EVALUATING PROPAGATION TECHNIQUES FOR *CANNABIS SATIVA* L. CULTIVATION: A COMPARATIVE ANALYSIS OF SOILLESS METHODS AND AEROPONIC PARAMETERS

A Thesis

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by

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ABSTRACT

Given the rapid growth of the industrial *Cannabis* sector, the necessity for a reliable source of starter plant with limited genetic variation and efficient growth is crucial to achieving reliable and successful cultivation results. This study presents a multi-faceted experiment series analyzing propagation techniques for evaluating proficiency in growth and development of *Cannabis* plants. The research encompasses various (1) soilless propagation methods ((i) aeroponics, (ii) horticultural foam, and (iii) rockwool) and (2) transplant timings, (3) aeroponic spray intervals, and (4) aeroponic reservoir nutrient concentrations to elucidate their impact on rooting and growth parameters amongst two cultivars. It was found that aeroponics can provide as or more effective root development and plant growth than soilless propagation substrates. Further, continuous spray intervals compared to intermittent and optimized nutrient concentrations result in better promotion of root initiation and plant growth in aeroponics. The effects of experimental treatment often depended on cultivar and sampling day. Cultivators should assess their specific genetics to pinpoint the optimal conditions for propagation. These findings offer valuable insights into how various propagation techniques and growth parameters can be tailored to enhance the cultivation process. These results hold critical implications for cultivators intending to achieve premium harvests through efficient propagule methods and optimization strategies in the competitive *Cannabis* industry.

BIOGRAPHICAL SKETCH

Matt recently earned a Master's degree in Integrative Plant Sciences from Cornell University, specializing in Cannabis Science and Mycology. He earned a Bachelor of Science degree in Human Biology and Economics from Michigan State University. Matt brings a unique combination of academic knowledge and practical experience to the cannabis industry. His journey has been shaped by a deep appreciation for nature and the life sciences. He has a three year background in medical practice administration in addition to the former owner, manager, and head grower of his multi-site CEA cannabis caregiver cultivation. Matt's trajectory then led him towards higher academic and industry goals. Matt’s capstone research at Cornell University was dedicated to the sustainable aspects and optimization of aeroponic propagation in *Cannabis sativa* L., focusing on the critical factors influencing growth and development.

With a dedication to advancing the field of cannabis science and mycology, Matt is committed to driving innovation and growth within the industry. His hands-on experience in cannabis production and processing has cultivated a deep understanding of the plant and its nuances, motivating him to contribute to the future of cannabis and its societal benefits. Matt is passionate about pushing the boundaries of what's possible in the cannabis industry and contributing to its sustainable development. The findings from his capstone research underscore the importance of efficient and more sustainable propagation methods, rooted in his comprehensive study on aeroponic techniques and their potential to enhance the cultivation process, providing cultivators with valuable insights into the intricate interplay between propagation techniques, genetics, and growth parameters.

ACKNOWLEDGMENTS

I extend my heartfelt appreciation for the incredible support of my friends and family, which has been an unwavering pillar throughout my academic journey, fuelling my accomplishments. My sincere gratitude goes to my advisor Heather Grab, whose expertise in statistical analysis, hemp and continuous support have been integral to the success of this research. Neil Mattson's invaluable greenhouse insights, along with the valueable of Dan Buckley and Marvin Pritz, have significantly enriched the quality of my work. I am also deeply thankful to Nick Kaczmar for providing the his insights and equipment for this project. In addition, I would like to express my appreciation to my fellow MPS grad students Kevin Hernandez and Matt Dobbs for their guidance, advice, and friendship that have accompanied me on this journey. Lastly, the contributions of Neil Mattson, Dan Buckley, Marvin Pritz, and Heather Grab and many others have left a lasting impact on my academic and research growth.

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INTRODUCTION

Cannabis (*Cannabis sativa* L.) is an herbaceous annual plant, cultivated for millennia, serving purposes for medicinal and recreational applications to the production of textiles, food, and other industrial commodities (Crini et al., 2020; Rehman et al. 2021). Recently, changes in laws, reduction in societal stigma, and advancements in newly permitted research have significantly increased its agricultural and medicinal value. Optimizing cultivation practices ensures ideal potency, yield, and quality consistency, especially as the market landscape becomes more competitive and subject to increasingly rigorous regulatory standards (Sambucci et al., 2023).

To meet these demands, a variety of propagation methods have been explored and adopted by *Cannabis* growers. These range from traditional methods, such as sowing seeds directly into the soil to rapid and regenerative techniques such as vegetative propagation, in which stem cuttings from a stock plant are stimulated to root and produce genetically identical plants (Coffman et al., 1979). Another prevalent method is the use of tissue culture, a sophisticated approach that enables the generation of multiple plantlets from a small piece of plant tissue (Monthony et al., 2021). Each propagation method comes with its own set of advantages and challenges. While seeds offer genetic diversity, their germination rates, sex ratios and genetic variation can be unpredictable (REF). Tissue culture, on the other hand, offers scalability and ensures disease-free propagules but requires expensive facilities and equipment as well as sterility and trained staff. Vegetative propagation through stem cuttings is low-cost compared to tissue culture and a more consistent outcome regarding genetics, quality and yield than seeds, but may risk the spread of pathogens.

Variation in rooting and growth success during vegetative propagation has been observed in previous research Variability can be attributed to several factors such as choice of propagation method and genetics (Hinesley et al.,1981, Miller et al. 1982; Campbell et al., 2019) in addition to: stock plant age/health (Muñoz-Gutiérrez et al., 2009), cutting technique (Dorrell, D. 1974; Haile et al., 2011; Caplan et al., 2018), VPD and temperature (De Andrés et al., 2004; Dorrell, D. 1974; Haile et al., 2011), hormone application (Caplan et al., 2018), and others. There are an array of propagation systems used to produce vegetative propagules. In commercial usage, these typically include: rockwool, horticultural foam and aeroponics (Nemati et al., 2021).Rockwool, derived from molten basaltic rock, excels in maintaining a balance between water and air retention, promoting robust root growth (Yafuso et al., 2019; Campbell et al., 2021; Zheng et al., 2022). Nonetheless, there's concerns about its sustainability through manufacturing, including regular water maintenance and single-use (Robertson et al., 2023). Horticultural foam, crafted from petroleum-based phenolic foam, shares similar advantages and maintenance demands. Both of these options are single-use (Milks et al., 1989; Nemati et al., 2021; Robertson et al., 2023). An alternative to these approaches is aeroponics, in which roots are suspended and sprayed or misted consistently with a nutrient fertigation. Several authors have claimed that aeroponics enhances nutrient absorption, oxygenation, minimizes disease transmission risks, along with conservation of water and other resources (AlShrouf, A., 2017), aligning it with the global shift towards sustainable agriculture, (Kumari et al., 2019; Ferrini et al., 2021; Zheng et al., 2022), though, to our knowledge, these claims have not been empirically evaluated. However, as the roots hang and are exposed to air, its substantial reliance on electricity poses a limitation

(Kumari et al., 2019). There is a lack of comparative studies evaluating aeroponics against other propagation systems or different aeroponic conditions, warranting further research in this area. In order to meet the growing *Cannabis* industry's need for sustainable and efficient cultivation methodologies, a series of propagation systems and aeroponic optimization studies were conducted. Experiment 1 compares rooting success and propagule growth among aeroponics, horticultural foam, and rockwool. Experiment 2 compares transplant at varied post-propagation intervals across the same systems. Experiment 3 investigates the effect of different aeroponic pump timer intervals. Lastly, Experiment 4 explores the impact of aeroponic reservoir nutrient strength.

MATERIALS AND METHODS

Greenhouse and Stock Plant Conditions:

Stock plants of a CBD (cannabidiol) dominant cultivar, 'TJ's CBD' (Stem Holdings, Boca Raton, FL, USA) and a CBG (cannabigerol) dominant cultivar, 'Janet's G' (The Hemp Mine, Fair Play, SC, USA), along with the propagation trials were maintained at Kenneth Post Greenhouses on Cornell University's campus in Ithaca, NY. A 14-hour photoperiod was provided with controlled supplemental canopy lighting from 400 W high pressure sodium lamps. Temperatures averaged 26.0C (+/- 7.9) during the day and 18.3 C (+/- .23) at night, with four days reaching above 32.0C for Trial 1 of Experiments 1 and 3 spanning April 12th through May 2nd. Once A/C in the greenhouse turned on, for the remainder of trials, temperatures fell in reasonable range averaging 26.1 C (+/- 3.7) during the day and 20.3 C (+/- 2.25) at night through the remainder into summer months. The closure of the fluctuating shade cloth depended on solar intensity, as exposure to 10 minutes of direct sunlight triggered its closure. After July 11th the shade cloth remained closed for reduced spectral intensity (Moher et al., 2022). Stock plants were potted in 5 gallon pots containing a Lambert LM-111 All Purpose Mix (Lambert, Rivière-Ouelle (QC), CA) potting media. The stock plants were ~4 months old at experiment commencement. The plants were fertigated with Jack's Professional LX All Purpose (JR PETERS Inc, #77990, Allentown, PA) [21-5-20 (NPK%)](2.1 EC) weekdays and clear-water (0.5 EC) on weekends (add pH). Stock plants were scouted and treated weekly for pests and disease with ZeroTol 2.0 (BioSafe Systems, #70299-12, East Hartford, CT), Cease (Bioworks, #264-1155-68539, Victor, NY), Milstop (Bioworks, #68539-13, Victor, NY), Ultra-Pure Oil (BASF, 68539-13, Mississauga (ON), CA), Suffoil-X (Bioworks, #48813-1-68539, Victor, NY).

Plant Culture and Treatment:

For all experiments, cuttings were taken from apical branches of stock plants at a length of ~15-20 cm, having 2-3 fully expanded leaves and 3-5 nodes (Caplan et al., 2018). Each cutting was dipped in a Clonex, 0.31% indole-3-butyric acid gel (Clonex, Growth Technology Ltd., UK), before being placed to a 5 cm depth in each propagation system.

Experiments

Experiment 1: Propagation System

This experiment compared 64-site aeroponic propagation systems featuring macro droplet spray nozzles (Clone King, ck64, Albuquerque, NM) to other soilless media treatments and was replicated twice. Although the aeroponic system in this research is a Clone King product, it shares common design elements found in many aeroponic systems (Edmonds et al., 2020). Two popular soilless propagation substrates: a horticultural foam and rockwool, were selected: ROOTCUBES® (Oasis Grower Solutions WEDGE® strips, Kent, OH, USA), and rockwool cubes (AO Cubes, Grodan, Milton, ON, Canada),

The aeroponic unit was set to spray continuously, with a fertigated dilution of one-part fertigation solution and three-parts clear-water, resulting in four gallons per aeroponic unit (1

EC). To maintain humidity in the rockwool and horticultural foam, 7 ½ in tall domes were used and plants were watered as needed with a 1:3 nutrient dilution. Domes were kept closed for the first 6 days, then incrementally opened until day 14. Sets of 32 cuttings per cultivar were then placed in an aeroponic cloner, horticultural foam, and rockwool. The horticultural foam and rockwool were arranged randomly in 4 domed trays, each tray having 16 replicates of each cultivar across 2 replicates of each treatment. Aeroponics units contained 32 replicates of each cultivar totalling 64 cuttings per unit. Each trial consisted of 192 cuttings total across all cultivars and treatments. Domes and reservoirs were randomly arranged within a greenhouse bench.

Cuttings were harvested at 14 and 21 days after propagation. A randomized selection of half of each treatment and cultivar were collected at each harvest date. Each plant was cut in half (5 cm from the bottom stem) to measure above and below-ground dry biomass, height, stem thickness at 2" from stem bottom, and root quality score (1-10). Successfully rooted cuttings were assigned to a classification based on degree of adventitious rooting; a root quality score score of 1-10 was assigned based on visual representation (Figure 1a-c). Propagules at day 14 were removed from rockwool and horticultural foam to better evaluate root quality score, and left on at day 21. The effect of the media was accounted for by subtracting the average dry weight of a sample set of rockwool and horticultural foam from the results.

Experiment 2: Propagation System – Transplant

A transplant experiment was conducted to evaluate the effect of the propagation system (aeroponics, horticultural foam, and rockwool) and timing on transplant success, through two replicated trials. All conditions were the same as Experiment 1 except the aeroponic unit was set to spray in 1 minute on : 1 minute off timed intervals. At 8, 10, 12, and 14 days after propagating, 8 clones from each cultivar and propagation system were randomly selected and transplanted into 4 inch pots filled with Lambert LM-111 All Purpose Mix potting media, totalling 48 propagules per transplant date. The transplants were maintained with Jack's Professional LX All Purpose [2.1 EC] once daily.

Plants were removed from pots at 21 days after propagation to assess height and root quality score. Successfully rooted cuttings were assigned to a classification based on degree of adventitious rooting; a root quality score of 1 - 4 was assigned based on visual representation (Figure 2).

Experiment 3: Aeroponics – Spray Interval

Aeroponic pump timing was investigated to understand the impact of continuous and intermittent pump spray interval timings on the rate and success of root initiation and development. All aeroponic conditions were the same as Experiment 1 except an additional four aeroponic systems with differentiating pump timing settings were compared across three trials. Trial 1 incorporated aeroponics systems with continuous, 1 minute on : 3 minutes off (1:3) and 1:9 timed intervals consisting of total 192 cuttings. Trial 2 incorporated continuous and 1:1 timed intervals, with 128 cuttings. Trial 3 incorporated four aeroponic systems with continuous, 1:1, 1:3, and 1:9 timer intervals, with 256 cuttings. Aeroponics systems contained 32 replicates of each cultivar totalling

64 cuttings per reservoir. Reservoirs were randomly arranged within a greenhouse bench. Data were collected as in Experiment 1.

Experiment 4: Aeroponics – Fertigation Dilutions

To assess how nutrient solution strengths in the aeroponics reservoir impact rooting and growth, two replicated trials were conducted in which electrical conductivity (EC) of the solution varied across three aeroponic systems with two replications. Each trial utilized an aeroponic system set to spray continuously with nutrient solutions at one of three EC concentrations; initially measured to .7 EC (equivalent to a 1:4 fertigation dilution), 1 EC (1:3), and 1.4 EC (1:2). Aeroponics systems contained 32 replicates of each cultivar with 64 cuttings per reservoir and 192 cuttings per trial. Data were collected as in Experiment 1.

Figure 1:

Figure 1a: Demonstrating the root quality score of aeroponics root scores 1-10

Figure 1b: Demonstrating the root quality score of Rockwool cubes root scores 1-8

Figure 1c: Demonstrating the root quality score of Horticulture Foam root scores 1-8

Figure 2:

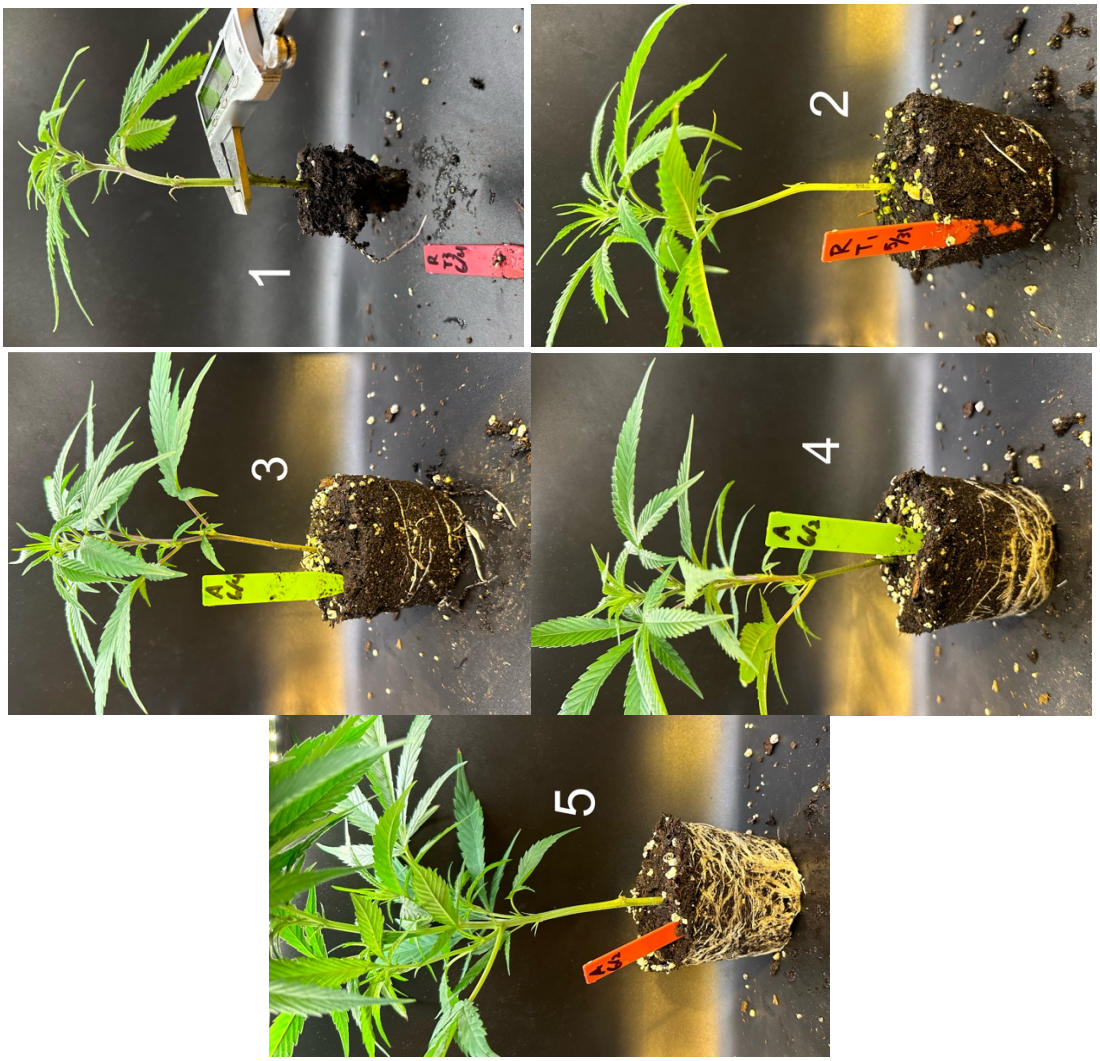

Figure 2: Demonstrates the root quality score of Experiment 2's out-of-pot transplanted root scores 1-5

Statistical Methods:

Data analysis was conducted using the R statistical software (R Core Team, 2023). Stem diameter was taken into account in mass measurements by dividing both above and below-ground masses by stem diameter. The analysis employed mixed-effects models through the 'lme4' and 'lmerTest' packages (Bates et al., 2015; Kuznetsova et al., 2017). In cases of count data, a Poisson distribution was utilized. The models included fixed effects for treatment, sampling day, and cultivar, along with their interactions.To account for trial-specific variability, trial was included as a random effect in all models. The 'Anova' function from the 'car' package was used for significance testing (Fox et al., 2019), employing a type II Wald Chi-squared test. Post hoc comparisons were conducted via the 'emmeans' package, applying Tukey's HSD test for pairwise comparisons (Lenth, 2023).

RESULTS

Experiment 1: Propagation System

Root Quality Scoring

Root quality varied depending on the choice of propagation system ($\chi^2 = 75.24$, df = 2, $P < 0.001$), cultivar ($\chi^2 = 43.85$, df = 1, $P < 0.001$), and sampling day ($\chi^2 = 181.74$, df = 1, $P < 0.001$). The effect of substrate was dependent on sampling day ($\chi^2 = 10.21$, df = 2, $P = 0.006$) and varied between sampling days and cultivars ($\chi^2 = 7.95$, df = 1, $P < 0.005$). On day 14, aeroponics had higher root scores in both cultivars when compared to horticultural foam, while only TJ's CBD exhibited superior root scores in aeroponics compared to rockwool (Figure 3). By day 21, Janet's G showed no differences in propagation systems, while TJ's CBD had higher root scores in aeroponics compared to bothhorticultural foam and rockwool (Figure 3).

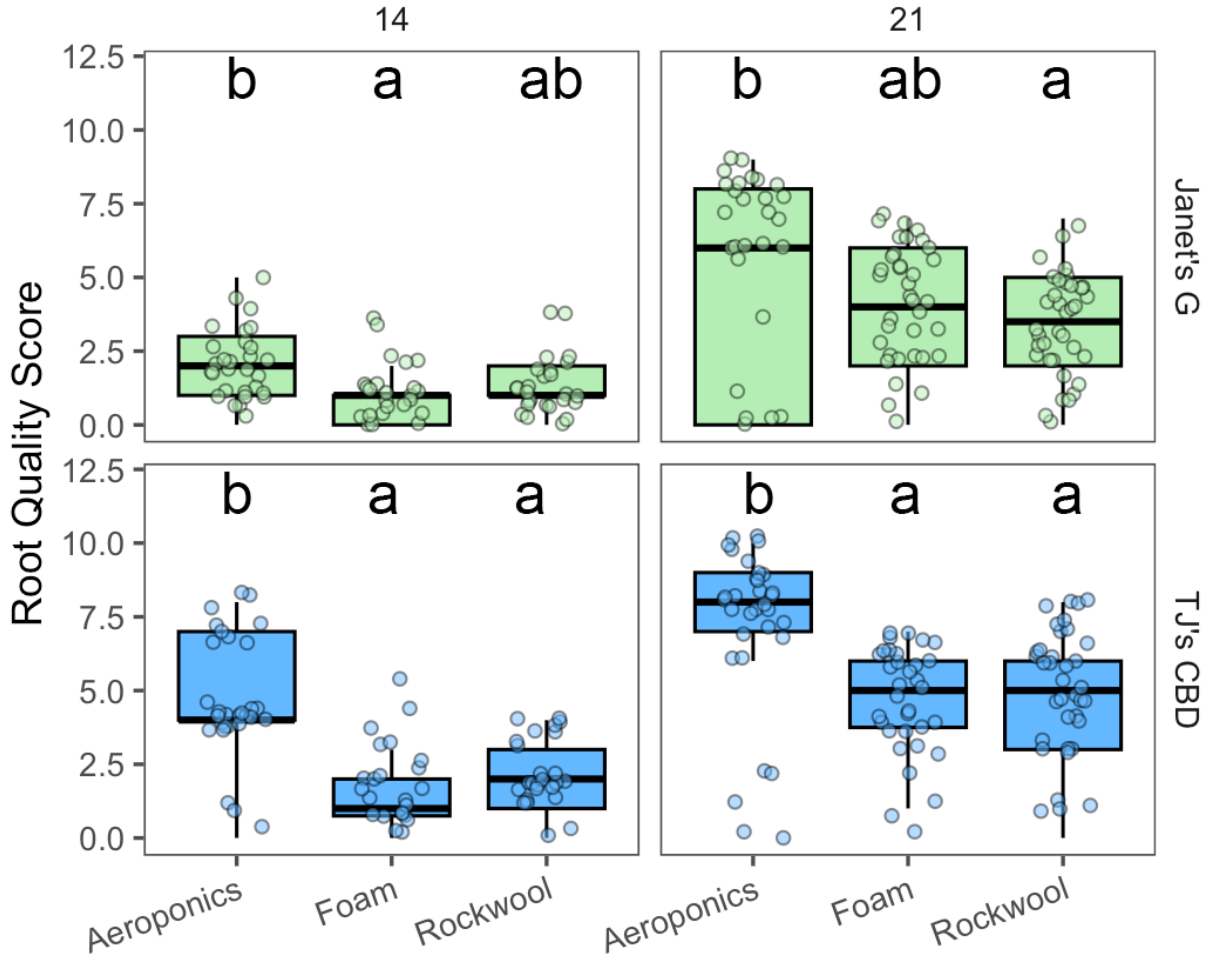

Figure 3: Root quality score (Figure 1) compared across propagation systems aeroponics, rockwool, and (horticultural) foam using *Cannabis sativa* L. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Height

Propagule height varied based on the propagation system ($\chi^2 = 28.12$, df = 2, $P < 0.001$), cultivar ($\chi^2 = 42.38$, df = 1, $P < 0.001$), and sampling day ($\chi^2 = 29.65$, df = 1, $P < 0.001$). The effect of substrate depended on sampling day ($\chi^2 = 11.57$, df = 2, $P < 0.005$) in addition to cultivar ($\chi^2 = 21.4$, df = 2, $P < 0.001$). Janet's G showed no difference in height among propagation systems at either 14 or 21 days, while TJ's CBD, although no clear height differences were observed on day 14, aeroponic led to taller plants on day 21 compared to both horticultural foam and rockwool (Figure 4).

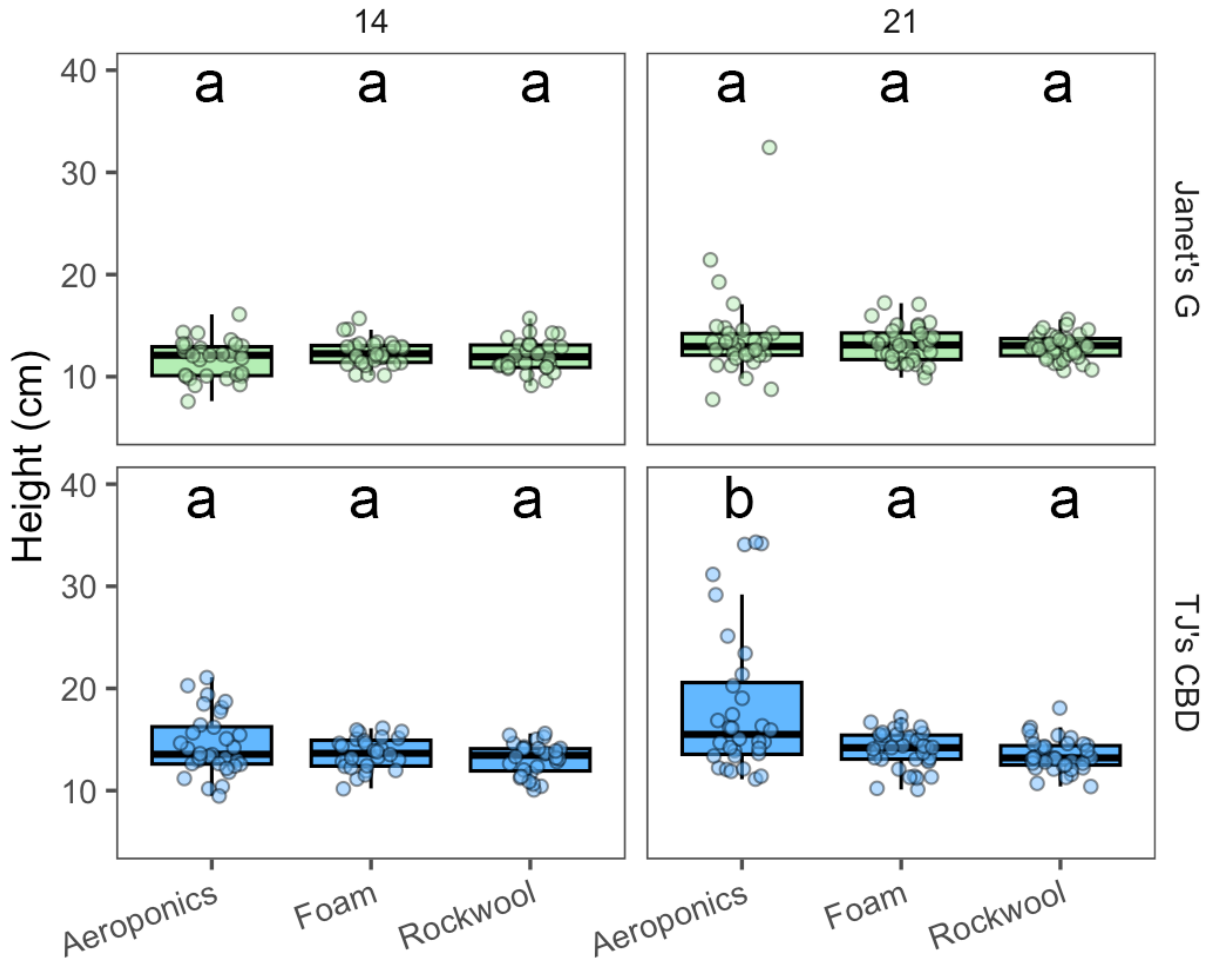

Figure 4: Height (cm) was compared across propagation systems aeroponics, rockwool, and (Horticultural) foam using *Cannabis sativa* L. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Above Ground Dry Mass

Above-ground dry mass to stem diameter ratios varied across propagation systems ($\chi^2 = 66.91$, df = 2, $P < 0.001$) and cultivar ($\chi^2 = 16.15$, df = 1, $P < 0.001$). Aeroponics consistently yielded

higher masses compared to Rockwool across both sampling days and cultivars (Figure 5). On day 21 of Tj's CBD, aeroponics exhibited heavier masses than horticultural foam (Figure 5).

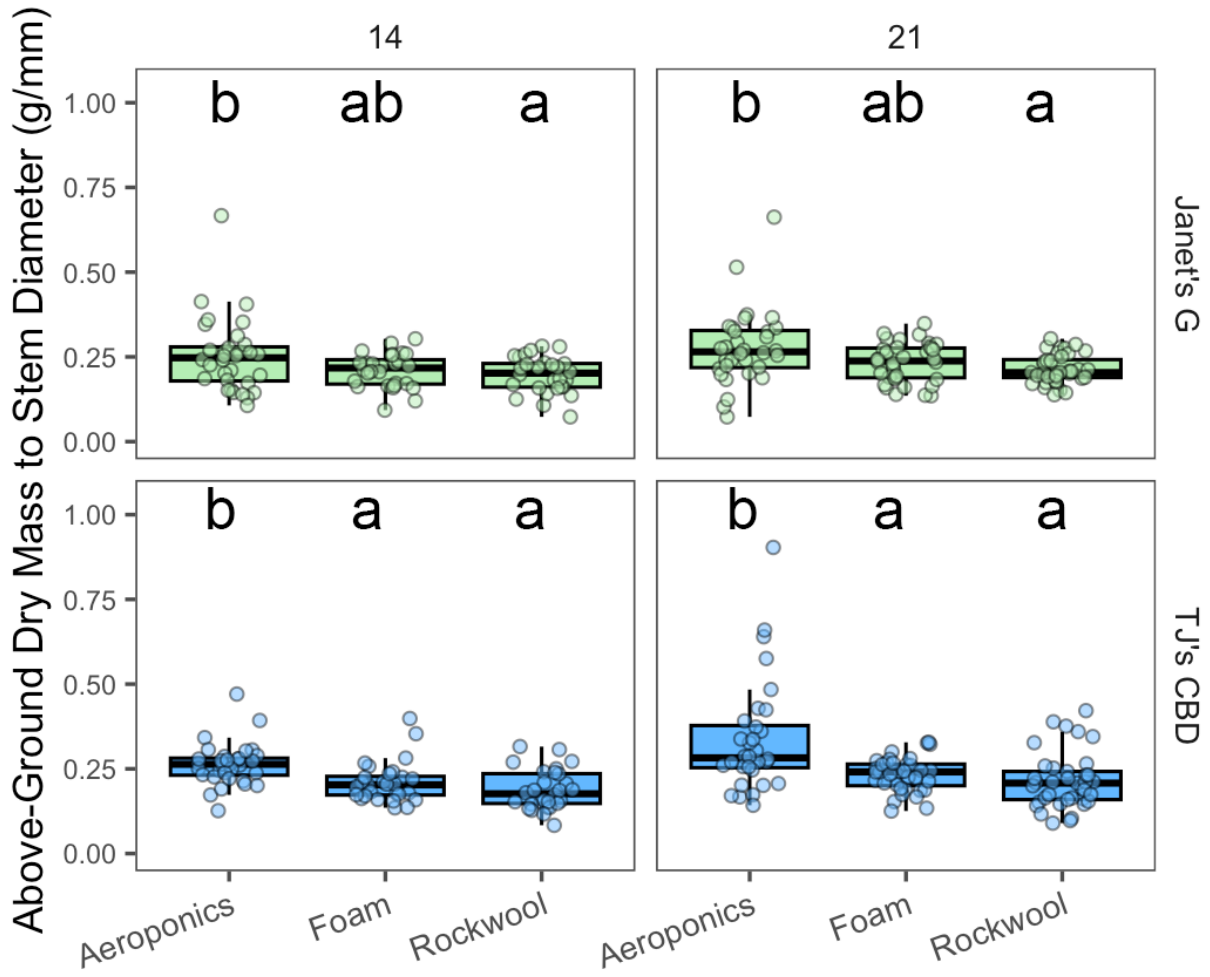

Figure 5: Above-ground biomass to stem diameter (g/mm) was compared across propagation systems aeroponics, rockwool, and (horticultural) foam using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing below-ground masses by stem diameter. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Below Ground Dry Mass

Below-ground dry mass to stem diameter was impacted by propagation system ($\chi^2 = 84.95$, df = 2, $P < 0.001$) and cultivar ($\chi^2 = 23.72$, df = 1, $P < 0.001$). Propagation system was shown to have interactions between sampling day ($\chi^2 = 37.7$, df = 2, $P < 0.001$) and cultivar ($\chi^2 = 10.81$, df = 2, $P < 0.005$). On day 14 for TJ's CBD, aeroponics had greater below-ground dry masses compared to horticulture foam but not rockwool while Janet's G showed no differences across propagation systems. On day 21, both Janet's G and TJ's CBD showed consistently higher below-ground dry masses in aeroponics compared to horticultural foam and rockwool (Figure 6).

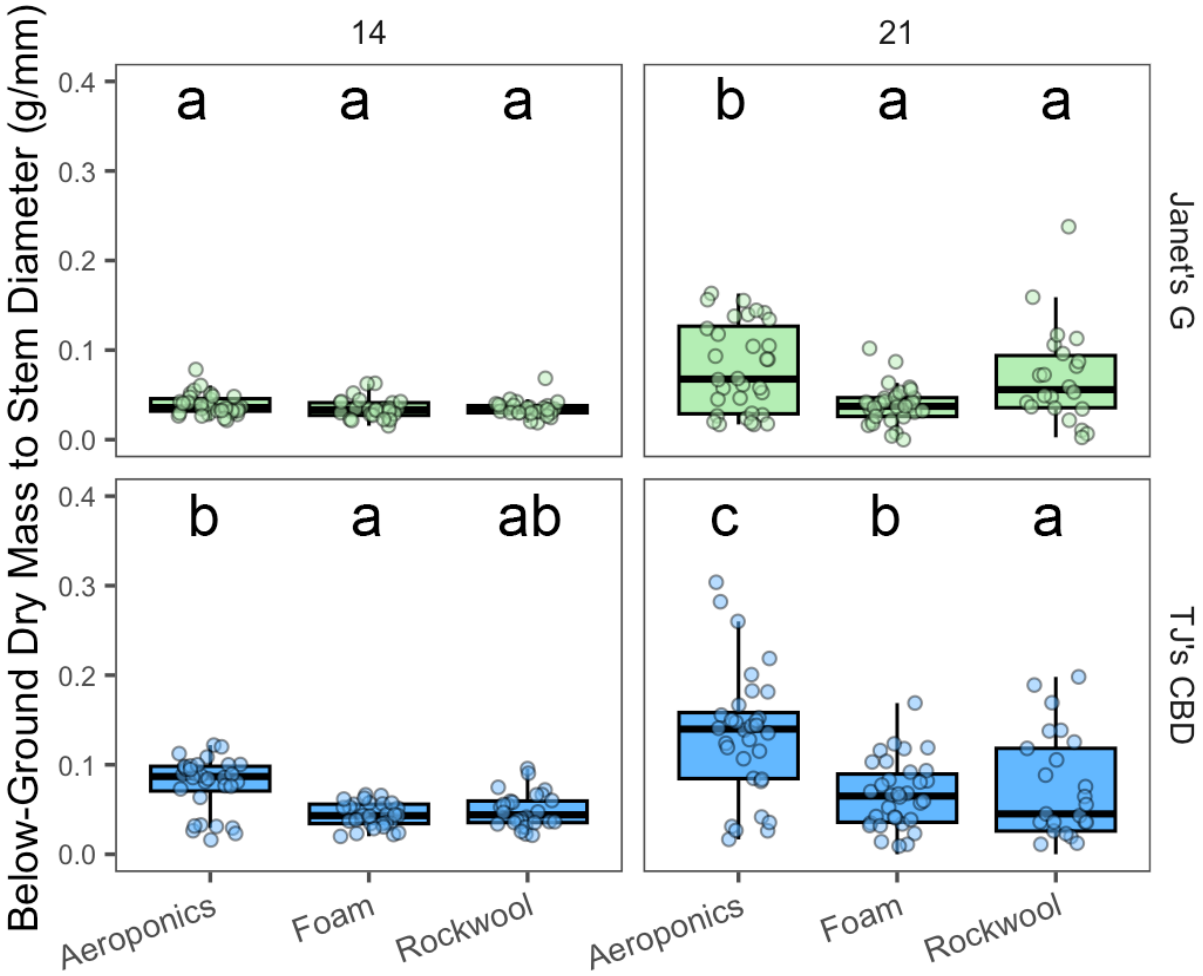

Figure 6: Below-ground biomass to stem diameter (g/mm) was compared across propagation systems aeroponics, rockwool, and (Horticultural) foam using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing below-ground masses by stem diameter. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Experiment 2: Propagation System – Transplant

Root Quality Scoring

The effect of the on root quality score varied on the cultivar ($\chi^2 = 125.36$, df = 21, $P < 0.001$) and propagation system ($\chi^2 = 23.29$, df = 2, $P < 0.001$). Interactions were shown between propagation system and cultivar ($\chi^2 = 12.43$, df = 2, $P < 0.005$) along with transplant day and cultivar ($\chi^2 = 8.46$, df = 3, $P < 0.05$). On day 14 for TJ's CBD, aeroponics had higher root scores compared to horticulture foam but not rockwool while Janet's G showed no differences across propagation systems. On day 21, both Janet's G and TJ's CBD showed consistently higher root scores in aeroponics compared to horticultural foam and rockwool (Figure 7)

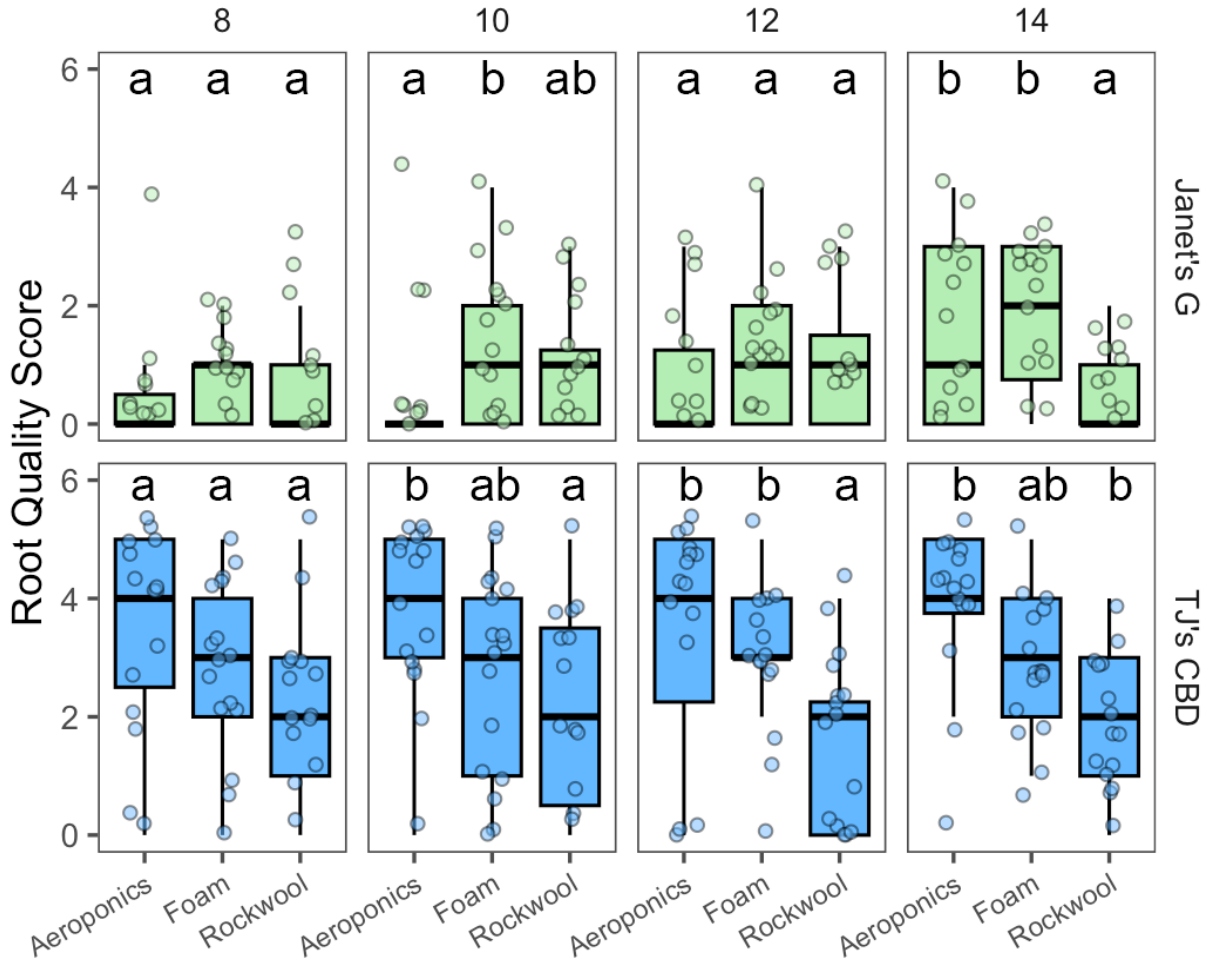

Figure 7: Root quality score (Figure 2 a-c) compared across transplanted days and propagation systems aeroponics, rockwool, and (Horticultural) foam using *Cannabis sativa* L. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Height

The effect of height varied on the cultivar ($\chi^2 = 146.1$, df = 1, $P < 0.001$), substrate ($\chi^2 = 18.62$, df = 2, $P < 0.001$) along with the interaction between cultivar and propagation system ($\chi^2 = 19.77$, df = 2, $P < 0.001$). TJ's CBD showed consistently taller plants in aeroponics compared to rockwool but only outperformed horticultural foam on transplant days 10 and 14 while Janet's G, although no clear differences among propagation systems, aeroponics had marginal height over horticultural foam on 10 transplant day (Figure 8).

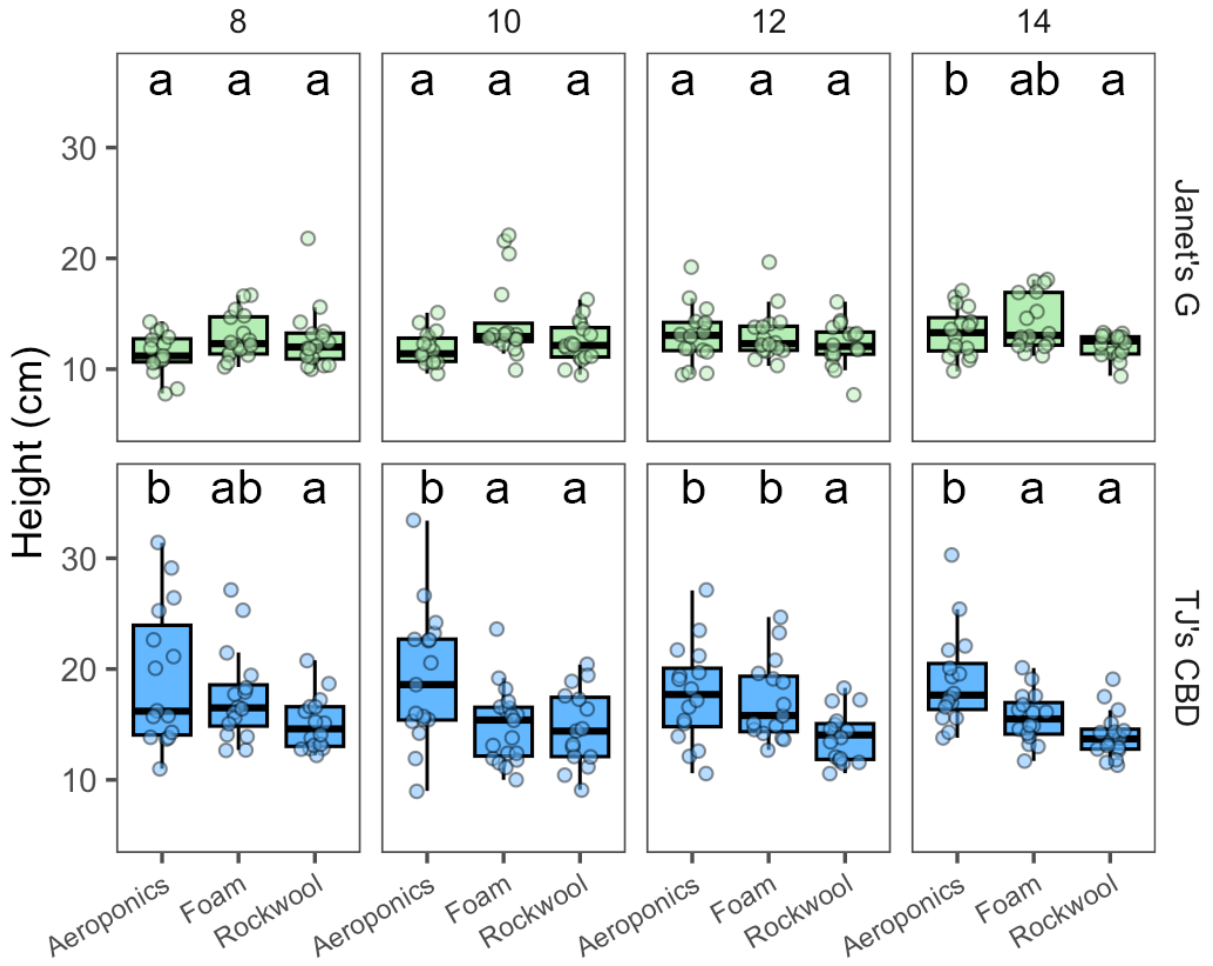

Figure 8: Height (cm) compared across transplanted days and propagation systems aeroponics, rockwool, and (Horticultural) foam using *Cannabis sativa* L. Mean separation across propagation systems is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Experiment 3: Aeroponics – Spray Interval

Root Quality Scoring

Root score varied on the cultivar ($\chi^2 = 111.83$, df = 1, $P < 0.001$), spray time interval ($\chi^2 = 32.71$, df = 3, $P < 0.001$), and sampling day ($\chi^2 = 286.65$, df = 1, $P < 0.001$). The effect of spray interval was dependent on sampling day ($\chi^2 = 9.41$, df = 3, $P < 0.05$) along with interactions between spray interval, sampling day and cultivar ($\chi^2 = 14.65$, df = 1, $P < 0.0001$). On Day 14 for both cultivars, constant spray showed elevated root score over 1 min on : 9 min off time interval (Figure 9). In addition, on day 14 for TJ's CBD, higher root scores were observed between different time intervals (1:1 vs. 1:9, and 1:3 vs. 1:9) (Figure 9). On sampling day 21 for TJ's cultivar, continuous spray outperformed 1:9 and marginally outperformed 1:3 time interval (Figure 9).

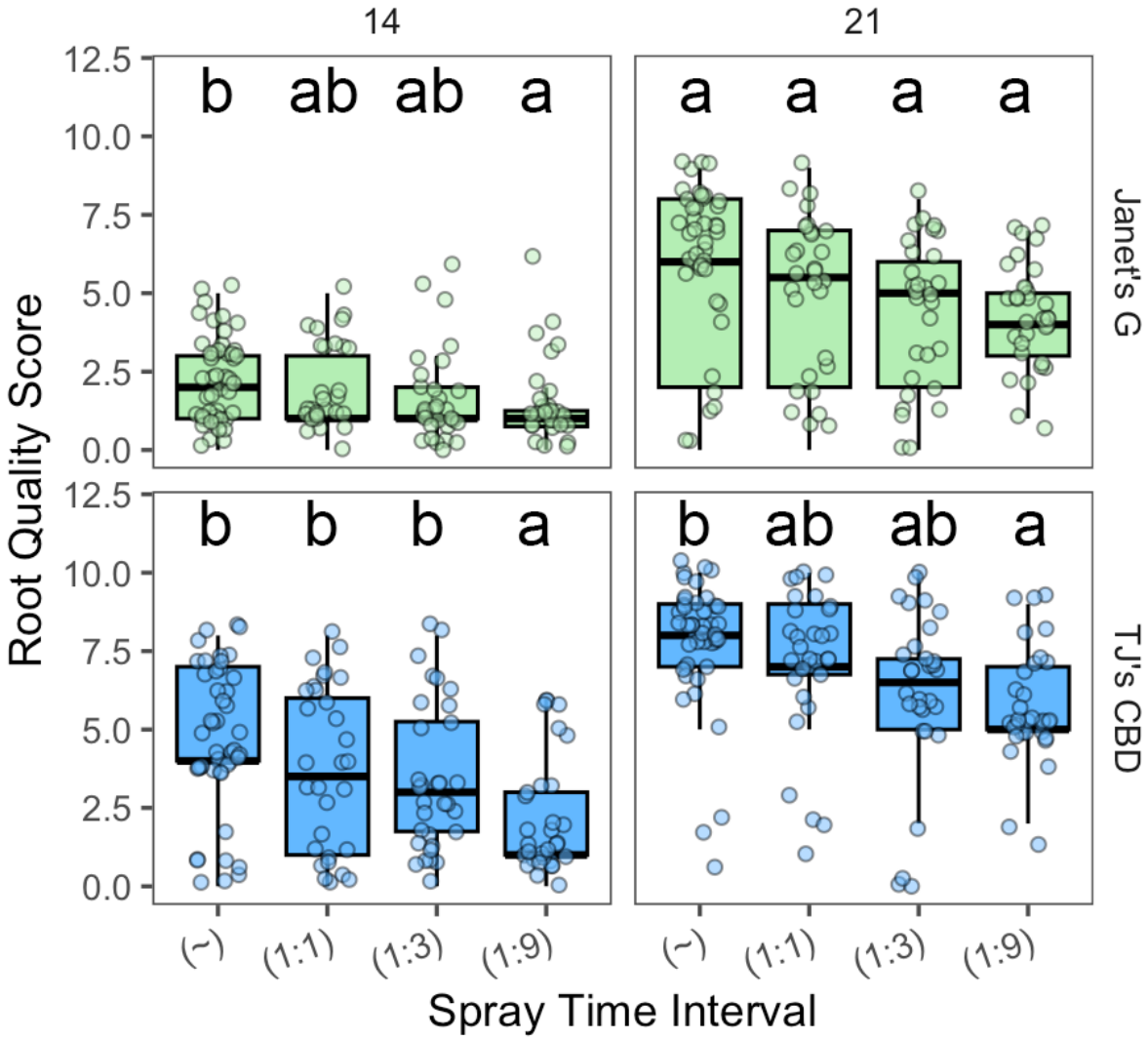

Figure 9: Root quality score (Figure 1) compared across spray time intervals (min) in aeroponics systems using *Cannabis sativa* L. Mean separation across spray time intervals is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Height

The effect of spray interval on the height varied on the spray interval ($\chi^2 = 15.26$, df = 3, $P <$ 0.001), cultivar ($\chi^2 = 105.38$, df = 1, $P < 0.001$) and sampling day ($\chi^2 = 129.34$, df = 1, $P <$

0.001). Interactions were seen between sampling day and spray interval ($\chi^2 = 27.77$, df = 3, $P < 0.001$) along with sampling day and cultivar ($\chi^2 = 31.13$, df = 1, $P < 0.001$). Although no clear differences in height were observed on day 14. On day 21 continuous spray displayed much taller plants than (1 min on : 9 min off) for both cultivars. On day 21 for Janet's G, continuous spray had marginally taller plants compared to (1:3). Day 21 for TJ's CBD, with an interaction of , both Aeroponics (1:1) and Aeroponics (1:3) also exhibited taller plants compared to Aeroponics (1:9) (Figure 10).

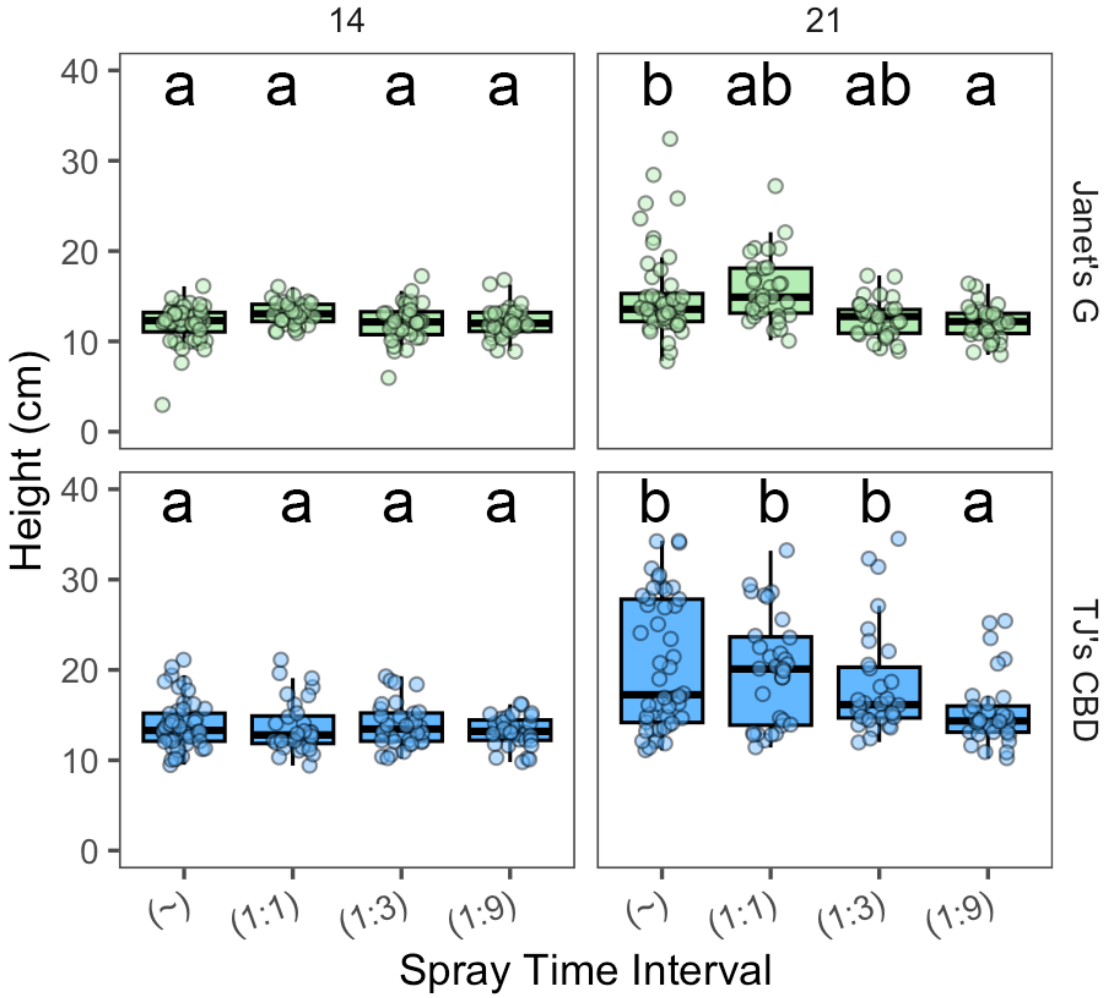

Figure 10: Height (cm) compared across spray time intervals (min) in aeroponic systems using *Cannabis sativa* L. Mean separation across spray time intervals is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Above Ground Dry Mass

The effect of the spray time interval on the above-ground dry mass to stem diameter depended on the choice of spray interval ($\chi^2 = 22.49$, df = 3, $P < 0.001$) and sampling day ($\chi^2 = 21.14$, df = 1, $P < 0.001$). Sampling day interactions were seen between spray time ($\chi^2 = 18.16$, df = 3, $P < 0.0005$) along with cultivar ($\chi^2 = 26.21$, df = 1, $P < 0.001$). On day 14 for Janet's G, both

continuous spray and (1 min on: 1 min off) displayed larger dry masses than (1:3). Day 21 for TJ's, continuous spray unanimously performed better than all other spray intervals (Figure 11),

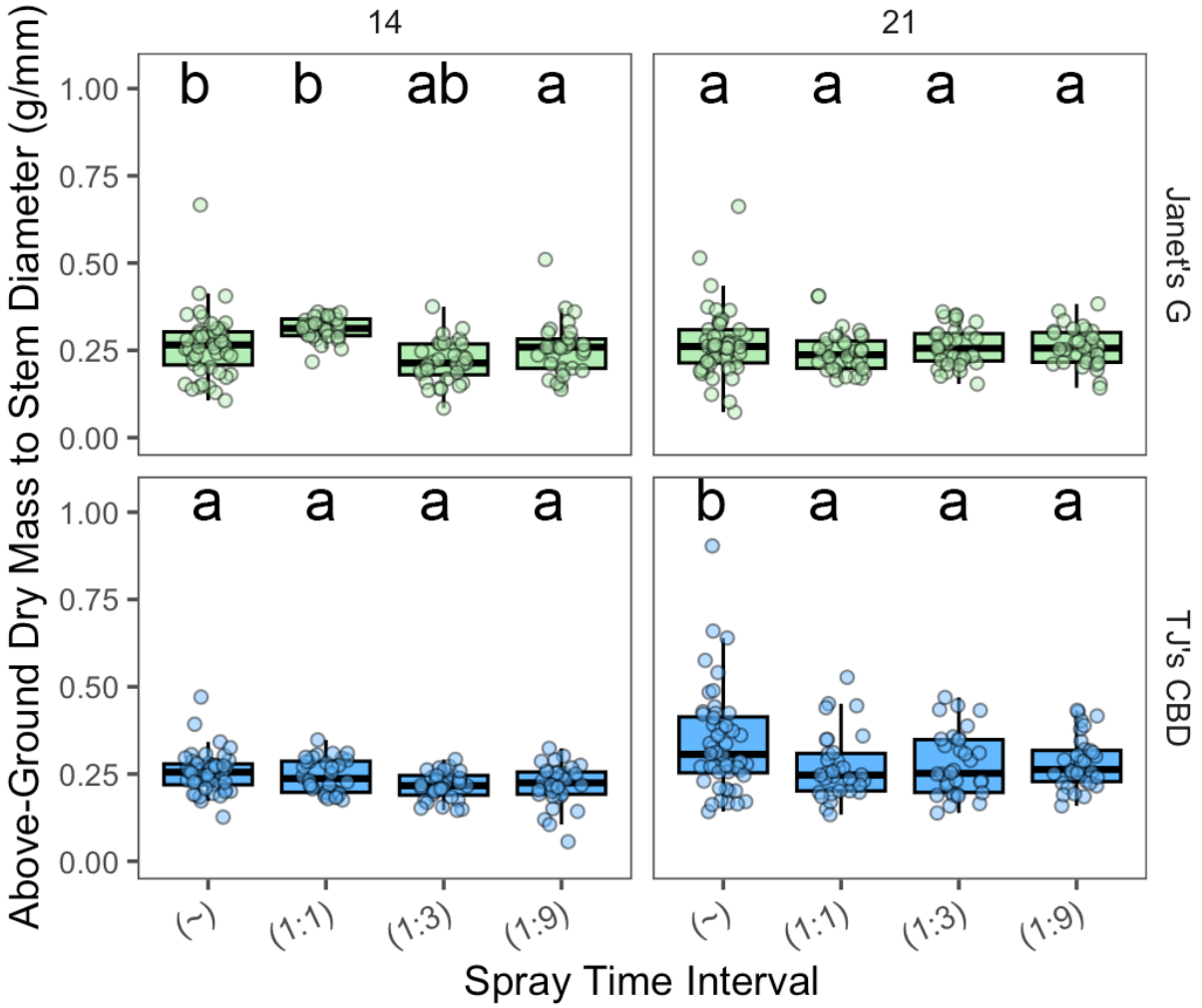

Figure 11: Above-ground biomass to stem diameter (g/mm) was compared across spray time intervals in aeroponic systems using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing below-ground masses by stem diameter. Mean separation across spray time intervals is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Root Dry Mass

The effect of the spray time interval on below-ground dry mass to stem diameter depended on the choice of spray interval ($\chi^2 = 32.52$, df = 3, $P < 0.001$), sampling day ($\chi^2 = 158.06$, df = 1, $P < 0.001$) and cultivar ($\chi^2 = 108.82$, df = 1, $P < 0.001$). The effect of spray intervals depended on sampling day ($\chi^2 = 13.79$, df = 3, $P < 0.005$) and cultivar ($\chi^2 = 12.7$, df = 3, $P < 0.005$) along with sampling days interaction with cultivar ($\chi^2 = 18.52$, df = 1, $P < 0.001$). On day 14 for Janet's G, aeroponics (1 min on : 1 min off) exhibited higher dry root mass than (1:3) (Figure 3.3d). On sampling day 21, (continuous) showcased marginally heavier dry root masses than (1:3) (Figure

12). TJ's CBD on both days clearly revealed that spray continuous resulted in larger dry root masses compared to Aeroponics (1:9) and (1:3) (Figure 12), additionally on day 21 continuous spray demonstrated heavier roots than (1:1) (Figure 12).

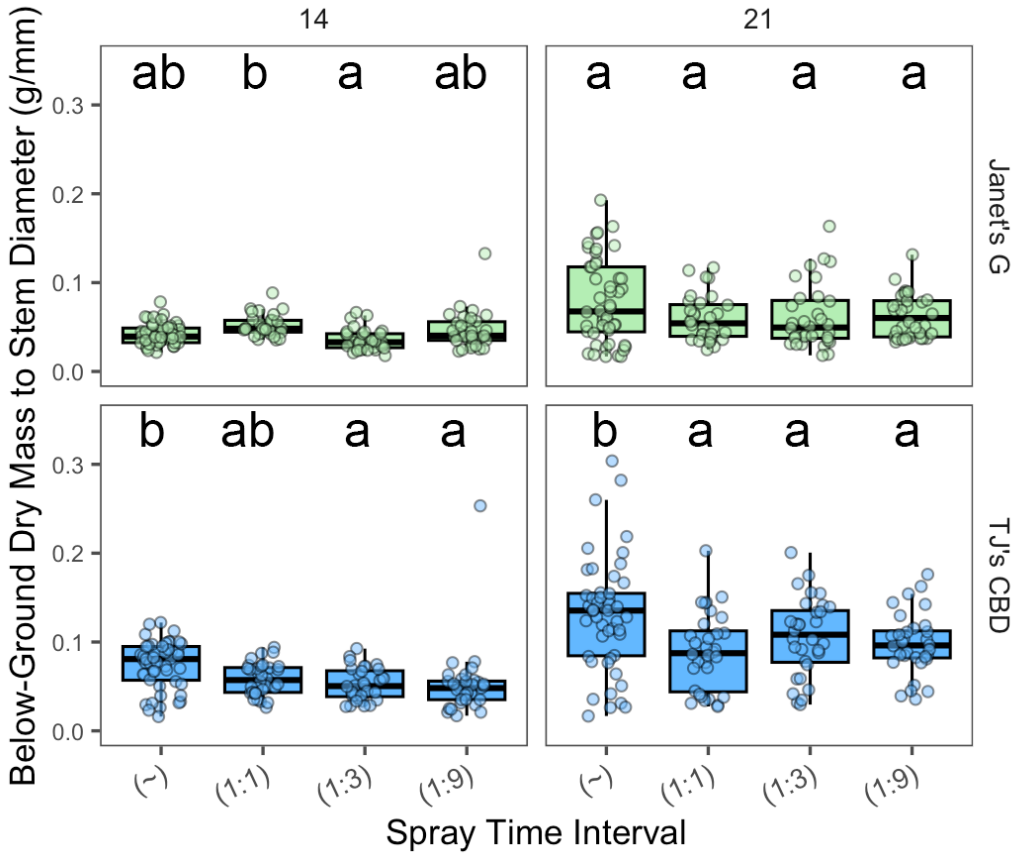

Figure 12: Below-ground biomass to stem diameter (g/mm) was compared across spray time intervals in aeroponic systems using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing below-ground masses by stem diameter. Mean separation across spray time intervals is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Experiment 4: Aeroponics – Fertigation Dilutions

Root Quality Scoring

The effect of fertigation EC concentration on root quality score varied on the concentration ($\chi^2 = 21.17$, df = 1, $P < 0.001$), sampling day ($\chi^2 = 126.32$, df = 1, $P < 0.001$) and cultivar ($\chi^2 = 193.65$, df = 1, $P < 0.001$). The effect of sampling day was dependent on cultivar ($\chi^2 = 11.23$, df = 1, $P < 0.001$). While Janet's G had no clear differences among EC concentrations (Figure 13), TJ's CBD, for both sampling days, demonstrated EC 1.4 with consistently higher root scores compared to both 1 EC and 0.7 EC, with marginally better scores on day 21 to 1 EC (Figure 13).

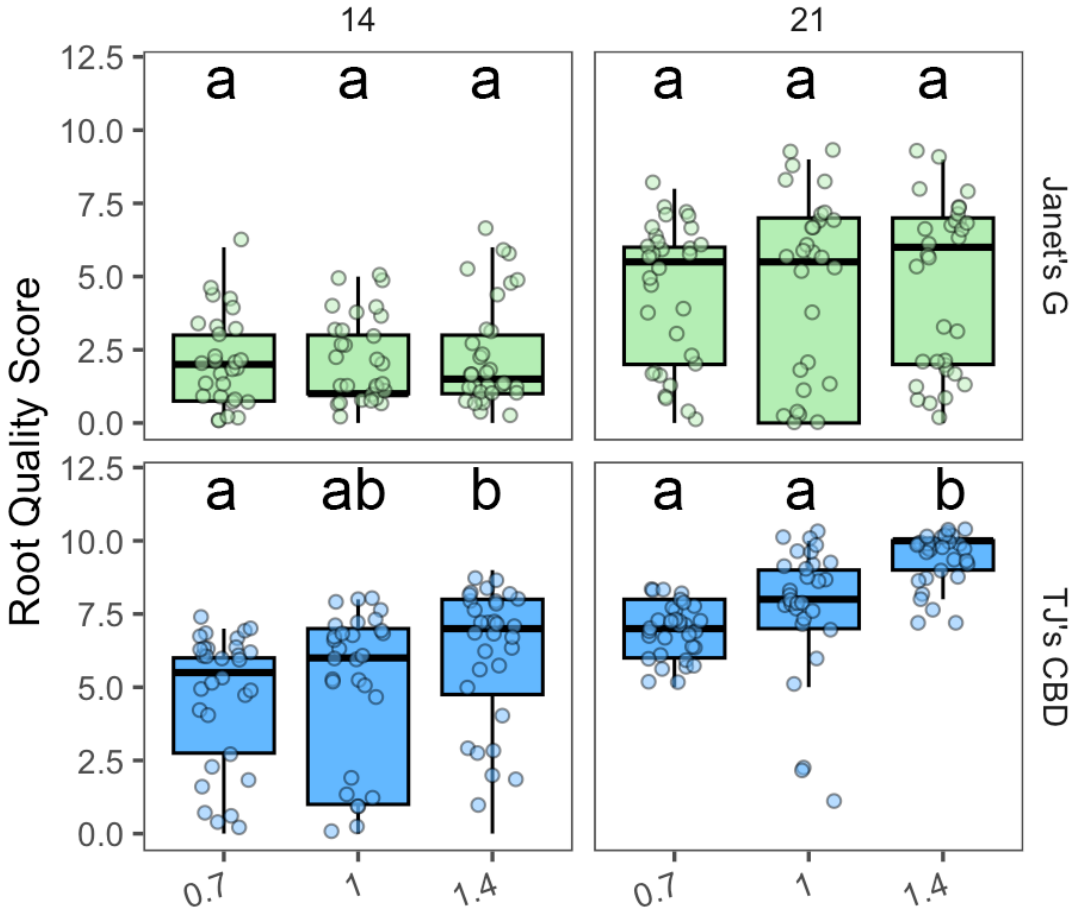

Figure 13: Root quality score (Figure 1) compared across fertilizer concentrations (EC) in aeroponic systems using *Cannabis sativa* L. Mean separation across fertilizer concentrations (EC) is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Height

The effect of fertigation EC concentration on the height varied on the EC concentration (χ^2 = 108.19, df = 2, $P < 0.001$), sampling day (χ^2 = 189.38, df = 1, $P < 0.001$) and cultivar (χ^2 = 184.39, df = 1, $P < 0.001$). The effect of EC concentration was dependent on sampling day (χ^2 = 35.31, df = 2, $P < 0.001$), cultivar (χ^2 = 39.47, df = 2, $P < 0.001$), along with sampling day and cultivar(χ^2 = 10.14, df = 2, $P < 0.05$). In addition, interactions were shown between sampling day and cultivar (χ^2 = 53.15, df = 1, $P < 0.001$). For day 21 Janet's G, and both TJ's CBD sampling days, 1.4 EC showed significant top growth compared to both 1 EC and 0.7 EC treatments (Figure 14). TJ's CBD on day 21 also showed taller plants for 1 EC than 0.7 EC (Figure 14).

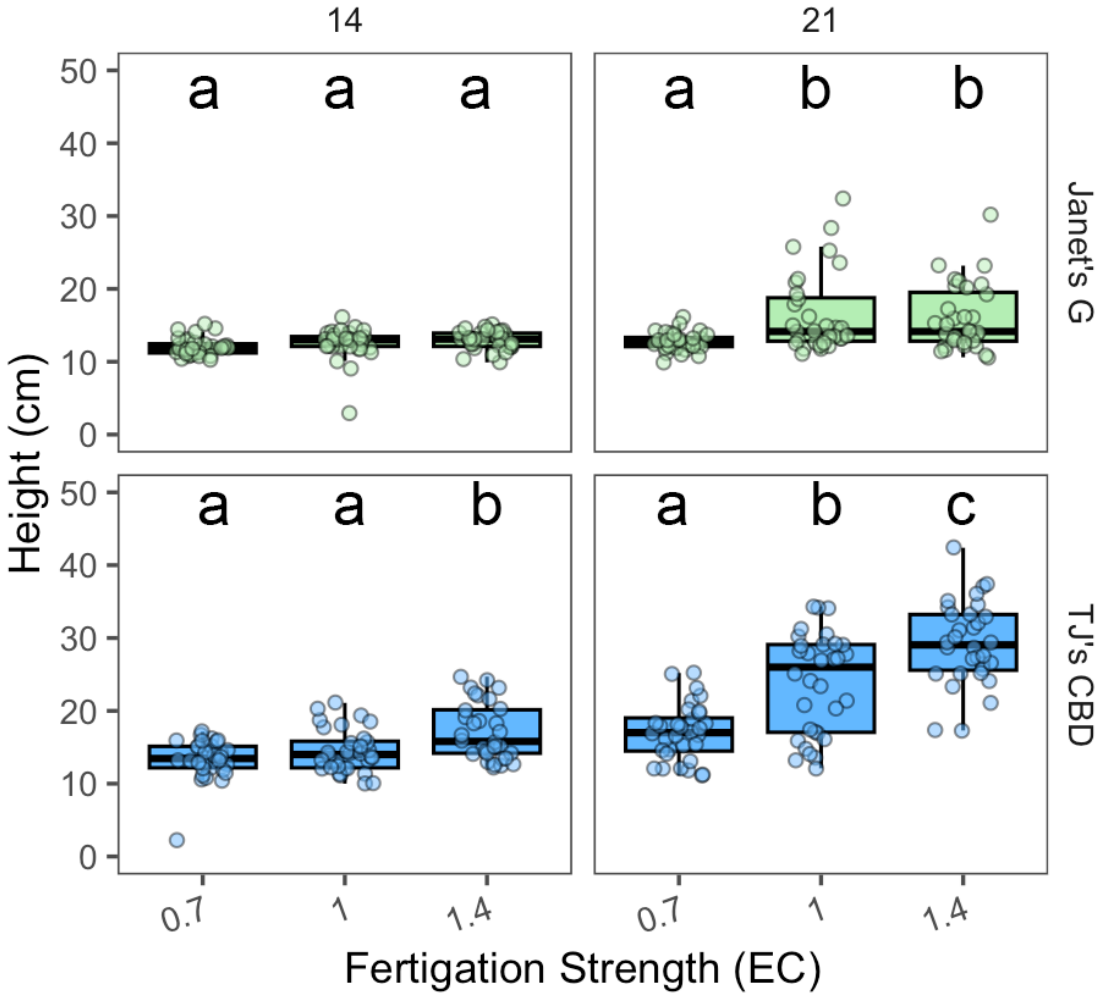

Figure 14: Height (cm) compared across fertilizer concentrations (EC) in aeroponic systems using *Cannabis sativa* L. Mean separation across fertilizer concentrations (EC) is indicated by letters using Tukey's Honest Significant Difference at P < 0.05.

Above Ground Dry Mass

Above-ground dry mass to stem diameter varied based on the choice of EC concentration (χ^2 = 33.68, df = 2, $P < 0.001$), sampling day (χ^2 = 47.3, df = 1, $P < 0.001$) and cultivar (χ^2 = 14.31, df = 1, $P < 0.001$). EC concentrations were shown to be dependent on cultivar (χ^2 = 11.05, df = 2, $P < 0.005$), sampling day (χ^2 = 9.13, df = 2, $P < 0.01$), along with cultivar and sampling day (χ^2 = 9.47, df = 2, $P < 0.01$). In addition, cultivar had effects with sampling day (χ^2 = 45.54, df = 1, $P < 0.001$). Although no clear differences were observed on day 14, TJ's CBD on day 21 showed heavier masses in both 1.4 and 1 EC compared to 0.7 EC (Figure 15).

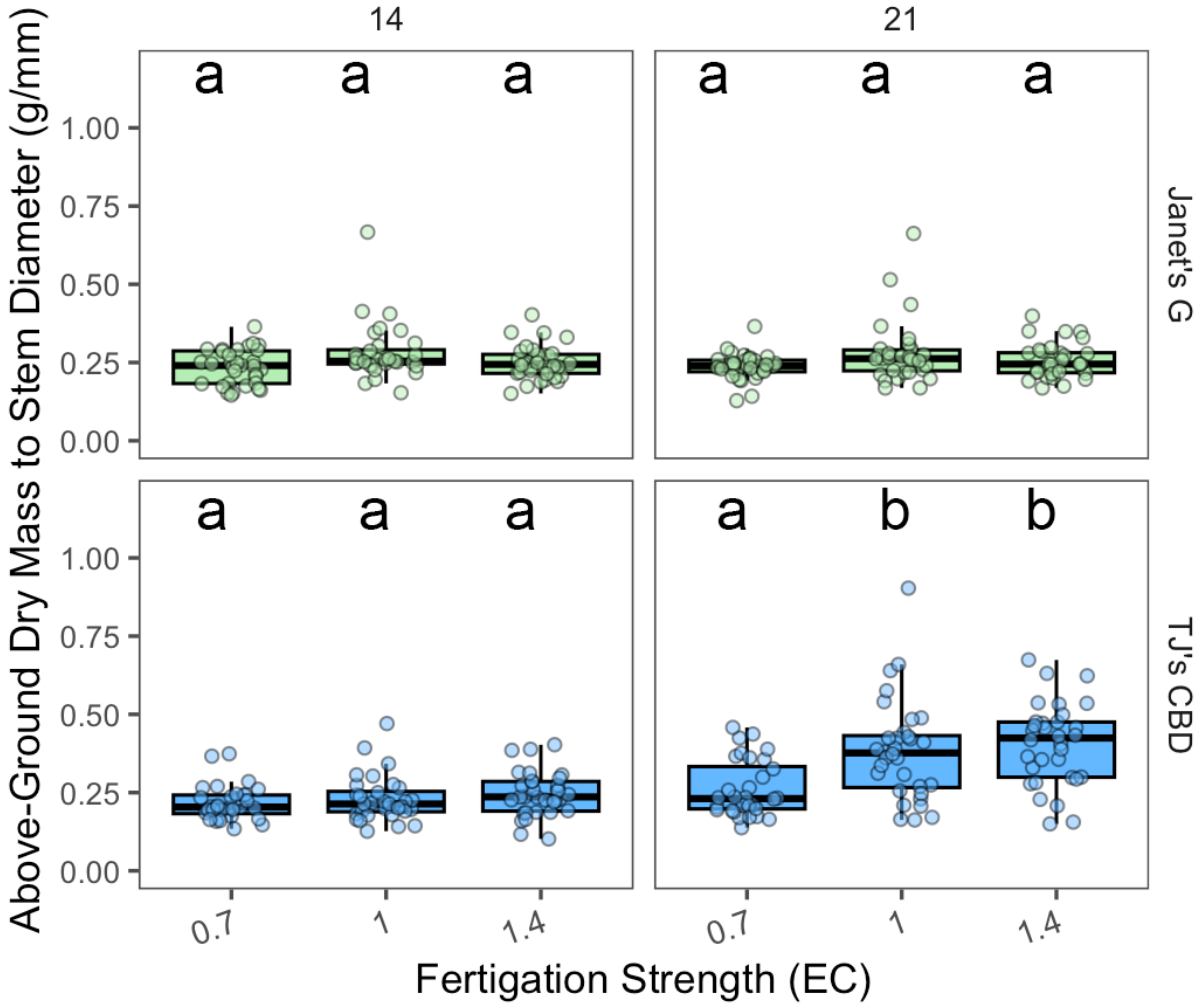

Figure 15: Above-ground biomass to stem diameter (g/mm) was compared across fertilizer concentrations (EC) in aeroponic systems using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing both above and below-ground masses by stem diameter. Mean separation across fertilizer concentrations (EC) is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

Root Dry Mass

Below-ground dry mass to stem diameter varied depending on EC concentration ($\chi^2 = 36.54$, df = 2, $P < 0.001$), sampling day ($\chi^2 = 145$, df = 1, $P < 0.001$) and cultivar ($\chi^2 = 166.76$, df = 1, $P < 0.001$). The effect of EC concentration was dependent on sampling day ($\chi^2 = 12.71$, df = 2, $P < 0.005$), cultivar ($\chi^2 = 11.71$, df = 2, $P < 0.005$), along with sampling day and cultivar ($\chi^2 = 5.92$, df = 2, $P < 0.005$). In addition to interactions between sampling day and cultivar ($\chi^2 = 37.41$, df = 1, $P < 0.001$). Although no clear trend was witnessed for Janet's G, TJ's CBD for day 21 showed 1.4 EC with consistently heavier masses than both 1 EC and 0.7 EC (Figure 3.4d), 1 EC also displayed larger dry root mass than 0.7 EC (Figure 16).

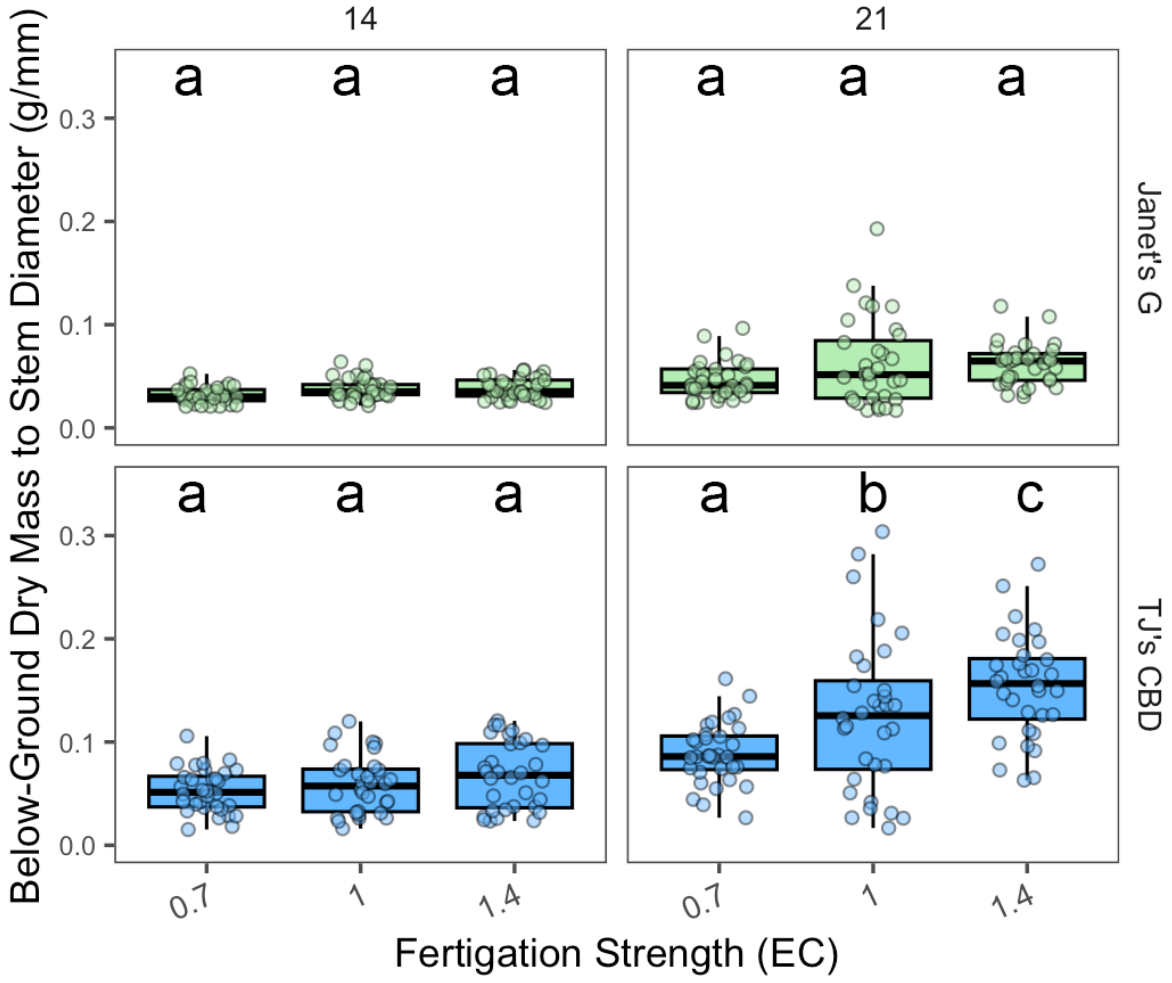

Figure 16: Below-ground biomass to stem diameter (g/mm) was compared across fertilizer concentrations (EC) in aeroponic systems using *Cannabis sativa* L. Stem diameter was taken into account in mass measurements by dividing both above and below-ground masses by stem diameter. Mean separation across fertilizer concentrations (EC) is indicated by letters using Tukey's Honest Significant Difference at $P < 0.05$.

DISCUSSION

As global demand for *Cannabis* products continues to rise, cultivators are pressed to scale production while mitigating evolving regulatory standards. The role of effective cultivation practices are necessary, as the quality and uniformity of plant propagation can dictate the success of an entire crop. Among emerging solutions, soilless propagation methods stand out, offering the potential to produce *Cannabis* plants with limited genetic variation and efficient growth profiles, qualities indispensable in today's competitive and tightly regulated market. While commonly used materials like rockwool and petroleum-based horticultural foam are effective, they raise concerns due to their resource-intensive production processes and single-use nature (Nemati et al., 2021; Robertson et al., 2023). As an alternative, this research evaluates aeroponics propagation and its impact on *Cannabis* growth and development under varied conditions. When comparing aeroponics to traditional soilless propagation methods, this study presents compelling evidence that aeroponics can yield equal or superior root development, plant growth and transplant success. These experiments show aeroponics offers a conservation sensitive alternative to resource-intensive substrates. Furthermore, the investigation reveals the effective aeroponics use of continuous spray intervals and the optimized nutrient concentrations in promoting root and overall plant growth.

Experiment 1: Propagation System

The findings of Experiment 1 investigated key distinctions between different propagation substrates and their impact on root development, plant height, above-ground dry mass, and root dry mass. Notably, the aeroponic propagation method performed as well or in some cases better than both horticultural foam and rockwool in terms of promoting root score, plant height, and both above-ground and root dry mass. This trend of aeroponics can be attributed to its efficient nutrient and advantageous oxygen delivery (Soffer et al., 1988), leading to enhanced root development and overall plant growth (Yafuso et al., 2019). These results align with previous studies that highlight the benefits of aeroponics in promoting rapid, robust root growth, and characterized as a more sustainable approach (Eldridge et al., 2020; Ferrini et al., 2021; Robertson et al., 2023).

Experiment 2: Propagation System – Transplant

Transplant success was influenced by substrate choice and cultivar selection, with aeroponics showing the greatest effect in enhancing transplant outcomes. Transplant day itself did not exhibit significant effects on root development, the choice of substrate and cultivar significantly influenced root scores. Aeroponics performed as effective if not more than both horticultural foam and rockwool with higher root scores indicating its potential in enhancing transplant success and reduced transplant shock. Aligning with Experiment 1's results and literature, these findings underscore the importance of selecting the right substrate and cultivar to achieve optimal transplant outcomes (Hinesley, et al., 1985; Kumari et al., 2019).

Experiment 3: Aeroponics – Spray Interval

The influence of aeroponic spray intervals on root development, plant height, above-ground dry mass, and root dry mass is undeniable. Extended dry intervals might compromise the necessary

hydration (Weathers et al.,1992) required during the early propagation stages in addition to limiting nutrient availability as roots are established. In contrast, aeroponic systems utilizing continuous spraying or 1:1 timer intervals generally produced equivalent or superior outcomes when compared to other timing intervals and fertigation methods. These results identified the importance of maintaining regular and consistent water spraying intervals to encourage root initiation and plant growth (Tunio et al., 2022).

Experiment 4: Aeroponics – Fertigation Dilutions

It's widely acknowledged that different growth stages of the same species can have distinct nutrient needs. When these demands are met, plant performance is enhanced (Raviv and Lieth, 2007; Wang, 2000). This is evident in cannabis, which has shown an ability to not only accept but, in some instances, benefit from higher growth nutrient concentrations. A 1.4 EC ratio often produced results that were as if not better in terms of root development and height when compared to other dilutions. Increased nutrition in the early stages might not only be acceptable but could be advantageous when properly managed (Abdou et al. 2014; Wei et al., 2023).

Variations and Future Directions:

Genetic variation existed among cultivars, Tj's CBD notably having better survival rates and quicker root initiation and growth (Campbell et al., 2021). Additionally, temperature fluctuations within the greenhouse along with the greenhouse effect, could be expected to impact both the microclimate in the dark colored reservoir along with the clear plastic clone domes. This was particularly noted in Experiment 1 Substrate and Experiment 3 Timer Trial 1's, 4/12 - 5/2/2023. This experiment occurred farther into summer when there were warmer outdoor temperatures. The remainder of the trials and experiments benefited from climate control having AC on and auto shade covered. This highlights the impact of temperature (Eldridge et al., 2020) regulation on root growth. Specifically, improved root development was observed when air conditioning and greenhouse shading were utilized. This could be seen as a decrease in root meristematic speed and initiation due to a heat stress response as shown in previous research (Gonzalez-Garcia et al., 2022; Lynch et al., 2012).

There are many variables which can be studied to optimize Cannabis growth and productivity. Future research directions could focus on further investigating optimizing factors and reducing destructive factors. Research potential exists in studying a broader range EC concentrations. Comparative studies could also be conducted to assess vegetative propagated root zone temperature preferences in *Cannabis*, or utilizing different varieties of cultivars.

CONCLUSION

The results of these experiments collectively contribute to the growing body of knowledge on optimizing cultivation techniques for *Cannabis* plants. These results shed light on the profound effects of various soilless propagation methods, transplant timings, aeroponic spray intervals, and electrical conductivity concentrations on growth attributes.Transplant success was influenced by substrate choice and cultivar selection, with aeroponics showing the greatest effect in enhancing transplant outcomes versus horticultural foam or rockwool. In aeroponics systems it was identified that the use of continuous spraying obtained maximal plant root initiation and overall growth. Optimized electrical conductivity ratios proved to positively impact root development and height. By considering the most suitable propagation systems and, in the case of aeroponics, spray time intervals and fertigation ratios, cultivators can use these findings to elevate proficiency, precision, and yield. Strategic selection of the most effective propagation systems and, in the case of aeroponics, the ideal spray intervals and fertigation concentrations, growers benefit. As the industry continues to expand and evolve, the cultivation of premium and consistent *Cannabis* products will be paramount. The findings of these presented experiments contribute to an expanding, robust knowledge foundation for future agricultural practices in the realm of *Cannabis* cultivation, thus ensuring the industry's continued commitment to quality control, sustainability, growth and success.

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