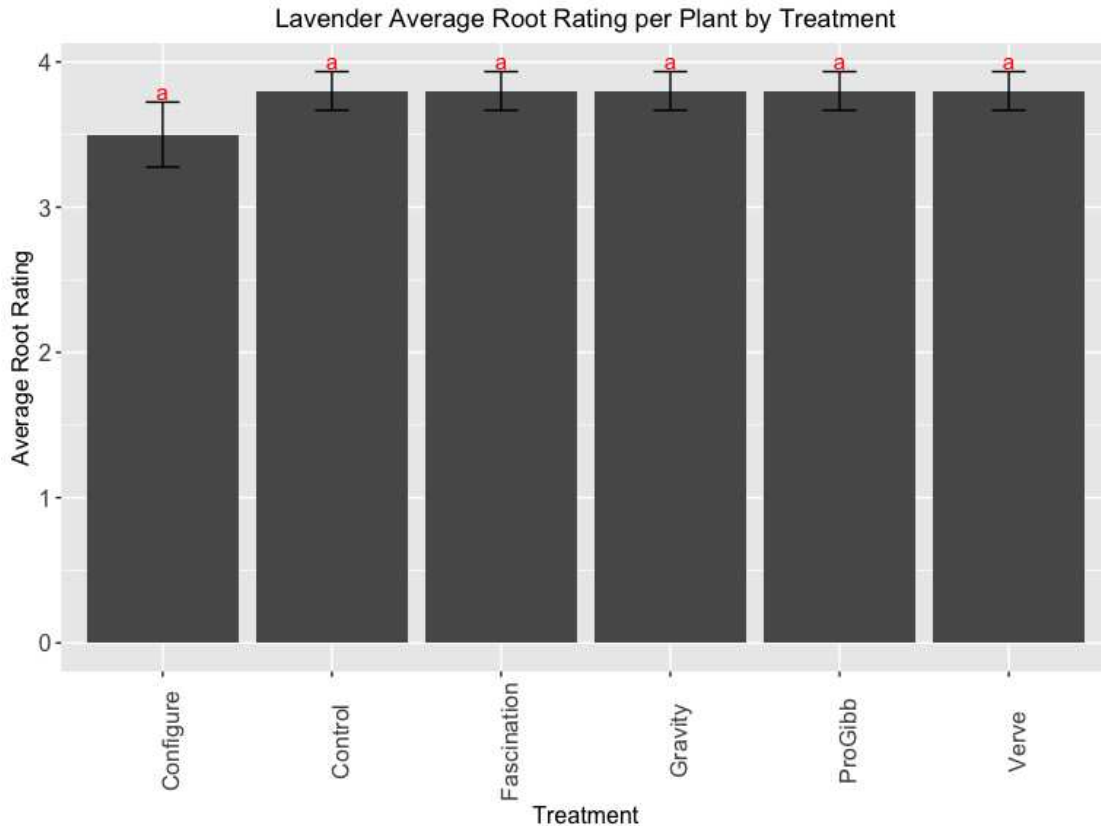
Figure 4.6.2 *Lavandula angustifolia* 'Wee One' Experiment #1 bar graph of average root rating per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a significant effect of treatment for the average root rating and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.6.3 and Table 4.6.2). The plants treated with Verve had the lowest root rating and plants treated with ProGibb had the largest root rating (Table 4.6.2 and Figure 4.6.4). While experiment #2 had significant difference while experiment #1 did not, the difference between experiments could show the effect of environmental conditions and time of year. Root rating of stock plants is important to show that after multiple applications of any treatment it is not causing any negative effects to the root system of that stock plant. Roots are important for uptake of nutrients and water. Negatively affected root systems could lead to reduced stock plant vegetative material

production. After four monthly applications of PGRs, no significant effects can be seen to the

rooting of these stock plants.

```
## Response: RootRating
##             Sum Sq Df F value    Pr(>F)
## Treatment      8  5    5.76 0.0002486 ***
## Residuals
```

Figure 4.6.3 *Lavandula angustifolia* 'Wee One' Experiment #2 one-way ANOVA table for root rating of stock plants with Treatment as predictor.

Table 4.6.2 *Lavandula angustifolia* 'Wee One' Experiment #2 root rating of stock plants and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment   emmean    SE df lower.CL upper.CL .group
##  Verve          2.9 0.167 54     2.57     3.23  a
##  Fascination    3.3 0.167 54     2.97     3.63  ab
##  Configure      3.5 0.167 54     3.17     3.83  abc
##  Gravity        3.5 0.167 54     3.17     3.83  abc
##  Control        3.7 0.167 54     3.37     4.03   bc
##  ProGibb        4.1 0.167 54     3.77     4.43    c
```
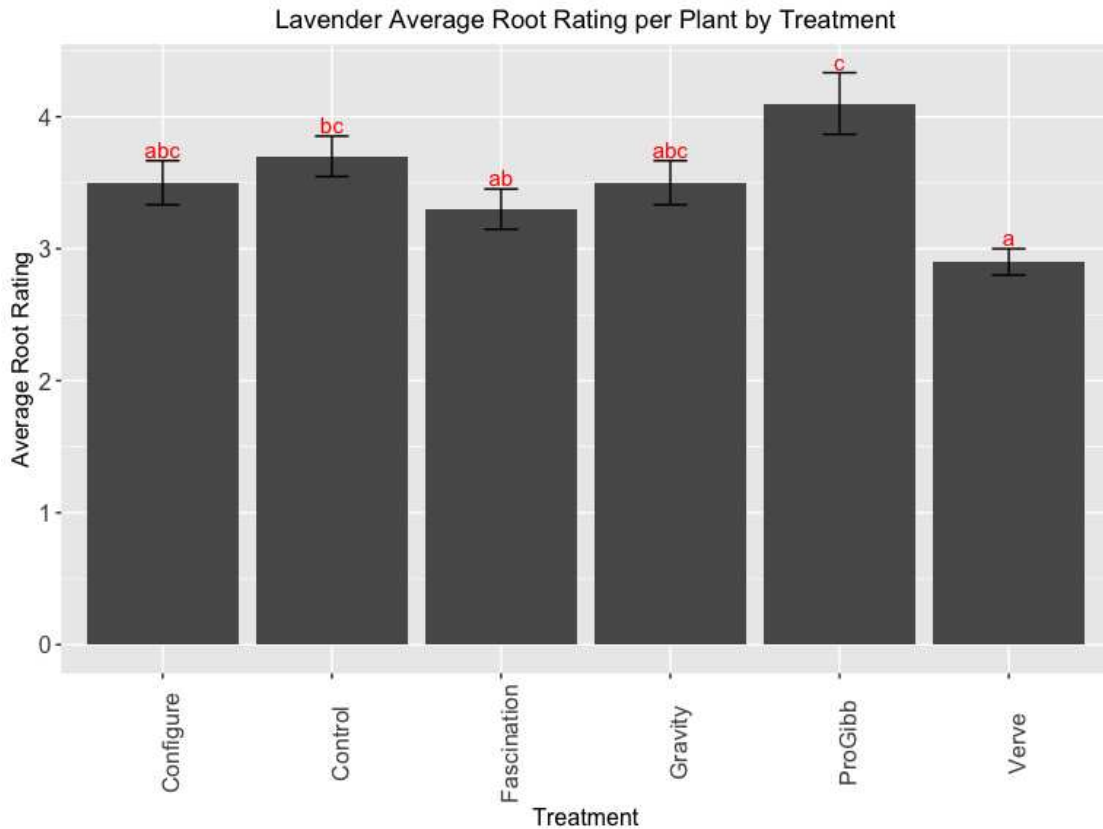

Figure 4.6.3 *Lavandula angustifolia* 'Wee One' Experiment #2 bar graph of average root rating per treatment. Standard error bars indicate a 95% confidence interval for the mean.

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4.7 Difference between Experiment #1 and #2

Differences in response between experiment #1 and #2 are partially due to the time of year the experiment was carried out. The first experiment was from February 2020 to June 2020 while the second experiment was July 2020 to December 2020. It is possible that fewer cutting and smaller plants were produced in the second study because of lower ambient temperatures and lower light intensities in the greenhouse. During the second experiment, Colorado was experiencing large wildfires. These wildfires were producing large amounts of smoke that covered a large portion of the state. Fort Collins was one of the cities that was highly effected with multiple poor air quality alerts being sent to residents during August through October 2020 (O'Donnell, 2020).

Figure 4.7.1 Photo taken on September 5, 2020 in Greeley, CO showing the edge of the smoke cloud created by wildfires.

4.8 Rooting Experiment Results

Rooting data was taken weekly starting after week two for a period of four weeks on the mist bench. The rooting data collected counted the number of visible roots to a total of 31 visible roots. Statistical analysis was performed using the visible number of roots averaged over the four-month time points for each experiment. A rooting percentage was calculated after 4 weeks on how many cuttings out of the ten for each plant growth regulator treatment and control rooted or not. Four harvest dates lead to 4 different sets of rooted cuttings. These rooting percentages were collected into a table for comparisons between harvests. There were some correlations between treatments applied and the rooting of those vegetative cuttings.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #1 revealed a significant effect of treatment for the average number of visible roots and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.8.1 and Table 4.8.1). The cuttings treated with ProGibb had the least amount of visible roots and the untreated cuttings had the most amount of visible roots (Table 4.7.1 and Figure 4.7.2).

```
## Response: AvgRoots
##            Sum Sq Df F value  Pr(>F)
## Treatment 174.07   5   4.497 0.00169 **
## Residuals 418.05 54
```

Figure 4.8.1 *Lavandula angustifolia* 'Wee One' Experiment #1 one-way ANOVA table for number of visible roots with Treatment as predictor.

Table 4.8.1 *Lavandula angustifolia* 'Wee One' Experiment #1 number of visible roots and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment     emmean   SE df lower.CL upper.CL .group
##  ProGibb         7.78 0.88 54     6.01     9.54  a
##  Configure      10.22 0.88 54     8.46    11.99  ab
##  Fascination    11.90 0.88 54    10.14    13.66   b
##  Verve          11.93 0.88 54    10.16    13.69   b
##  Gravity        12.50 0.88 54    10.74    14.26   b
##  Control        12.62 0.88 54    10.86    14.39   b
```
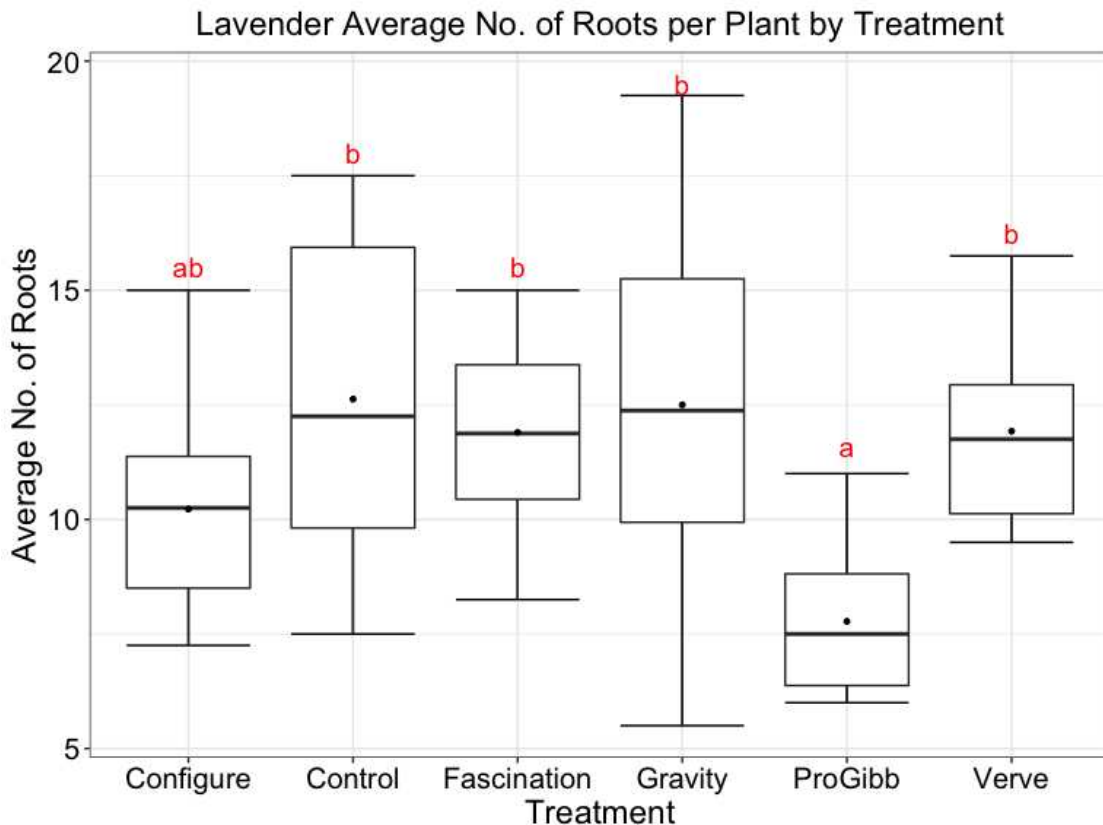

Figure 4.8.2 *Lavandula angustifolia* 'Wee One' Experiment #1 boxplot of average number of roots per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Analysis of variance for *Lavandula angustifolia* 'Wee One' Experiment #2 revealed a significant effect of treatment for the average number of visible roots and the pairwise comparisons showed a significant difference at the significant level of 0.05 (Figure 4.8.3 and Table 4.8.2). The cuttings treated with Configure had the least amount of visible roots and the

cuttings treated with ProGibb T&O had the most amount of visible roots (Table 4.8.2 and Figure 4.8.4). The trend between experiment #1 and experiment #2 for the number of roots was similar with the exception of ProGibb T&O. ProGibb T&O had the least number of roots in experiment #1 and the most in experiment #2. The significance of this difference can be seen in the rooting percentage of Harvest 3 where ProGibb T&O had poor rooting in experiment #1 compared to experiment #2 (Table 4.7.3 and Table 4.7.4). ProGibb T&O and Configure have the active ingredient of gibberellic acid. Applications of gibberellic acid can lead to shorter and thinner roots produced (Fonouni-Farde et al., 2019). Because of the differences between experiment #1 and #2 with ProGibb T&O and other PGR's with gibberellic acid as the active ingredient, it is not possible to state that applications of GA caused negative effects to rooting of cuttings and root formation. Further research needs to be completed to see if continued applications of GA shows these negative effects as noted in other research.

```
## Response: AvgRoots
##             Sum Sq Df F value    Pr(>F)
## Treatment  94.693  5  8.4124 6.167e-06 ***
## Residuals 121.569 54
```

Figure 4.8.3 *Lavandula angustifolia* 'Wee One' Experiment #2 one-way ANOVA table for average number of roots with Treatment as predictor.

Table 4.8.2 *Lavandula angustifolia* 'Wee One' Experiment #2 average number of roots and 95% confidence intervals for each PGR treatment. Means with different significance groups are statistically different at the significance level of P<0.05.

```
##  Treatment    emmean    SE df lower.CL upper.CL .group
##  Configure     2.85 0.474 54     1.90     3.80  a
##  Fascination   3.50 0.474 54     2.55     4.45  ab
##  Verve         4.17 0.474 54     3.22     5.13  abc
##  Control       5.28 0.474 54     4.32     6.23   bcd
##  Gravity       5.67 0.474 54     4.72     6.63    cd
##  ProGibb       6.45 0.474 54     5.50     7.40     d
```
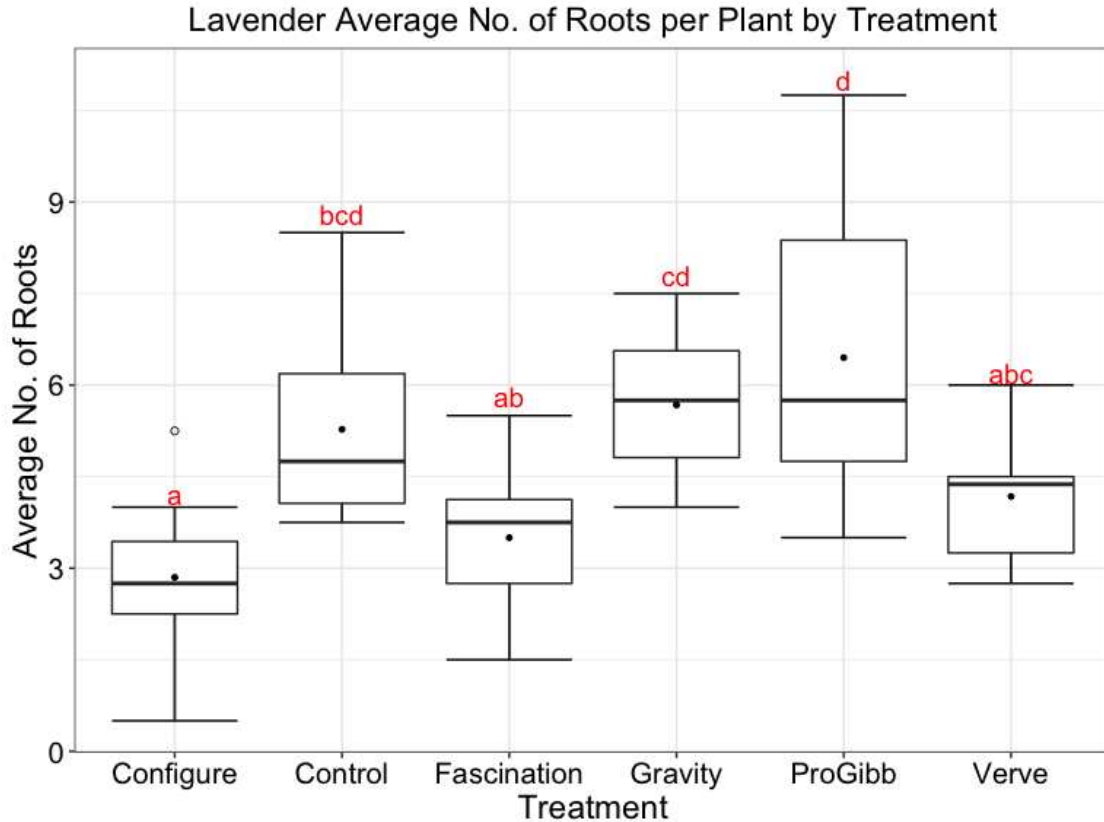
Figure 4.8.4 *Lavandula angustifolia* 'Wee One' Experiment #2 boxplot of average number of roots per treatment. Standard error bars indicate a 95% confidence interval for the mean.

Rooting percentage was calculated as a binary response for each cutting at the end of

four weeks. A cutting with visible roots was marked as 1 and a cutting with no visible roots was

marked as 0. These binary response numbers of either 1 or 0 were added together for each

treatment and divided by the total number of cuttings in that treatment then multiplied by 100

to get a percent ((2/10)*100). In the ideal situation we are striving for 80 to 90% rooting in any

treatment. In the rooting percentages for each harvest of *Lavandula angustifolia* 'Wee One'

Experiment #1 and #2, a trend can be seen during Harvest 3, where rooting percentages

dropped for Configure and Fascination. This is a significant find as it has been reported in

research that applications of GA can have a negative effect on rooting. GA causes cell expansion

of the plant but GA also resulted in shorter and thinner roots (Fonouni-Farde et al., 2019).

Lavender shows the effects of treatment with gibberellic acid plant growth regulators by having lower number of roots and lower rooting percentages when compared to the control. Overall, rooting percentages were above 50% for each treatment and the control except for Harvest 1 in Experiment #2. Difference in rooting percentage of experiment #2 in Harvest 1 was due to environmental differences that occurred in the greenhouse at that time. As explained previously, Colorado was effected by large wildfires during the second experiment that caused changes in ambient temperatures and light intensities in the greenhouse.

Table 4.7.3 *Lavandula angustifolia* 'Wee One' Experiment #1 rooting percentage each harvest by treatment((number of cuttings with visible roots for each treatment / 10)* 100)

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Control	100%	100%	80%	100%
Configure	100%	90%	60%	100%
ProGibb	70%	100%	50%	100%
Verve	100%	100%	100%	100%
Gravity	100%	100%	70%	90%
Fascination	100%	90%	100%	100%

Table 4.7.4 *Lavandula angustifolia* 'Wee One' Experiment #2 rooting percentage each harvest by treatment((number of cuttings with visible roots for each treatment/ 10)* 100)

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Control	50%	100%	100%	100%
Configure	40%	100%	80%	0%
ProGibb	70%	100%	100%	70%
Verve	30%	100%	80%	80%
Gravity	60%	100%	100%	100%
Fascination	80%	100%	80%	20%

CHAPTER 5: Conclusion for Plant Growth Regulator Study

5.1 Response to Plant Growth Regulator Treatments

Stock plants of *Lavandula angustifolia* 'Wee One' responded to plant growth regulator treatments in similar trends depending on the time of year it was grown. Experiment #2

resulted in smaller stock plants and reduced number of cuttings. More in-depth research will need to be performed to determine which physiological and environmental traits are involved in that growth response. During the first experiment, ProGibb T&O treatments resulted in larger plants. The average number of cuttings from the ProGibb T&O treatment were close to the mean number of cuttings for all PGR treatments and produced more than the control. The average fresh and dry weight per cutting for ProGibb T&O treatments were higher in the first experiment. The increased amount of cuttings from stock plants grown in the first experiment can be attributed the effects of gibberellic acid on lateral branching and stem elongation of Lavender.

During the second experiment, ProGibb T&O treatments resulted in larger plants and more cuttings per plant. The average fresh and dry weight per cutting for ProGibb T&O was close to the mean weight for all PGR treatments and resulted in no small cuttings. The same trends remained for both experiments which showed a strong correlation for determining the best PGR treatment which resulted in the most propagation material.

The application of Gravity, Configure, Verve and Fascination resulted in varying growth on the stock plants and the number of cuttings produced. In the second experiment, Verve applications resulted in the smallest stock plants which could be attributed to the time of year going into the fall and environmental conditions in the greenhouse.

5.2 Propagator Recommendations

Despite some differences between the first and second experiment, it is possible to make some recommendations to perennial propagators for future stock plant care and rooting of *Lavandula angustifolia* 'Wee One'. Based on the research conducted, stock plants would

result in more cutting material with the addition of monthly applications of ProGibb T&O at a rate of 100 ppm. Our experiments did not last as long as many growers keep their stock plants, therefore, no claims can be made about the longevity of the stock plant in relation to additional treatments of PGR.

After completing the rooting study, it can be recommended that growers follow the propagation protocols described in Chapter 2 which resulted in successful rooting of 100% during both experiments. There was no correlation between the number of roots and rooting percentages of cuttings applied with any of the PGR treatments. This finding shows that we cannot conclude that applications of ProGibb T&O will not decrease a propagator's rooting percentage or number of visible roots on *Lavandula angustifolia* 'Wee One' cuttings.

UNIT 2: PROPAGATION TECHNIQUES STUDY

CHAPTER 6: Literature Review

6.1 Literature Review on *Pterocephalus depressus*

Pterocephalus depressus(Archibald) is in the Dipsacaeae family and native to Morocco (Peris et al., 1999). This small rock outcropping perennial is relatively small in growth habit. *Pterocephalus depressus* also produces a soft pink flowers on top of a very compact plant late in the summer. It was attractive to Plant Select® because of the drought tolerance and ability to survive in cold environments. Due to the relatively small growth habit, *Pterocephalus* was noted by Plant Select® growers for having low rooting percentages and small vegetative growth (*Plant Select*, 2021).

Research on the genus of *Pterocephalus* is numerous due to the potential medicinal value of this herbaceous perennial. *Pterocephalus* has been researched in the medical community because of its' use in traditional Tebetan medicine (Wang et al., 2019). The potential of using *Pterocephalus* for medicinal purposes has driven research in the chemical compounds found in the plant tissues. Beyond the medical research, propagation and stock plant management research is very limited. Research has been completed at Colorado State University on *Pterocephalus depressus* by a fellow graduate student. This research looked at the effects of plant growth regulators on stock plant management and the location of these plant growth regulators in the tissue (Markovic & Klett, 2021).

6.2 Literature Review on *Osteospermum* species

Osteospermum(L.) species is a long blooming herbaceous perennial with evergreen-like foliage. The *Osteospermum* sp. of focus in this research is native to the Drakensberg Mountains

of South Africa. *Osteospermum* sp. is part of the Asteraceae family and generally called African

Daisy in the horticulture industry (*Plant Select*, 2021).

Osteospermum sp. was selected due to the spreading growth habit of nearly evergreen

foliage that is covered with flowers in late spring. Selections made by Plant Select® are also

noted for having increased disease resistance and more blooms than other hardy selections.

Plant Select® has two different cultivars that have either a white or purple bloom (*Plant Select*,

2021).

Some research has been conducted with *Osteospermum* that focuses on the use of plant

growth regulators to produce compact plants. Gibson reported that growth retardants caused

phytotoxicity when foliar applications were used applied but drench applications were more

effective with no toxicity (Gibson & Whipker, n.d.). Additional research has been reported on

the nutrition and soil requirements for optimal growth of *Osteospermum* (Nowak, 2001).

Pathogen research has been extensive on *Osteospermum*. The plant has been a host for many

problematic pathogens in the horticulture industry. A collaborative of universities have

researched a specific pathogen strain of *Ralstonia solanacearum* that has been found to affect

Osteospermum sp. and many other cultivated crops (Weibel et al., 2016). Numerous areas of

research have been reported in the literature but research on the propagation of cuttings is

lacking for the clones promoted by Plant Select®.

6.3 Literature Review on *Epilobium canum subsp. garrettii* 'PWWG01S'

Epilobium canum(Greene) is part of the Onagraceae family. Hummingbird trumpet or

California Fuchsia is native to the California foothills and coastal areas. The natural growth habit

of this flowering herbaceous perennial is generally under a 46 cm in height and spreads 61 – 91

cm in width. The natural reproduction is by seed or spreading through the rhizomes. (California Native Plant Society, 2014)

Epilobium canum subsp. *garrettii* 'PWWG01S' (formerly labeled as *Zauschneria garrettii* 'PWWG01S') is a rapidly spreading groundcover. A selection was made from seed collected in Idaho to have high survivability in a high elevation. The plant has been noted for the large mass of orange-red flowers late in the summer. The mass of blooms attracts many native species of pollinators and hummingbirds. This subspecies was selected due to increased winter hardiness compared to the native form (*Plant Select*, 2021).

6.4 Vegetative Propagation Techniques

Vegetative propagation techniques refers to the many methods used to manipulate the environment and treatment of a specific plant material during production. Research was done at Colorado State University to increase rooting percentages by manipulating rooting hormone, root zone heating temperatures, and application methods.

6.4.1 Rooting Hormone – Auxin

Indole-3-acetic acid (IAA) is the main form of auxin naturally found in plants. IAA is synthesized mainly in the leaf primordia, young leaves and developing seeds (Davies, 2010). These cells are a large source of auxin creation but auxin is used throughout a plant. Auxin movement is facilitated by the vascular cambium. The vascular cambium moves nutrients, hormones, and water from the top of the plant to the bottom of the plant. Auxin has many effects in plant cells such as cell enlargement, cell division, fruit growth, and root initiation. The use of auxin in this research was to help induce root initiation of stem cuttings. When auxin is applied to stem cuttings, root growth is encouraged (Hartmann et al., 2011).

Two readily available forms of auxin used in the horticulture industry are indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) (VanDerZanden, 2012) . These synthetic compounds can be used in different formulations such as salts or as a liquid solution. These synthetic compounds are desired to use as they do not degrade in light like IAA does when not in a plant cell (Kroin, 2008).

6.4.2 Root Zone Temperature

Root zone temperature refers to the additional application of heat to the root zone area of the propagation growth substrate to encourage root growth (Hartmann et al., 2011). Temperature influences root development especially when increased root zone temperature is maintained at a slightly higher temperature than the air. The goal is to encourage root growth to occur faster than shoot growth (Runkle, 2006).

6.4.3 Application Methods

Application methods refers to how a rooting hormone is applied to a cutting and what part of the cutting tissue is exposed to the hormone. Different tissues of the cutting are exposed to the hormone depending on the application technique. Hormones can be applied by foliar spray, dip, immersion, or a soil drench (Hartmann et al., 2011). Foliar spray exposes the foliage of a cutting to the hormone. Dip and soil drench applications expose the basal tissue of the cutting to the hormone. Immersion application exposes the entire cutting tissue to the hormone. Depending on plant species, certain application methods may be preferred to limit phytotoxicity (Moorman, 2011). Phytotoxicity can occur with any chemical applied to a plant. Incorrect application methods can cause phytotoxicities with any chemical that may not occur if the correct application method was used (Moorman, 2011).

6.5 Study Objectives

The objectives of the propagation techniques study for *Osteospermum species*, *Epilobium canum subsp. garretti*, and *Pterocephalus depressus* was to determine the optimal combination of rooting hormone, root zone heating temperature and application methods that result in successful rooting of cuttings with high rooting percentages (above 80%) in a four week period. The rooting study also helps determine if any of the treatment methods would result negative effects on the rooting percentage for the cuttings. The final objective is to develop a propagation protocol for growers to improve their propagation production.

CHAPTER 7: Methods and Materials

7.1 Propagation Techniques Study

This study was conducted at Colorado State University Horticulture Center which is located at 1707 Centre Avenue, Fort Collins, CO. The first experiment was performed starting in July 2019 with data collected for three replications until October 2019. The second experiment was performed starting in February 2020 with data collected for two replications until April 2020.

Research was conducted to examine three herbaceous perennials from the Plant Select® program; *Osteospermum* species, *Epilobium* canum subsp. *garretti* 'PWWG01S', and *Pterocephalus depressus*. Stock plants were started as plants of uniform size (72 plug tray) that was purchased from a local greenhouse (Gulley Greenhouse, Fort Collins, CO). A total of 5 to 10 stock plants were maintained for cutting collections. These stock plants were placed on a greenhouse bench and received no additional treatments beyond fertilized watering with 20-10-20 and pruning to encourage new growth.

7.1.1 Stock Plants used for Cutting Harvest

The stock plants were grown in a 4.5" black square, black #1 (2.84L), or black #5 (14.55L) containers. *Pterocephalus* was planted in smaller containers due to the small growth habit. The larger #5 containers were stock plants that were upshifted to prevent root gridling. All containers were prepared by being soaked in a disinfecting anti-fungal, anti-bacterial and anti-algae solution for ten minutes prior to use to prevent contamination from previous use.

The greenhouse used to hold the stock plants was run by a Wadsworth control system. The greenhouse, number 118, was heated by natural gas and forced air heater. The greenhouse was cooled passively by automatic ridge vents and automatic pulled shade cloths and actively by a pad and fan system. The Wadsworth system had preset daytime temperatures that were maintained between 22.7-23.9°C. The temperatures at night were maintained between 16.8-18.9°C.

Stock plants were automatically watered each week with a fertilizer solution. The fertilizer regimen was a 20-10-20 fertilizer at 200 ppm nitrogen continual feed. Using 4 liter per hour emitters, the irrigation ran twice a week for 30 minutes, for a total of 4 liters of fertilized water per week per plant. Every two weeks, all stock plants were watered with clear water to minimize the build-up of salts in the media.

7.1.2 Rooting Study

Cuttings were harvested when ample amounts of vegetative cuttings became available. Rooting hormone treatments, root zone heating temperature and application methods were based on recommendations on the product label, conversations with Plant Select® growers and literature review.

The first experiment focused on two rooting hormone concentrations applied to a cutting and placed on two different root zone heating temperatures. The first experiment had three replications completed. The first experiment treatments included two rooting hormone concentrations of Dip N Grow and two root zone heating temperatures. The first replication was treated on July 22, 2019. The second replication was treated on August 16, 2019 and the third replication was treated on September 13, 2019.

The second experiment included two rooting hormones (Dip N Grow at 500 ppm and Hortus IBA at 500 ppm) applied to a cutting and two application methods for exposing that cutting to the rooting hormone. Cuttings were either quick dipped for 30 seconds or immersed for 3 minutes in the desired treatment solution. The second experiment had two replications completed. The first replication treatments were applied to cuttings on February 10, 2020. The second replication of this experiment was started on March 4, 2020.

The media used for each rooting experiment was a Jiffy Performa plug in 26-strip plug trays. The media in a Jiffy Performa plug is a blend of coco coir and peat moss with a small amount of binder. Plug trays were purchased from Gulley Greenhouse in Fort Collins, CO.

The propagation greenhouse used for this study was run by a Wadsworth control system. Greenhouse 117 at the CSU Horticulture Center was heated by natural gas and forced air heater. The greenhouse was cooled passively by automatic ridge vents and automatic pulled shade cloths, and actively by a pad and fan system. The Wadsworth system was preset to daytime temperatures of 22.7°C F. The night temperature was set at 16.8°C.

The propagation bench was a single roll top greenhouse bench with dimensions of 1.54

m by 12.19 m. Our propagation benches are surrounded by a hanging sheet of plastic to

control water evaporation and to maintain even distribution of mist over bench.

Figure 7.1.1 Propagation bench located at the CSU Horticulture Center at 1707 Centre Avenue,

Fort Collins, CO.

The propagation bench top had three Redi-Heat propagation mats placed on them and

were controlled by two Redi-Heat digital thermostat. Each thermostat was preset to a different

root zone heating temperature for experiment one and then set to the same temperature for

experiment two. The one propagation bench had two overhead irrigation zones mounted

directly to the bench. The misting overhead irrigation system was controlled by a Nova 1626 ET

six zone misting control system. The misting nozzles were Dramm Pin-Perfect nozzles in the 2.8

mm size. Each nozzle could release up to 3.5 to 4 liters of water per minute. The mist times on

the bench were adjusted weekly; week one was 10 seconds every 15 minutes, week two was 10

seconds every 30 minutes, week three was 20 seconds every 60 minutes, and week four was 20 seconds every 60 minutes. This schedule was active for the entire 24 hour period each day.

7.2 Cutting Protocols

7.2.1 Step by Step Protocol of Pterocephalus

1. Take all ideal vegetative cuttings from the stock plant.

    - Ideal cuttings should be at least 4 centimeters in length or longer.

    - Cuttings should also be of a healthy stem caliber between 1.5 to 2 mm.

2. Do not remove more than a 1/3 of growth off the stock plants. Do not remove any woody cuttings.

3. Continually clean stock plant of any dead stems and leaves.

Figure 7.2.1 Photograph of *Pterocephalus* cutting protocol from Gulley Greenhouse, Fort Collins, CO.

7.2.2 Step by Step Protocol of Osteospermum sp.

1. Take all ideal vegetative cuttings from the stock plant.

    - Ideal cuttings should be at least 6 centimeters in length or longer.

    - Cuttings should also be of a healthy stem caliber between 2.5 to 3.5 mm.

2. Do not remove more than a 1/3 of growth off the stock plants. Do not remove any woody

cuttings.

3. Continually clean stock plant of any dead stems and leaves.

Figure 7.2.2 Photograph of *Osteospermum* cutting protocol from Gulley Greenhouse, Fort Collins, CO.

7.2.3 Step by Step Protocol of Epilobium

1. Take all ideal vegetative cuttings from the stock plant.

    - Ideal cuttings should be at least 4 centimeters in length or longer.

    - Cuttings should also be of a healthy stem caliber between 0.60 to 1.00 mm.

2. Do not remove more than a 1/3 of growth off the stock plant. Do not remove any woody

cuttings.

3. Continually clean stock plant of any dead stems and leaves.
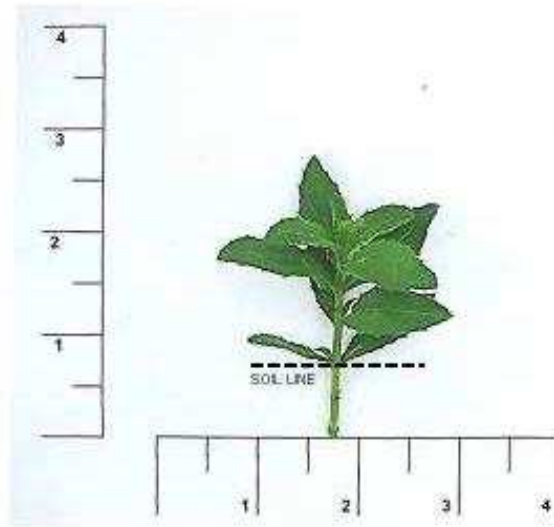
Figure 7.2.3 Photograph of *Epilobium* cutting protocol from Gulley Greenhouse, Fort Collins, CO.

7.3 Data Collection

Cuttings were harvested for each experiment when enough vegetative cuttings could be collected from the stock plants. Cuttings were taken at the same time of day, in the morning, and stuck in trays of 26-strip filled with Jiffy Performa media and placed under mist. Cuttings were taken from all stock plants maintained in the greenhouse. Ten randomly selected cuttings were chosen and treated based on experiment. Rooting data of average number of roots and rooting percentages was then collected every week for four weeks. Photographs were taken of the cuttings each week to show root development and overall cutting health.

7.4 Data Analysis

Data analysis was done using R version 4.0.4 and Microsoft Excel. Response variables include: rooting percentage of each week for each cutting and number of roots per plant during each week. Terms included in the model were predictor variables matching to the selected experiment treatments. Rooting percentages were analyzed using Microsoft Excel. Number of

roots per plant during all 4 weeks were analyzed using R. These were analyzed using a one-way

Anova and least square means using the eemeans package for each response variable.

Significant differences were noted using α =0.05 and 95% confidence intervals.

CHAPTER 8: Results and Discussions
8.1 *Pterocephalus depressus*

Experiment one looked at the effects of two concentrations of Dip N Grow and placed

on two root zone heating temperatures of either 20°C or 23.9°C. Results are displayed as the

average number of roots by week for each treatment combination and rooting percentage of

the ten cuttings in each treatment by week. Cuttings of *Pterocephalus depressus* in week 2 at

both concentrations at the higher root zone heating temperature of 23.9 °C showed a

consistent average number of roots for each replication in week 2. In the following week 3,

cuttings in 1000 ppm at 23.9°C shows an average number of roots reaching the maximum of 31

visible roots (Figure 8.1.1). In Figure 8.1.1, any concentration of Dip N Grow at either

temperature had a higher average number of roots compared to the control. The figure also

shows that in week 3, the average number of roots per cutting are reaching the maximum

amount of 31. Cuttings of *Pterocephalus* treated with either concentration and held on the

23.9°C root zone heating mat showed a higher rooting percentage in week 2 (Table 8.1.1 and

Table 8.1.2). In table 8.1.1, the rooting percentages are above the optimal 80% in week 2 for

the treated cuttings compared to the control. In table 8.1.2, the rooting percentages are above

the optimal percent 80% in week 2 for 1000 ppm and in week 3 for 500 ppm compared to the

control. These results support that any treatment with an auxin based rooting hormone shows

an increase in number of roots and higher rooting percentages (Hartmann et al., 2011). Our

results show that application of a rooting hormone increased the rate of root induction and

growth of those roots compared to the control. An interesting result that we can report is that all cuttings even the control rooted by week 4. This result is different from what Plant Select® growers were sharing with our research group. Differences may be due to our greenhouse conditions and the time of cutting harvest.
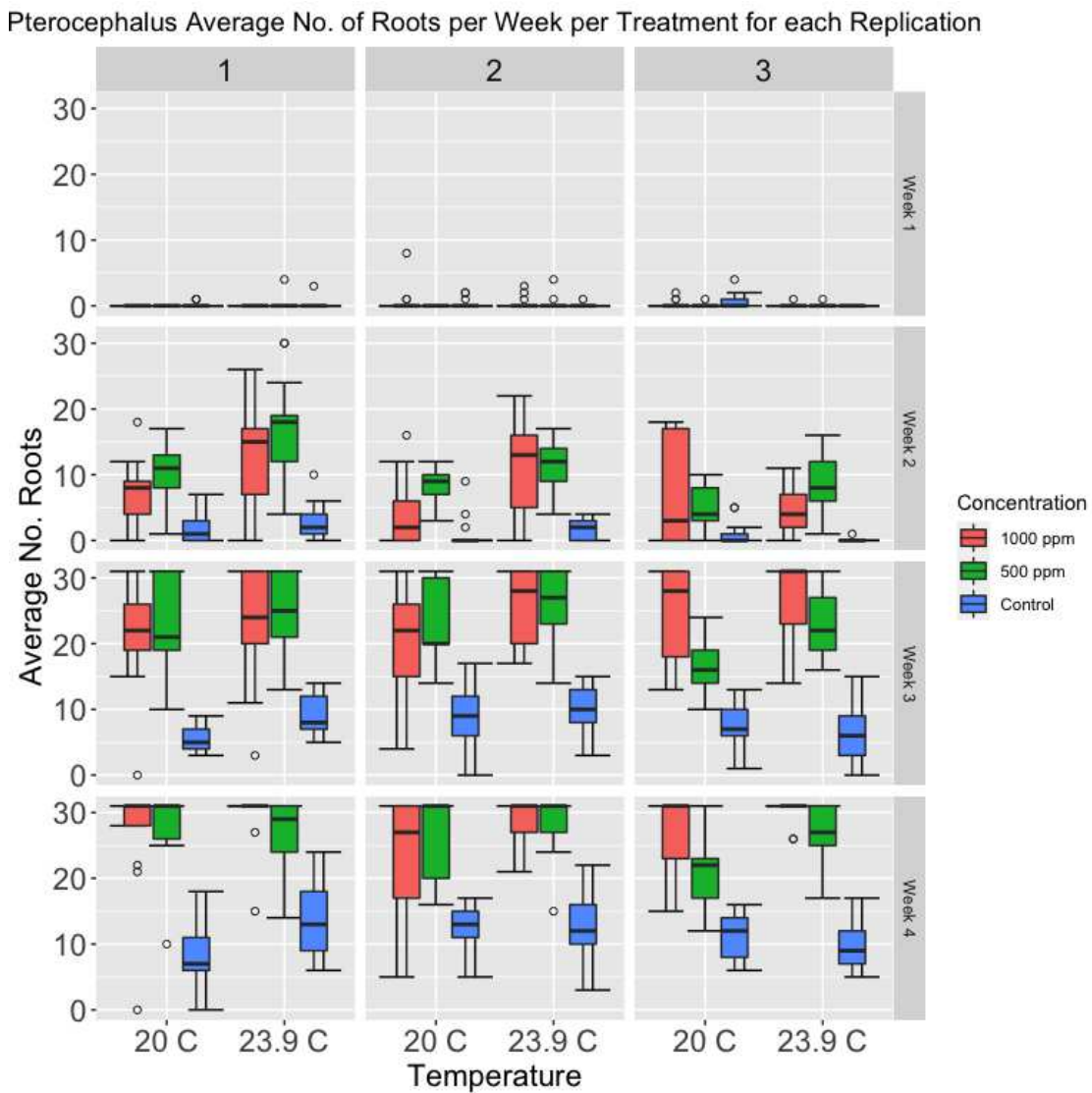
Figure 8.1.1 *Pterocephalus depressus* Experiment #1 boxplot for average number of visible roots each week color coded by concentration of Dip N Grow (orange for 1000 ppm, green for 500 ppm and blue for control) and split by root zone heating temperatures of either 20°C or 23.9°C. These boxplots show the three replications side by side to use for comparisons. Standard error bars indicate a 95 % confidence interval for the mean.

Table 8.1.1 *Pterocephalus depressus* Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 23.9°C root zone heating.

1000 ppm - 23.9°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		0%	92%	100%	100%
	Rep 2		23%	92%	100%	100%
	Rep 3		8%	92%	100%	100%
Control - 23.9°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		8%	69%	100%	100%
	Rep 2		8%	54%	100%	100%
	Rep 3		0%	8%	92%	100%
500 ppm - 23.9°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		8%	100%	100%	100%
	Rep 2		15%	100%	100%	100%
	Rep 3		8%	100%	100%	100%

Table 8.1.2 *Pterocephalus depressus* Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 20°C root zone heating.

1000 ppm – 20°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		0%	85%	92%	92%
	Rep 2		23%	62%	100%	100%
	Rep 3		23%	69%	100%	100%
Control - 20°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		23%	69%	100%	100%
	Rep 2		23%	23%	92%	100%
	Rep 3		38%	38%	100%	100%
500 ppm - 20°C			Week 1	Week 2	Week 3	Week 4
	Rep 1		0%	100%	100%	100%
	Rep 2		0%	100%	100%	100%
	Rep 3		8%	85%	100%	100%

Experiment two looked at the effects of two rooting hormones (Dip N Grow and Hortus IBA) and applied by dip or immersion methods. Results are displayed as the average number of roots by week for each treatment combination and rooting percentage of the ten cuttings in each treatment by week. Cuttings of *Pterocephalus* treated with either rooting hormone applied with the dip method had a higher number of roots in week 3 for both replications

(Figure 8.1.2). In Figure 8.1.2, either hormone applied by dip showed a higher average number of roots starting in week 2 when compared to the immersion method. A difference can be seen between replication 1 and replication 2 as the cuttings did not produce as many roots in the second replication. These replications were started in February and March 2020, respectively. The differences in root production may be due to time of year that the cuttings were taken and environmental conditions. Cuttings treated with either hormone and applied with the immersion method showed deformities in the top growth of the vegetative material (Figure 8.1.3). The method of hormone application can cause an effect to the growth of vegetative material such as we noted with *Pterocephalus*. Deformation of the vegetative material due to a chemical shows phytotoxicity caused by the application method (Moorman,2011). Cuttings treated with Hortus IBA and applied with the dip method had a higher rooting percentage in week 4 (Table 8.1.3). In Table 8.1.3, rooting percentages were high for Hortus IBA applied by immersion in week 3 but due to leaf deformities found on the top growth, this application method would not be recommended until further research was completed. *Pterocephalus* foliage may be sensitive to auxins applied through immersion methods. The addition of rooting hormones and application method has an effect on the *Pterocephalus depressus* cuttings being rooted for production.

Figure 8.1.2 *Pterocephalus depressus* Experiment #2 boxplot for the average number of visible roots for each week color coded by hormone (orange is Dip N Grow at 500 ppm and blue is Hortus IBA at 500 ppm) and split by application method of either dip of 30 seconds or immersion of 3 minutes. These boxplots show the two replications side by side to use for comparisons. Standard error bars indicate the 95% confidence interval for the mean.

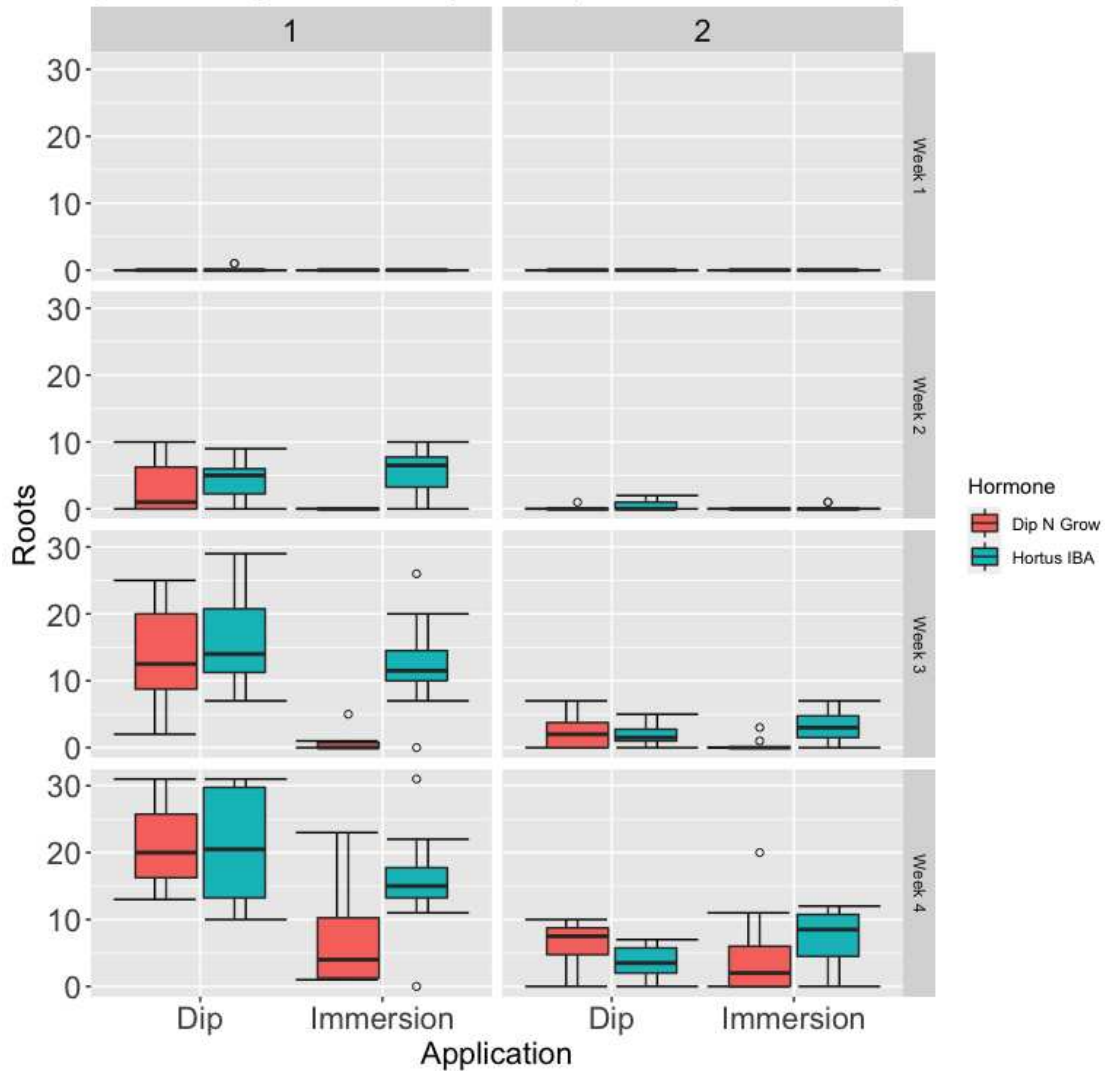**Figure 8.1.3** *Pterocephalus depressus* cuttings during experiment #2 showing deformed leaf tissues.

Table 8.1.3 *Pterocephalus depressus* Experiment #2 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment.

Dip N Grow - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	60%	100%	100%
	Rep 2	0%	10%	60%	90%
Dip N Grow - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	0%	30%	100%
	Rep 2	0%	0%	20%	60%
Control		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	60%	90%	100%
	Rep 2	0%	40%	70%	90%
Hortus IBA - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	20%	90%	100%	100%
	Rep 2	0%	40%	80%	90%
Hortus IBA - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	90%	90%	90%
	Rep 2	0%	20%	90%	90%

8.2 *Osteospermum* sp.

Experiment one looked at the effects of two concentrations of Dip N Grow and placed

on two root zone heating temperatures of either 20°C or 23.9°C. Results are displayed as the

average number of roots by week for each treatment combination and rooting percentage of

the ten cuttings in each treatment by week. In week 3, *Osteospermum* cuttings treated with

either concentration at the lower root zone temperature of 20°C lead to a higher number of

roots for all 3 replications (Figure 8.2.1). In Figure 8.2.1, the average number of roots remain

consistent at the lower temperature of 20°C when compared to the control in all three

replications. Replication 3 shows almost no data for treatments of either concentration and the

control placed on the root zone heating temperature of 23.9°C. From research notes taken

during collections, these cuttings showed evidence of stem rot in the third replication. Strong

trends can be seen in the first and second replication that supports findings mentioned above.

Higher rooting percentages of 86% and above was observed for cuttings of *Osteospermum*

treated with either hormone and rooted with a root zone heating temperature of 20°C (Table

8.2.1 and Table 8.2.2). In Table 8.2.2, rooting percentages were above the optimal 80% for

either hormone placed on the 20°C root zone heating temperature when compared to the

control. Similar to *Pterocephalus*, we noted that all cuttings rooted at the end of week 4 for

Osteospermum.
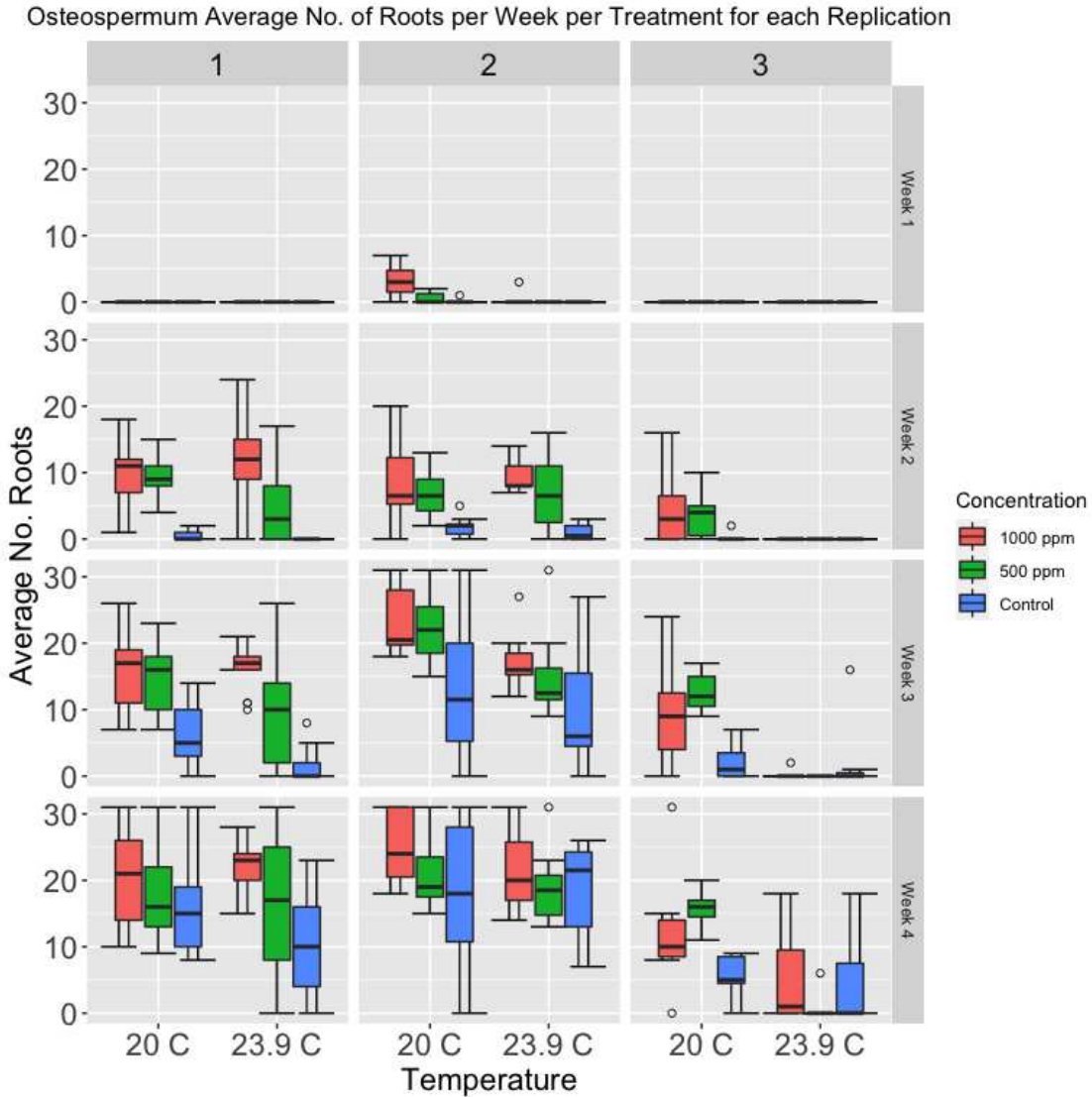
Figure 8.2.1 *Osteospermum* Experiment #1 boxplot for average number of visible roots each week color coded by concentration of Dip N Grow(orange for 1000 ppm, green for 500 ppm and blue for control) and split by root zone heating temperatures of 20°C and 23.9°C. These boxplots show the three replications side by side to use for comparisons. Standard error bars indicate a 95% confidence interval for the mean.

Table 8.2.1 *Osteospermum* sp. Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 23.9°C root zone heating.

| 1000 ppm - 23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	92%	100%	100%
	Rep 2	13%	100%	100%	100%
	Rep 3	0%	0%	14%	57%
Control -23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	0%	46%	85%
	Rep 2	0%	50%	88%	100%
	Rep 3	0%	0%	29%	43%
500 ppm - 23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	62%	85%	92%
	Rep 2	0%	88%	100%	100%
	Rep 3	0%	0%	0%	14%

Table 8.2.2 *Osteospermum* sp. Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 20°C root zone heating.

1000 ppm - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	75%	88%	100%	100%
	Rep 3	0%	57%	86%	86%
Control - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	46%	92%	100%
	Rep 2	13%	75%	88%	88%
	Rep 3	0%	14%	57%	86%
500 ppm - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	38%	100%	100%	100%
	Rep 3	0%	71%	100%	100%

Experiment two looked at the effects of two rooting hormones (Dip N Grow and Hortus IBA) and applied by dip or immersion methods. Results are displayed as the average number of roots by week for each treatment combination and rooting percentage of the ten cuttings in each treatment by week. Cuttings of *Osteospermum* treated with Dip N Grow and applied with the immersion method had higher number of roots in week 3 for both replications (Figure

8.2.2). In Figure 8.2.2, all cuttings started to have roots in week 2 but some differences between replication 1 and 2. In week 3, the trend is set with the average number of roots being the highest when cuttings were treated with Dip N Grow and applied by the immersion method. That application of auxin shows a response in the cuttings to encourage root formation (Davies, 2010). Cuttings of *Osteospermum* treated with either rooting hormone and applied with either as a dip or immersion caused a higher rooting percentage than the control as seen in week 3 (Table 8.2.3). In Table 8.2.3, rooting percentages in week 3 for both treatment combinations and control were above the optimal 80% rooting that we were striving for with this research. We have seen interesting results that show untreated control cuttings can root as well as cuttings treated with a hormone. These results may show the necessity of having clean stock plants with no viruses when producing cuttings. *Osteospermum* has been known to be affected by many pathogens such as *Ralstonia* (Weibel et al., 2016). Plant Select® growers will need to maintain clean stock plants to have optimal rooting percentages. Overall, the addition of rooting hormones and application method has an effect on the *Osteospermum* cuttings being rooted for production.

Figure 8.2.2 *Osteospermum* sp. Experiment #2 boxplot for the average number of visible roots for each week color coded by hormone (orange for Dip N Grow at 500 ppm and blue for Hortus IBA at 500 ppm) and split by application method of either dip of 30 seconds or immersion of 3 minutes. These boxplots show the two replications side by side to use for comparisons. Standard error bars indicate a 95 % confidence interval for the mean.
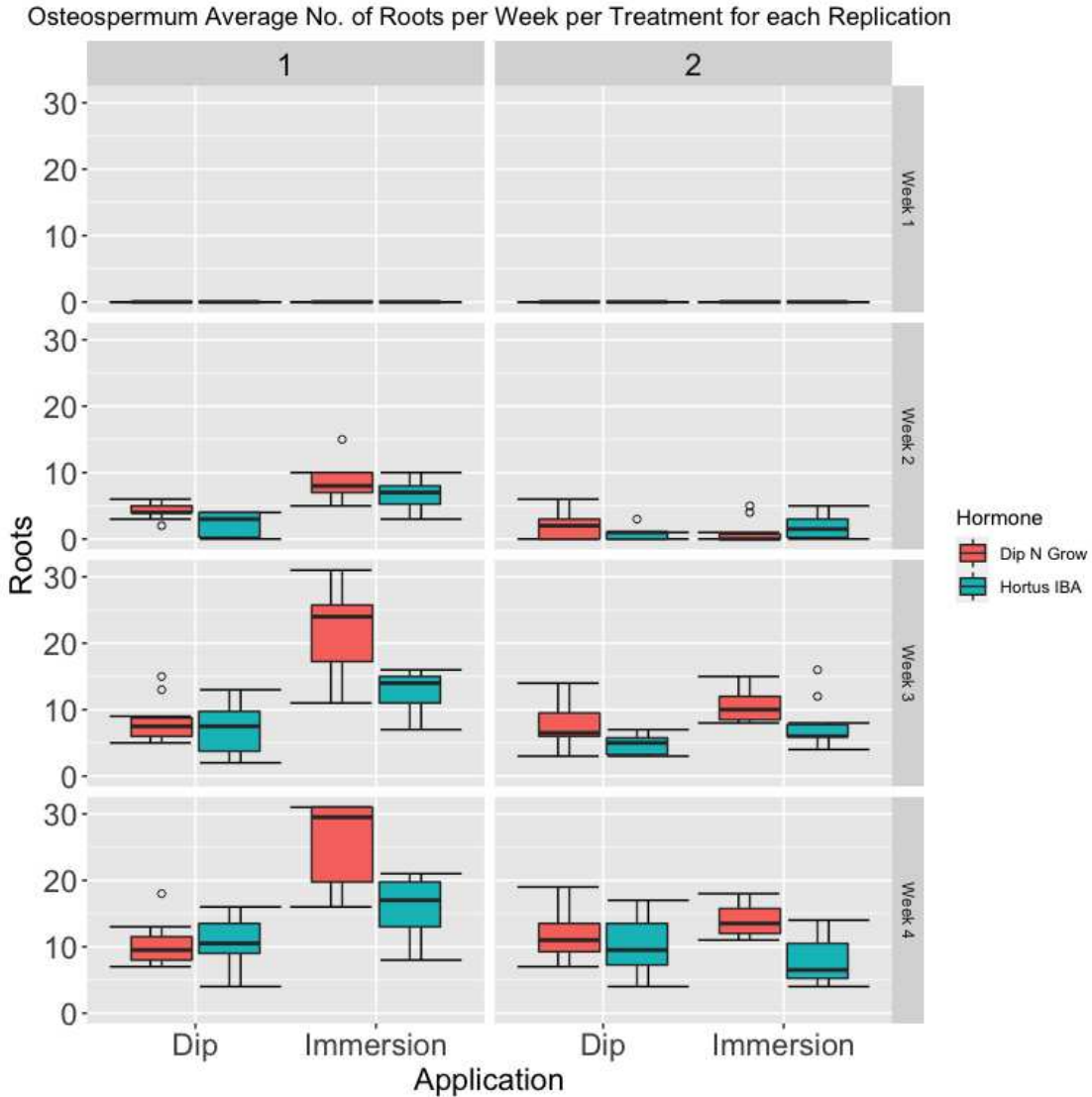
Table 8.2.3 *Osteospermum* sp. Experiment #2 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment.

Dip N Grow - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	0%	60%	100%	100%
Dip N Grow - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	0%	30%	100%	100%
Control		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	30%	90%	100%
	Rep 2	0%	10%	90%	100%
Hortus IBA - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	70%	100%	100%
	Rep 2	0%	60%	100%	100%
Hortus IBA - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	0%	70%	100%	100%

8.3 *Epilobium canum* subsp. *garrettii* 'PWWG01S'

Experiment one looked at the effects of two concentrations of Dip N Grow and placed

on two root zone heating temperatures of either 20°C or 23.9°C. Results are displayed as the

average number of roots by week for each treatment combination and rooting percentage of

the ten cuttings in each treatment by week. Cuttings of *Epilobium* treated with a 500 ppm and

rooted on 20°C root zone heating temperature had a higher average number of roots for the

first 2 replications (Figure 8.3.1). In Figure 8.3.1, replication 3 shows different results when

compared to replication 1 and 2. The difference may be due to time of year that cuttings were

harvested and environmental conditions. The same cuttings of E*pilobium* had higher rooting

percentages in week 4 compared to the other treatments and the control (Table 8.3.1 and

Table 8.3.2). In Table 8.3.2, rooting percentages are lower than the optimal of 80% for both

treatment and control. These results are also reflected in the average number of roots

produced for experiment one. When referencing data collection notes, these *Epilobium* cuttings

showed signs of reproductive tissues. Reproductive tissues that have flowering buds and will

not root as well as vegetative cuttings (Hartmann et al.,2011). These cuttings in experiment one

were harvested in July, August, and September 2019. Epilobium is noted for having showy

displays of flowers late in the summer and early fall (California Native Plant Society, 2014). An

important note for Plant Select® growers would be to ensure that harvest of cuttings occurs

from vegetative tissues and not reproductive tissues.
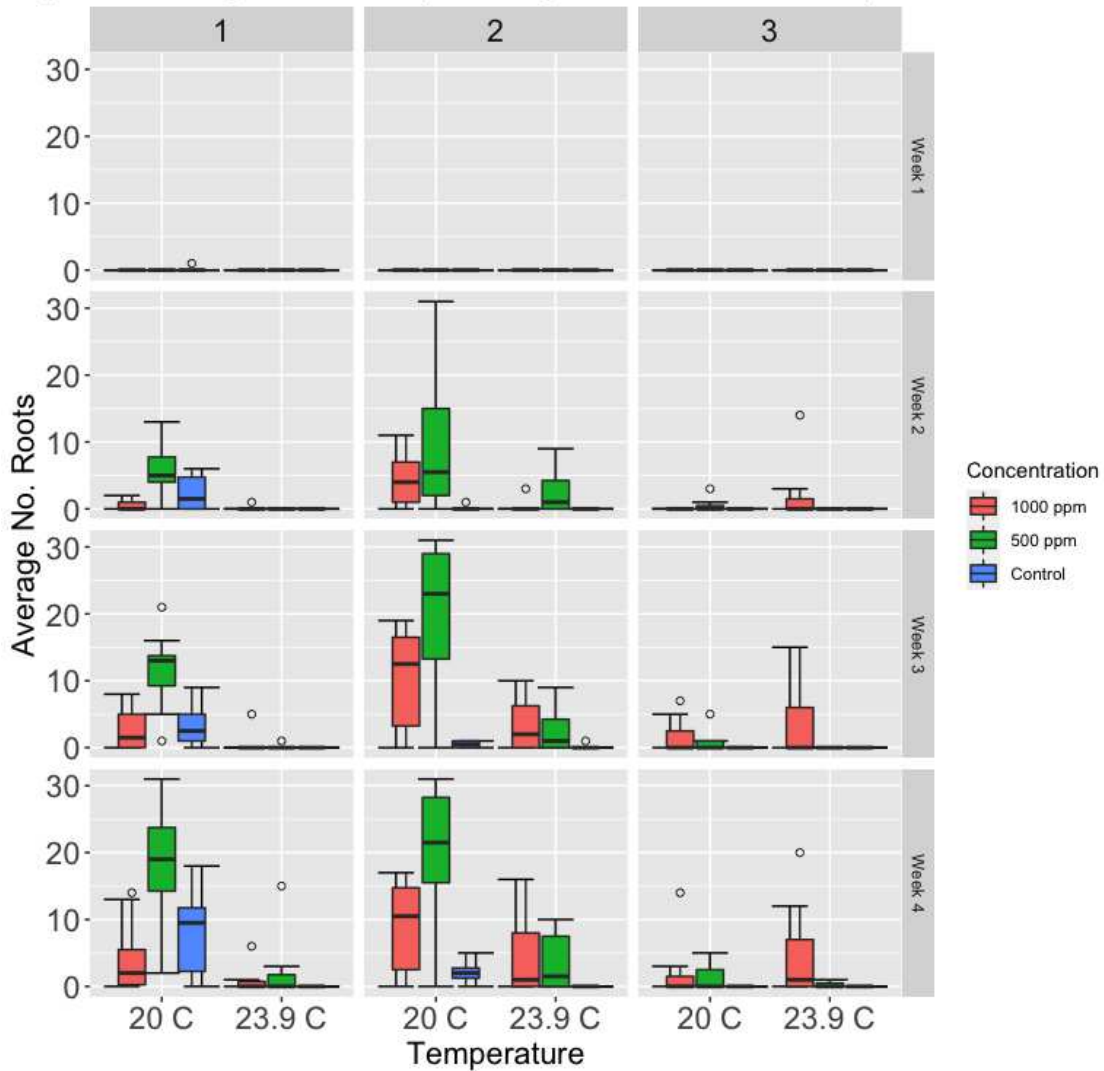
Figure 8.3.1 *Epilobium canum* Experiment #1 boxplot for average number of visible roots each week color coded by concentration of Dip N Grow(orange for 1000 ppm, green for 500 ppm and blue for control) and split by root zone heating temperature of 20°C or 23.9°C. These boxplots show the three replications side by side to use for comparisons. Standard error bars indicate a 95 % confidence interval for the mean.

Table 8.3.1 *Epilobium canum* Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 23.9°C root zone heating.

1000 ppm – 23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	10%	10%	30%
	Rep 2	0%	17%	50%	50%
	Rep 3	0%	29%	43%	57%
Control - 23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	0%	0%	0%
	Rep 2	0%	0%	17%	17%
	Rep 3	0%	0%	0%	0%
500 ppm - 23.9°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	0%	10%	40%
	Rep 2	0%	50%	50%	50%
	Rep 3	0%	0%	0%	29%

Table 8.3.2 *Epilobium canum* Experiment #1 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment at 20°C root zone heating.

1000 ppm - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	40%	60%	70%
	Rep 2	0%	67%	83%	67%
	Rep 3	0%	0%	29%	29%
Control - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	10%	60%	80%	90%
	Rep 2	0%	17%	50%	83%
	Rep 3	0%	0%	0%	0%
500 ppm - 20°C		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	100%	100%	100%
	Rep 2	0%	83%	83%	83%
	Rep 3	0%	29%	43%	43%

Experiment two looked at the effects of two rooting hormones (Dip N Grow and Hortus IBA) and applied by dip or immersion methods. Results are displayed as the average number of roots by week for each treatment combination and rooting percentage of the ten cuttings in each treatment by week. Cuttings of Epilobium treated with Dip N Grow and applied with the

dip method resulted in higher number of rooted in week 2 for both replications (Figure 8.3.2).

In Figure 8.3.2, the average number of roots was shown reaching the maximum of 31 for

cuttings treated with Dip N Grow and applied with the dip method for replication 1 and 2 in

week 2. The dip method of application for Dip N Grow results in a faster production of roots

compared to the other treatment combinations. Higher rooting percentages occurred in

Epilobium cuttings treated with either rooting hormone and applied with the dip method (Table

8.3.3). In Table 8.3.3, higher rooting percentages above the optimal of 80% are reported for

the dip application method compared to the control. Similar to the two other perennials

researched, all cuttings had roots by the end of week 4. These results may suggest that time of

year when cuttings are harvested and stock plant management have an important role in

continued successful rooting.
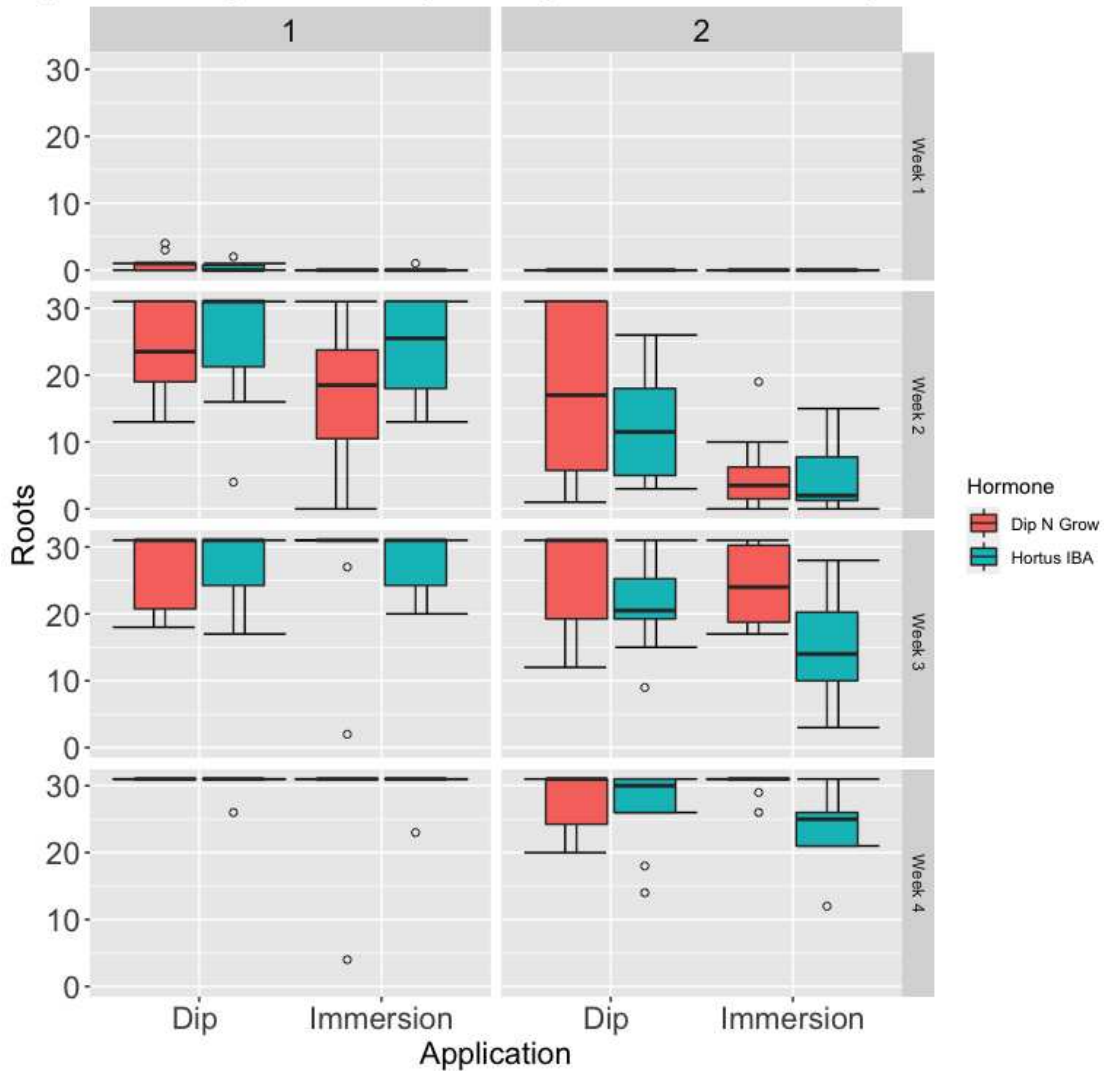
Figure 8.3.2 *Epilobium canum* Experiment #2 boxplot for the average number of visible roots for each week color coded by hormone (orange for Dip N Grow at 500 ppm and blue for Hortus IBA at 500 ppm) and split by application method of either dip of 30 seconds or immersion of 3 minutes. These boxplots show the two replications side by side to use for comparisons. Standard error bars indicate a 95 % confidence interval for the mean.

Table 8.3.3 *Epilobium canum* Experiment #2 table of rooting percentages ((number of cuttings with visible roots for a treatment/total number of cuttings in that treatment)*100) each week by treatment.

Dip N Grow - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	50%	100%	100%	100%
	Rep 2	0%	100%	100%	100%
Dip N Grow - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	0%	90%	100%	100%
	Rep 2	0%	80%	100%	100%
Control		Week 1	Week 2	Week 3	Week 4
	Rep 1	10%	100%	100%	100%
	Rep 2	0%	80%	100%	100%
Hortus IBA - Dip		Week 1	Week 2	Week 3	Week 4
	Rep 1	30%	100%	100%	100%
	Rep 2	0%	100%	100%	100%
Hortus IBA - Immersion		Week 1	Week 2	Week 3	Week 4
	Rep 1	10%	100%	100%	100%
	Rep 2	0%	90%	100%	100%

CHAPTER 9: Conclusions For Propagation Techniques Study

9.1 Response to Propagation Techniques

All treated cuttings for each perennial species responded to the multiple propagation techniques used during this study. Applications of a rooting hormone to a cutting caused increased rooting percentages and number of roots compared to the untreated control cuttings. Rooting cuttings while supplying heat to the root zone system with a heating mat encouraged faster rooting of those cuttings. Application methods of a rooting hormone caused varying results depending on the species. Generally a basal dip of the cutting into a rooting solution is enough to encourage roots but species like *Osteospermum* prefers immersing the entire cutting in a rooting solution.

9.2 Propagator Recommendations

Based on the two experiments and multiple replications, it is possible to make some recommendations to perennial propagators for techniques to used when rooting cuttings for perennial production on these three Plant Select® perennials.

9.2.1 Propagator Recommendations for Pterocephalus depressus

Pterocephalus depressus prefer quick dip application (30 second exposure) with either hormone at 500 ppm at a 23.9°C root zone heating temperature. When given these inputs, 95% rooting of all cuttings was seen in the third week.

9.2.2 Propagator Recommendations for Osteospermum species

Osteospermum species prefer an immersion application (3 minutes exposure) with Dip N Grow at 500 ppm at a 20°C root zone heating temperature. When given these inputs, 100% rooting of all cuttings was seen in the second week.

9.2.3 Propagator Recommendations for Epilobium canum subsp.garrettii 'PWWG01S'

Epilobium canum subsp.garrettii 'PWWG01S' prefer a quick dip application (30 second exposure) with either hormone at 500 ppm with a root zone heating temperature of 20°C. When given these inputs, 100% rooting of all cuttings was seen in the third week.

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