ABSTRACT

HUMPHREY, SAM. Controlled Environment Strawberry Propagation: CO_2, Light Intensity, and Daughter Plant Rooting Experiments. (Under the direction of Dr. Ricardo Hernández).

The US strawberry industry has a market value of over 2 billion USD and produces over a million tons of strawberries every year. This fruit production industry depends on strawberry nurseries to annually provide new plant material. However, the conventional strawberry nursery system is plagued with many challenges and threats, including low availability and increasing costs of labor, increasing regulations on pesticides, and the high risk of disease. Controlled environment agriculture (CEA) may be a solution to these many challenges. The environmental manipulation and optimization of CEA could produce many more strawberry daughter plants per mother plant, compared to conventional systems. But can CEA be an economically feasible solution?

We investigated the effects of CO_2 enrichment and light intensity on the mother plants, comparing overall growth rate and the number of daughter plants grown per mother. Walk-in growth chambers at the NCSU Phytotron contained three CO_2 treatments: 500, 850, and 1200 μmol mol^{-1}, and two treatments of light intensity: 250 and 500 μmol m^{-2} s^{-1}. We found that a greater CO_2 concentration could increase the daughter plant yield by as much as 48%, and greater light intensity could increase the yield by as much as 28%. The economic analysis indicates that it may be economical to produce daughter plants indoors, even with the ongoing costs of electricity and CO_2 consumption. These results indicate that controlled environments with elevated CO_2 concentration and light intensity are able to produce many more daughter plants than field conditions.

In a second experiment, we investigated the effect of daughter plant size. In greenhouses and controlled environments, strawberry mother plants produce daughter plants in a range of sizes, with a few daughters that are very large, a few that are medium sized, and many daughters that are

small or very small. The objective of this study was to investigate whether the small and very small daughters can successfully root and grow, and to quantify the growth rate differences between daughters of different sizes. We collected a large number of daughter plants, and split them into size categories based on their number of “peg roots”, then rooted them in a growth chamber for 28 days. We found that the small and medium plants had the best rooting rate and survival rate, while the very small and large plants had a slightly lower rate of success by day 28. Overall, the rooting and survival rate was higher than expected, and indicated that the majority of plants of all sizes (90%+ overall) can be successfully rooted and sold. Taken together, these two experiments provide evidence that CEA can produce usable strawberry daughter plants more efficiently than in the field, especially when CO_2 is elevated, light intensity is increased, and daughter plants of all sizes are harvested.

Controlled Environment Strawberry Propagation: CO_2, Light Intensity, and Daughter Plant Rooting Experiments.

by
Sam Humphrey

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Horticultural Science

Raleigh, North Carolina
2024

APPROVED BY:

_____	_____
Dr Ricardo Hernández Committee Chair	Dr. Mark Hoffmann
_____	
Dr. Daniel Tregeagle	

BIOGRAPHY

Samson Humphrey was born in the suburbs of Orlando, Florida, where his fascination with dinosaurs and wild animals led him to join the Veterinary Science FFA program in high school. However, when his agriculture teacher Tim App encouraged him to compete in the forestry competition he fell in love with plant science. With encouragement from Tim App and Caela Paioff, and endless support from Valerie Lantigua, he pursued college degrees in Plant Science, from Santa Fe Community College (AA) and the University of Florida (BS). At Santa Fe, Sam's advisor Bobby Hom encouraged and supported Sam's entries into research paper writing competitions, where Sam became enamored with science writing. At the University of Florida, Dr. James Estrada taught Sam's first plant physiology class, leading Sam down the path of becoming a plant physiologist. Sam had multiple chemistry and engineering projects in his undergraduate years, and these projects could not have been successful without the support and teamwork of many of his peers, including Wenbo Peng, Megan Wnek, Tyler Less, Brooke Gaster, Shane Lovello, Emerick Larkin, Jacob Mass, Jacob York, Oren Anderson, Seann Romero, Stephen Lantin, Kai Rasmussen, Luke Concollato, Dr. Rafael Loureiro, and Dr. Colette Jacono. Additionally, Sam's undergraduate research at would not have been possible without the opportunities and support given to him by his PIs, Dr. Edward Phlips, Dr. Stephen Enloe, Dr. Diane Rowland, and Dr. Celina Gómez. These people all provided opportunities, wisdom, kindness, and generosity necessary for Sam's success in all his future endeavors.

During this master's degree, Sam had the support of many more researchers, and teammates, including members of the PIP-CAP Strawberry Team, his *Plants People Science* podcast team, and too many friends to count. After graduation, Sam will continue his career in plant physiology by joining Dr. Kellie Walters' Lab at the University of Tennessee Knoxville.

ACKNOWLEDGMENTS

Thank you to the USDA-NIFA Specialty Crop Research Initiative (SCRI) (award nr: 2021-51181-35857) for funding this research, which made this important work possible. Thank you to HortAmericas for their generous donation of the light fixtures used for this experiment.

Thank you to the members of the PIP-CAP Strawberry Grant, and other scientists I collaborated with, especially Dr. Mark Hoffmann, Dr. Celina Gómez, Dr. Chieri Kubota, Dr. Jennifer Boldt, Dr. Peter Nitzsche, Dr. Heidi Schweitzer, Dr. Melanie Yelton, and Dr. Gerald Holmes for their support and guidance. Thank you to Morgan Frankel and Adam Tripp at Plenty Vertical Farm for their support and wonderful research discussions.

And thank you to all my teammates and friends who helped make this experiment possible, including S. Partin Thompson, Cristian Collado, Moein Moosavi-Nezhad, Victor Meras Marquez, Cristiane da Silva, Sofia Ruiz Vega, Emma Volk, Xiaonan Shi, Michael Palmer, Elissa Boster, Eshwar Ravishankar, Ibraheem Olasupo, Jerry Yu, Joy Johnson, Morgan Moran, David Dupree, Chuck Gibbs, and Jacob Stuart. Their help and labor were crucial to this work, and their company helped me overcome the most difficult moments of my masters.

Lastly, thank you to Dr. Ricardo Hernández for your advice, guidance, and opportunities to succeed. I chose to attend NCSU because of Dr. Hernández, and I’m so happy I did.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1: Controlled Environment Strawberry (*Fragaria ×ananassa* cv. Monterey) Propagation Produces More Daughter Plants Under Elevated Carbon Dioxide Concentrations and Higher Light Intensities

Chapter 2: Rooting Efficacy of Different Size Strawberry (Fragaria ×ananassa cv. Monterey) Daughter Plants in a Controlled Environment

LIST OF FIGURES

Chapter 1: Controlled Environment Strawberry (Fragaria ×ananassa cv. Monterey) Propagation Produces More Daughter Plants Under Elevated Carbon Dioxide Concentrations and Higher Light Intensities

Chapter 2: Rooting Efficacy of Different Size Strawberry (Fragaria ×ananassa cv. Monterey) Daughter Plants in a Controlled Environment

CHAPTER 1

Controlled Environment Strawberry (*Fragaria* ×*ananassa* cv. Monterey) Propagation Produces More Daughter Plants Under Elevated Carbon Dioxide Concentrations and Higher Light Intensities

Abstract

Strawberry nurseries are facing unprecedented challenges of disease risk, fumigation regulations, and labor availability, so nursery managers are considering alternative methods for propagation. Controlled environment (CE) agriculture is a potential alternative to address those challenges. One limiting factor to improve the economic feasibility of CE propagation is the daughter plant yield: the total number of daughter plants produced per mother plant. The present research investigates the effect of CO_2 enrichment (500, 850, and 1200 µmol mol^{-1}) and daily light integral (14.4 mol m^{-2} d^{-1} and 28.8 mol m^{-2} d^{-1} DLI) on strawberry mother plant growth, daughter plant production, morphology, and development. Strawberry (*Fragaria ×ananassa* Duch. cv. Monterey) mother plants were grown in three controlled environment growth chambers for 70 days under combinations of CO_2 and light intensity treatments under 26°C, 65% relative humidity, and a 16-hour photoperiod. On average, plants under 500 PPFD (photosynthetic photon flux density) produced 18% more daughters than plants under 250 PPFD. Furthermore, the increase in CO_2 concentration linearly increased the production of daughter plants. Under a CO_2 concentration of 1200 µmol mol^{-1}, there were 40% more daughter plants than under 500 µmol mol^{-1} (60 compared to 84 daughter plants). The combined increase of CO_2 and light (28.8 mol m^{-2} d^{-1} DLI and 1200 µmol mol^{-1}) increased daughter plant production by 70% when compared to 14.4 mol m^{-2} d^{-1} DLI and 500 µmol mol^{-1} CO_2. The increase in the number of daughter plants due to the rise in CO_2 concentration is attributed to the 38% increase of stolon development under elevated CO_2 on stolon development (11.3 to 15.55). Simple economic analysis of CO_2 enrichment and electrical lighting is also presented, with a cost of CO_2 enrichment being less than $0.01, compared to the cost of electricity for electrical lighting being $0.03 to $0.06. Overall, CO_2 enrichment and increasing light intensity are methods that could be used in indoor strawberry nurseries to improve daughter plant yield.

Introduction

The Nursery Industry

The US strawberry industry produced 13.9 million tons of strawberries in 2022, with an annual market value of over three billion USD (Wu, Guan, and Whidden 2018, Samtani et al. 2019; Shi et al. 2021b; Shahbandeh 2023; Wade et al. 2024; Holmes 2024). Strawberry fruit growers all acquire their plants from a small number of strawberry nurseries (estimated 8 to 15 in total by Hoffmann, personal communication, July 10th, 2024), which are mostly located in California and Canada (Hoffmann 2020, Holmes 2024). In California alone, strawberry nurseries produce 1.5 billion plants annually (Holmes 2024). These open-field nurseries propagate strawberry plants asexually, outdoors in rows (Hoffmann 2020, Holmes 2024). In this system, a small number of mother plants are repeatedly multiplied in screenhouses and propagation fields for three to five years before the plants are shipped and sold to strawberry fruit growers. Conventional outdoor strawberry propagation is highly labor- and time-intensive, and additional challenges include difficult disease prevention, increasing costs of transportation, and logistical challenges of meeting demand for new cultivars (Hoffmann 2020).

Of those challenges, a key issue is disease prevention. With the current outdoor multi-location, multi-year nursery system, disease transmission is unavoidable. Common nursery diseases include powdery mildew (caused by Podosphaera aphanis), anthracnose fruit rot (caused by Colletotrichum acutatum) and anthracnose crown rot (caused by C. gloeosporioides) Paulus 1990, Baggio et al. 2021). Other diseases of concern include Botrytis grey mold (caused by Botrytis cinerea), Macrophomina root rot (caused by Macrophomina phaseolina), Phytophthora crown rot (caused by Phytophthora spp.), Pestalotia Fruit Rot (caused by Neopestalotiopsis spp.), and Fusarium wilt (caused by Fusarium oxysporum) (Holmes, 2024). Even though these nurseries apply chemical soil fumigants, asymptomatic carriers of plant pathogens can lead to crop loss (Samtani et al. 2019, Holmes 2024). Strawberry nurseries have repeatedly been linked to disease outbreaks on fruit farms. For example, Neopestalotiopsis spp. samples from Canada and Florida were traced back to a nursery in North Carolina (Baggio et al. 2021, Zuniga et al. 2023). Furthermore, the most reliable fumigation tools (such as methyl bromide (CH3Br), 1,3-dichloropropene (C3H4Cl2), chloropicrin (CCl3NO2)) may not always be available due to ever-evolving fumigant regulations and restrictions (Hoffmann 2020). Methyl bromide was phased out in 2005, however the California strawberry industry was awarded exemption to the full

phase-out until 2016, and methyl bromide is still being used in strawberry nurseries under exemption (Holmes 2020). Currently popular fumigant alternatives to methyl bromide and 1,3-dichloropropene products would warrant additional plant inspections, which are expensive, so there is industrywide interest in developing non-fumigated methods for strawberry propagation (Muramoto et al. 2014, Fennimore 2018, Samtani et al. 2019). The current strawberry nursery industry is operating under the threat of losing methyl bromide as soil fumigant. If this happens, the industry immediately would be in need of alternative propagation and fumigation methods that can reliably and efficiently produce large numbers of clean stock plants (Samtani et al. 2019, Baggio et al. 2021).

Controlled Environment (CE) Solutions

Controlled environment (CE) propagation could mitigate several challenges facing these conventional strawberry nurseries. Controlled environment propagation can enable farmers to produce more plants in a smaller amount of space, with fewer chemical inputs, and with less labor needed to grow the plants (Vatistas et al. 2022, Despommier 2011, X. Xu and Hernández 2020). Most importantly propagating strawberries in controlled environments could completely mitigate soil-borne pathogens and diseases (Kroggel and Kubota 2017, Stanley and Hammond 1998). Controlled environments are also space efficient, producing more plants per square meter, and they can be located closer to the final plant destination (fruiting fields), reducing land use and transportation costs (Samtani et al. 2019, Hoffmann 2020, Vatistas et al. 2022). They also provide the ability to accurately control environmental conditions and can be adjusted to the ideal conditions for strawberry propagation. For example, nurseries in North Carolina typically produce about 220,000 daughters per acre (54 daughter plants per m^2), but a greenhouse experiment almost doubled this output, yielding 104 daughter plants per m^2. Furthermore, CE experiments have yielded over 100 daughters per plant, and over 520 daughters per m^2, surpassing field production by nearly an order of magnitude (Hoffmann 2020, X. Xu and Hernández 2020, Shi, et al. 2021b).

CO_2 and Light Intensity Affects Strawberry Growth

In CEs, CO_2 can rapidly become limiting unless it is supplemented, therefore CO_2 enrichment is necessary in CE systems. Carbon dioxide enrichment can have a number of

positive effects, including increased water-use efficiency, increased photosynthetic rate, and increased growth rate (Eamus 1991, Allen Jr et al. 2011, Balasooriya et al. 2018). Specifically in strawberry, elevated CO_2 increases strawberry growth rate and fruit yield (Itani et al. 1998, Oda 1997, Keutgen et al. 1997, Jun et al. 2017, Y. Mochizuki et al. 2013, Desjardins et al. 1988, Tagawa et al. 2022). For example, Itani, Y. Yoshida, and Fujime 1998 reported that CO_2 enrichment increased total leaf area and increased total yield by up to 50%. Tagawa et al. (2022) reported an increase of 25% in marketable fruit yields when CO_2 was increased to 800 µmol mol^{-1}. CO_2 enrichment also has some beneficial effects for fruit quality, where elevated CO_2 increased the levels of dry matter-content, fructose, glucose, and total sugar (Sun et al. 2012, Tagawa et al. 2022). Photosynthetic research has also shown that strawberry photosynthetic rate increases with increasing CO_2 concentration, however the "optimal" concentration varies depending on light intensity, temperature, and other factors (Keutgen et al. 1997, Jun et al. 2017, Balasooriya et al. 2018, Tagawa et al. 2022, Tagawa et al. 2022). Furthermore, all the CO_2 research on strawberry plants demonstrates the effect of CO_2 enrichment on fruiting strawberry plants or single leaves, with no published reports of the effects of CO_2 elevation on strawberry mother plants and propagation yields.

Research reports also show that increasing light intensity increases strawberry daughter number (Xu and Hernández 2020) and plant growth (Hidaka et al. 2013, Stadler 2017, X. Xu and Hernández 2020, Lee et al. 2020, Choi et al. 2016). The increase in plant growth under greater light intensity includes increased photosynthetic rate, increased dry mass, and improved fruit quality (such as increased soluble solids content of fruit) (Hidaka et al. 2013, Xu and Hernández 2020). For example, Xu and Hernández (2020) reported a linear increase in daughter plant production with increasing light intensity, with light treatments of 250, 350, and 450 µmol m^{-2} s^{-1}. With a 12-week growth period, the number of daughter plants produced was 38.7, 45.7, and 56.7, for these three light intensity treatments, respectively. Overall, this is a 47% increase in total number of daughter plants when light intensity is increased by 80% (200 µmol m^{-2} s^{-1}). The study also demonstrated a production rate ranging from 0.29 to 1.29 daughter plants per mole of light, with a maximum production rate of 107 plants per mother plant over a longer, 21-week cycle. However, the combined effects of CO_2 and light intensity on daughter plant production have not been investigated.

In the present study, the objectives are 1: to evaluate the effect of 500, 850, and 1200 µmol mol^{-1} CO_2 concentration on daughter plant production in strawberry cultivar 'Monterey' and 2: to determine how CO_2 concentration and light intensity affect daughter plant production of 'Monterey' when applied in different combinations.

We hypothesize that 1: CO_2 enrichment increases plant growth in 'Monterey' and therefore will increase the size and number of daughter plants, 2: increasing light intensity will increase the total number of daughter plants per mother, and 3: the combination of high CO_2 concentration and high light intensity will promote faster growth and development than other treatment combinations.

Materials and Methods

Source of Mother Plants

This experiment was replicated twice over time. The original greenhouse stock plants were sourced from Lassen Canyon Nursery (Redding, CA, USA) and grown at North Carolina State University in a plastic greenhouse from October 2022 to February 2023. On December 7th, daughter plants were harvested from the greenhouse, transplanted into ~70-mL wells, and moved into a reach-in growth chamber at the Phytotron facility, NCSU. In this growth chamber, temperature and light were maintained at 23°C and at 200 µmol m^{-2} s^{-1} respectively. High relative humidity was maintained using translucent plastic domes. After rooting for 35 days, the plug plants were transplanted into 250-mL wells (7311 10-Hole Strawberry Tray; Beekenkamp Verpakkingen, Maasdijk, ZH, NL) and grown for an additional 52 and 33 days for replicate 1 and 2, respectively, until they reached the minimum size threshold. The plug plants were evaluated for leaf number and crown diameter before transplanting. Only single-crown mother plants were used, and the target size of mother plants was 10 mm crown diameter. The mean initial crown diameter was 10.5 ± 1.17 mm. On the same day, plants were transplanted into 2-L pots and were randomly placed into growth chambers, under treatment conditions. Forty-eight strawberry plants per replication in time were moved into their treatment conditions in large walk-in growth chambers at the North Carolina State Phytotron.

Chamber Setup and Experimental Design

Three Phytotron growth chambers each provided a CO_2 concentration treatment: 500, 850, and 1200 µmol mol^{-1}. Environmental conditions in all chambers were maintained at 65% RH (±10%), 25°C day/25°C night temperature, and 16-hour photoperiod (See Table 1). The first and second rep were initiated on March 4th, 2023, and November 17th, 2023, and were run for 70 days and 71 days, respectively. The experimental design followed a split-plot approach, with the primary factor being CO_2 treatment (chamber) and the secondary factor being light intensity (250 PPF vs. 500 PPF). The experiment was repeated twice with different growing cycles (temporal repetitions). For each light intensity (secondary factor), 4 plants (observational units-OU) were sampled in the first repetition and 8 OU in the second repetition, resulting in 8 and 16 plants per chamber (primary factor CO_2) in repetitions one and two, respectively. The average of the OU in each repetition represented the sample size (n = 2).

For all chambers the photoperiod was set to 16 hours, and CO_2 concentration was set to fluctuate during the photoperiod: During the day the CO_2 setpoint would be maintained according to each treatment (500, 850, and 1200 µmol mol^{-1}), but at night each chamber was brought down to 500 µmol mol^{-1} CO_2. Day/night temp was set to 26°C, with days being warmer than nights by less than 0.5 degrees (See Table 1). Carbon dioxide application was controlled by a TC2 Microcontroller with two time-of-day-activated closed-loop process control channels (Environmental Growth Chambers, Chagrin Falls, OH, USA). This microcontroller was connected to a Vaisala CARBOCAP GMM112 compact diffusion aspirated CO_2 module inside a GMW115 diffusion transmitter (Vaisala, Vantaa, FI). The CO_2 sensor was calibrated using a CO_2/H_2O Trace Gas Analyzer (LI-7815; LI-COR Biosciences, Lincoln, NE, United States) before the start of each replication.

As shown in Figure 1, each (2.44 x 1.27 m) growth chamber was split in half, such that one half of the growth chamber was under a low light treatment (250 µmol m^{-2} s^{-1}), and the other half was under a high light treatment (500 µmol m^{-2} s^{-1}) in a split plot design, as shown in Figure 3. The light spectrum was white light (BRV ARIZE Lynk; General Electric Current, Boston, MA, USA) (Figure 2). Two light intensity treatments were deployed in each chamber: 250 µmol m^{-2} s^{-1} (250 PPFD, photosynthetic photon flux) and 500 µmol m^{-2} s^{-1} (500 PPFD). In order to maintain the treatment intensity at canopy height during the experiment, analog dimmers (0-10V/0-22mA Analog Simulator; TKXEC, CN) were installed to adjust the light intensity and to

ensure that the average light intensity was similar (within 5% total intensity) between chambers, treatments, and plant growth stage. Light measurements using a quantum sensor (LI-190R; Li-COR, Inc., Lincoln, NE, USA) attached to a light sensor logger (LI-1500; Li-COR, Inc., Lincoln, NE, USA), light intensity was measured at canopy level within each chamber. Several measurements were collected per pot position, and all measurements were averaged to yield the average treatment intensity. Then, the dimmer was adjusted, and measurements were taken again until the average light intensity was either 250 or 500 µmol m^{-2} s^{-1}.

These two light intensity treatments within each chamber were separated by a 60 cm-wide aisle. In the center of the aisle was a reflective polyethylene film (Pandafilm, Jiangsu Leader Greenhouse Equipment Co., CN) curtain, folded in half so that the white side reflected light back toward both sides of the chamber, with the black surface of the curtain hidden. This curtain still allowed airflow above and below it, while greatly reducing light contamination. Within each high-light (500 µmol m^{-2} s^{-1}) or low-light (250 µmol m^{-2} s^{-1}) plot, eight plants were arranged in two rows of four (Figure 3). Plants were spaced equally from each other, at a density of 7.18 plants per m^2. They were placed on custom-made benches, 1.2 m above the ground to allow the maximum amount of growing room for stolons under the benches. This experiment was replicated twice over time, with 48 total plants per repetition. Within each treatment half of the 'Monterey' strawberry plants were used for measurements at the end of the experiment.

Growing Conditions

Environment Conditions

Throughout the growth period, CO_2 sensors (CARBOCAP GMM112; Vaisala, Vantaa, FI), and Lascar temperature and humidity sensors connected to a Lascar datalogger (Easylog EL-GFX-2, Lascar Electronics Ltd., Erie, PA, USA) were placed inside a custom-built metal aspirated box and used to measure CO_2 concentration, temperature, and relative humidity from each growth chamber every 5 minutes. The humidifier (Trion CB777 Atomizing Humidifier; On Time Mall Inc., AZ, USA) and dehumidifier (FFAD5033R1; Frigidaire Appliance Company, NC, USA) were attached to a controller (IHC-200; INKBIRD, Shenzhen, GD, CN), which had its built-in humidity sensor located in the same aspirated box. Relative humidity was maintained around 65% (±10%). The aspirated box was located underneath a bench, within the daughter plant canopy.

Two fine-wire thermocouples (type T, wire diameter of 0.13 mm, Omega, Inc., Stamford, CT, USA) per treatment combination of CO_2 and light intensity were used to collect crown temperature data. The thermocouples were fixed on stakes to allow them to be adjusted to within 5 mm of the crown/apical meristem of the strawberry plant.

Irrigation and Substrate

treatment combinations of CO_2 and light intensity. This was necessary to provide similar soil moisture conditions between the various treatments, regardless of the rate of plant water uptake. For each CO_2/light treatment combination, an EC-05 capacitance volumetric water content sensor (ECH_2O EC-5; Decagon Devices, Inc., Pullman, WA, USA) was placed within one pot (6 sensors total). The sensor readings were manually calibrated before the start of repetition 1, to maximize consistency between sensors. The triggering threshold of the sensors to start irrigation was 40 cm3/cm3 (volume of water per volume of substrate) and the length of time was adjusted (9-15 minutes) to achieve 30% leachate volume for each CO_2/light treatment combination.

Plants were irrigated with a hydroponic nutrient solution containing 83.8 mg L^{-1} N, 7.78 mg L^{-1} P, 121 mg L^{-1} K, 45.7 mg L^{-1} Ca, 9.54 mg L^{-1} Mg, 11.2 mg L^{-1} S, 0.83 mg L^{-1} Cl, and micronutrients (Table 6). The EC and pH of the nutrient solution were recorded for both the supply solution and the leachate, using a portable solution sensor (HI 9813-6, Hanna Instrument Inc., Woonsocket, RI, USA) (Table 1).

The substrate was custom-mixed containing two parts coarse perlite, one part sphagnum peat moss, and one part coconut pith (Shur 2024). This substrate had a pH of 5.2, EC of 0.04, airspace of 22.8%, container capacity of 52.3%, and bulk density of 0.11 g cm^{-3}. The capacitance sensors used to trigger irrigation were calibrated to this substrate, to provide more accurate soil moisture measurement.

Data Collection

Throughout the growth period, mother plants were evaluated daily for inflorescence development. Inflorescences were removed as soon as they were large enough to pluck without damaging adjacent leaves, and at this point the inflorescences were approximately 1 to 2 centimeters long. Every time inflorescences were removed, we recorded the number of

inflorescences and the mother plants they grew from, to track the rate of new inflorescence growth per mother.

Stolons were evaluated daily for the presence of daughter plants. A new daughter plant was recorded when they developed a leaf greater than 1 cm long, with one or more leaflets partially or fully opened. Each daughter plant was labelled on the plant, using a white piece of tape with a handwritten label describing the position of the daughter plant, as a unique identifier code. This data was not collected for the full duration of replication 1 due to the labeling process taking too much time, but due to improvements in our data management system, new daughters were logged daily during repetition 2.

Primary stolons (developing from the mother plant crown), secondary stolons (developing from primary stolons), and tertiary stolons (developing from secondary stolons) were recorded on the date when they developed their first daughter plant. We created the parameter "stolon number", which indicates the order in which the stolons developed their first daughter plant. Stolon number is not necessarily the same order as when the stolons first began elongating from the main stem and was only recorded when daughter plants began to develop on each stolon.

Destructive Measurements

At ~10 weeks the experiment was terminated. During harvest, daughter plants were evaluated for leaf number, number of dead (dry) leaves, fresh mass, and leaf area as previously discussed. Mother plants were evaluated for crown diameter (averaged from two measurements), fresh mass of leaves, fresh mass of stems, number of leaves, number of dead (dry) leaves, height of the tallest leaf, and leaf area as previously discussed. Both the daughter and mother plants were bagged, then dried in a horizontal airflow drying oven (VWR-1685; Avantor Inc. PA, USA) at 70°C for at least 1 week before they were weighed again for their dry mass (MS104TS, Mettler Toledo, Greifensee, Switzerland). The dry mass of daughter plants was collected for the full daughter plant (leaves, petioles, and stems), but separate measurements of mother plant dry mass were collected for leaves and stems.

Economic Analysis

Costs of CO_2 Supplementation

The equation (Equation 1) and methods described in Ohyama and Kozai 1998 and Huber et al 2021 were used to estimate the usage of CO_2 consumed during the growth period. Setpoints and measurements from the present experiment were used for the estimation (Table 2). We also made generous assumptions for the consumption of CO_2 and leaf area per m^2, to demonstrate how even a potential overestimate of CO_2 consumption yields a low total cost. One underlying assumption is that the CO_2 leakage is proportional to the difference between the CO_2 concentration inside and outside the chamber (B. Acock 1989). All measurements used for these equations are listed in Table 2.

The Pn, CO_2 mass consumed per leaf area per hour, was estimated from the total leaf area of mothers and daughter per m^2 of growing area, and from our estimate of the photosynthetic rate reported in the literature. References state strawberry photosynthetic rates between 2 and 8 µmol $m^{-2}\,s^{-1}$ (Yanagi, Okamoto, and Takita 1996), or between 6 and 8 µmol $m^{-2}\,s^{-1}$ (Tabatabaei, Fatemi, and Fallahi 2006), or between 4 and 14 µmol $m^{-2}\,s^{-1}$ (Choi and Jeong 2020). For this calculation, the high photosynthetic rate of 14 µmol $m^{-2}\,s^{-1}$ was used to account for a potential increase under elevated CO_2. The total leaf area was then calculated within one m^2 of growing space by multiplying the average total leaf area of one plant under 1200 µmol mol^{-1} CO_2 by the plant density: 0.4591 m^2 multiplied by 7.18 plants per m^2 yields 3.29634 m^2 leaf area per m^2 growing area. Using the photosynthetic rate of 14 µmol $m^{-2}\,s^{-1}$, after unit conversions this would yield a total Pn of 0.007312 kg $m^{-2}\,h^{-1}$.

$$B = (A \times P_n + K_m \times E_a \times V\,(C_{in} - C_{out})) \times P \quad (1)$$

Then, the total cost of CO_2 supplementation is calculated in Equation 2, where the total consumption of CO_2 per day per m^2 is multiplied by D, the number of days per growing period, then multiplied by CC, the cost of CO_2 per kg ($0.58 per kg, Airgas, Radner, PA, United States).

$$TCC = B \times D \times CC \quad (2)$$

Costs of Lighting

These calculations are based on equations from Aldrich and Bartok 1994 and Kubota et al. 2016 and are also demonstrated in X. Xu and Hernández 2020. The cumulative cost of electricity (USD kWh^{-1}) increases linearly with the number of photons provided to the plants (mol $m^{-2}\,d^{-1}$). The yield in terms of number of daughter plants can be compared with this cumulative cost of electricity to estimate the cumulative cost of lighting per daughter plant. For this example, we use the repetition 2 data to demonstrate how the electricity cost per m^2 decreases as the cumulative photon number (mol $m^{-2}\,d^{-1}$) increases. We calculate these values separately for the high intensity and low intensity treatments (500 and 250 µmol $m^{-2}\,s^{-1}$).

To calculate the number of hours required to provide 1 mol of photons over 1 m^2 growing area, we can use Equation 3. The equation input PPF indicates photosynthetic photon flux, the desired light intensity over the growing area (µmol $m^{-2}\,s^{-1}$). Using inputs from the light fixture specifications sheet (MF, EM, and E), and the additional variable UF, we can reformulate the Aldrich and Bartok 1994 equation to calculate the amount of energy in kilowatts required per m^2 (Equation 4). Within Equation 4, the input E is the fixture power consumption in Watts (32 W, for our fixtures), EM is the emission rate of the fixture (70 µmol s^{-1}), and MF is the maintenance factor, which accounts for the rate decay of light output over time. A MF value of 0.9 was used for this calculation because these fixtures are rated for a 10% light output decay over the 36,000-hour fixture life (equivalent to 6.16 years of use at 16h per day). For the utilization factor (UF), we assumed that 90% of the light emitted was captured by the plants.

$$Hours\ mol^{-1} = \frac{1}{PPF \times 0.0036} \tag{3}$$

We can calculate the total energy required to produce 1 mol of photons over 1 m^2. Then, by multiplying this equation by the cost per kWh of energy, we generated Equation 4, which yields the cost per kWh per mole of light used to reach the desired PPF over a growing area of 1 m^2. In our own equations, we used \$0.14 kWh^{-1}, which is the average cost of electricity within North Carolina in 2024.

$$\$USD\ kWh^{-1}\ mol^{-1}\ m^2 = \frac{C \times E \times 0.2\overline{77}}{EM \times UF \times MF} \tag{4}$$

As demonstrated in X. Xu and Hernández 2020, this cost per m^2 can be divided by the propagation efficiency of strawberry (number of daughter plants per m^2 of growing area) to yield the cost of energy per daughter plant. We used the same equation (shown in Equation 5) to yield the total electricity cost per daughter plant.

$$Electricity\ cost\ per\ daughter\ plant = \frac{Electricity\ cost\ per\ mole\ of\ light}{Propagation\ efficiency\ of\ strawberry} \quad (5)$$

Statistical Analysis

Statistical analyses were conducted to compare the treatments using R version 4.2.1 (R Core Team 2021) and these results were corroborated using JMP version 16.0.0 (SAS Institute Inc. 2023). The analyses confirmed the absence of significant interactions between the treatment and replications over time. Linear regression was applied to the quantitative response to increasing CO_2 at each light intensity. For most variables, no interaction between light and CO_2 was observed, so one-way ANOVA tests were performed to evaluate the impact of light intensity. However, some variables showed a significant interaction between CO_2 and light intensity.

To evaluate the effect of CO_2 treatment on dependent variables, a linear model was fitted using Type III sums of squares. Global contrast settings were configured to use sum contrasts for unordered factors and orthogonal polynomial contrasts for ordered factors by specifying base::options(contrasts=c("contr.sum","contr.poly")), from R Core Team 2021. The CO_2 variable was centered around its mean to reduce the collinearity of this model (JMP Note 575394 2022). Centering was achieved by applying the scale function (base::scale) to the CO_2 variable, with centering enabled and scaling disabled. A linear regression model was fitted to the data to examine the effects of replication, CO_2 treatment (numeric data, centered), light treatment (categorical data), and their interaction on the response variables, using the lm() function from base R (R Core Team 2021).

In a separate analysis, to investigate the effects of CO_2 treatment on each variable under different light treatments, the dataset was filtered into two subsets based on the light treatment

levels 250 and 500 µmol m^{-2} s^{-1}, using dplyr::filter (Wickham et al. 2023). Separate one-way ANOVA tests were performed for each light treatment, using the aov() function from the car package (Fox and Weisberg 2019). The ANOVA summaries provided F-statistics and p-values, which were used to assess whether the differences in variables between CO_2 treatments were statistically significant within each light treatment condition.

Results

Impact of CO_2 and Light Intensity on Daughter Plants

As shown in Figure 4, the number of daughter plants linearly increased with increasing CO_2 concentration, by 33% for the high light intensity or 49 % for the low light intensity. The total number of daughter plants was significantly impacted by lighting treatments, where greater light intensity increased the total number of daughter plants by 28%, 11%, and 14% under 500, 850, and 1200 µmol mol^{-1} CO_2, respectively.

The total leaf number in the canopy formed by the stolons (number of daughter leaves) per mother plant was significantly impacted by CO_2 concentration, and light intensity. As shown in Figure 5, as CO_2 concentration increased, the total number of daughter leaves increased by 37% under the low light intensity treatment and by 21% under the high light intensity treatment. The high light intensity treatment had 27%, 16%, and 12% more daughter leaves than the low light treatment under 500, 850, and 1200 µmol mol^{-1} CO_2, respectively.

The total number of stolons (primary, secondary, and tertiary) significantly increased with increasing CO_2 concentration, where increasing the CO_2 concentration from 500 to 1200 µmol mol^{-1} increased the stolon number by 44% under the low light treatment and 32% under the high light treatment (Figure 6 and Table 5). We analyzed each stolon type and found that secondary stolons ($p = 0.0262$) showed the strongest response to the increase in CO_2, compared to primary ($p = 0.0702$) and tertiary ($p = 0.37$) stolon counts.

The stolon fresh mass was significantly impacted by CO_2 concentration ($p = 0.0029$), light intensity ($p = 0.0324$), and the interaction between CO_2 and light intensity ($p = 0.0224$). Increasing CO_2 concentration from 500 to 1200 µmol mol^{-1} increased the stolon fresh mass by 21% under low light intensity, but CO_2 concentration did not significantly impact the stolon fresh mass under high light conditions (Table 5).

Impact of CO_2 and Light Intensity on Mother Plants

Mother plant height (cm) was significantly affected by light intensity ($p = 0.0158$), where the low light treatment was roughly 3 cm taller than the high light treatment (Table 3).

CO_2 enrichment from 500 to 1200 µmol mol^{-1} increased the total number of leaves per mother plant by 54% under low light intensity ($p = 0.009$) but had no significant effect under high light conditions ($p = 0.321$) (Figure 7). Similarly, the stem and petiole fresh mass per mother was impacted by the interaction of CO_2 and light ($p = 0.0487$), likely due to the close correlation between number of petioles and number of leaves. In this case, when CO_2 concentration was increased from 500 to 1200 µmol mol^{-1}, stem and petiole fresh mass increased by 44% under the low light treatment. There was no observed significant effect of CO_2 or light intensity on the fresh mass of mother plant leaves (Table 3).

The dry mass of the mother plants (stems, petioles, and leaves) was summed with the dry mass of every daughter attached to that mother to yield the dry mass of each whole plant, excluding the stolon mass. Plant dry mass was significantly impacted by light intensity ($p = < 0.0001$), where plants under the high light intensity had a 29% greater dry mass than plants under the low light intensity (Figure 8). There was also a significant effect of the interaction of CO_2 and light intensity ($p = 0.00935$), where plants under the low light intensity treatment had a 31% increase in total dry mass when CO_2 concentration was increased from 500 to 1200 µmol mol^{-1} ($p = 0.016$) (Figure 8). However, plants in the high light intensity treatment were not impacted by CO_2 concentration ($p = 0.207$).

Impact of CO_2 and Light Intensity on Fresh Mass, Leaf Area, and Inflorescences

Although CO_2 concentration impacted the total number of daughters, it did not significantly impact the total fresh mass or dry mass of daughter plants ($p = 0.605$ and $p = 0.724$, respectively). Rather, fresh mass was impacted by light intensity ($p = 0.00303$), where plants under high light intensity had 40%, 31%, and 22% greater total fresh mass of daughters per mother under 500, 850, and 1200 µmol mol^{-1} CO_2 than plants in low light intensity. Daughter plant dry mass followed the same pattern in response to light intensity ($p < 0.0001$), where daughter plants under low light intensity produced 49%, 35%, and 30% lower dry mass under 500, 850, and 1200 µmol mol^{-1} CO_2 than plants in the high light intensity treatment (Figure 9). This difference between values was much less pronounced for fresh mass compared to dry mass,

where high light intensity produced 30.6% more fresh mass and 61.1% more dry mass than the low light intensity treatment.

Similar to daughter fresh mass, light intensity significantly increased ($p = 0.000198$) the leaf area of daughter plants from all positions (primary $p = 0.00598$, secondary $p = 0.000288$, tertiary $p = 0.014$). Daughter plant leaf area was 45.3% greater in the high light intensity treatment (3156 ± 1121 cm^2) compared to plants in the low light intensity treatment (2172 ± 731 cm^2). Mother plant leaf area was also significantly affected by light intensity ($p = 0.00127$), but with an opposite trend: the mother plants under the high light intensity treatment had 28% lower leaf area compared to the low light intensity treatment (1627 ± 472 cm^2 and 2251 ± 545 cm^2, respectively) (Figure 11). Neither daughter nor mother leaf area were significantly affected by CO_2 treatment ($p = 0.81$ and 0.91, respectively) (Figure 11).

Plants in the low light treatment also had 45% greater total number of inflorescences that developed throughout the experiment compared to the high light treatment ($p = 0.0163$) (Table 3). There was no significant impact of CO_2 concentration on the total number of inflorescences (Table 3).

Discussion

Growth and Development

Three hypotheses were proposed for this study: 1. Increasing light intensity will increase the yield of mother plants. 2. Increasing CO_2 concentration will increase the yield of daughter plants. 3. The benefit of CO_2 enrichment will be higher under higher light intensity.

Hypothesis 2: Increasing light intensity will increase the total number of daughter plants per mother.

In the present experiment, when light intensity was doubled from 250 to 500 µmol m^{-2} s^{-1}, the number of daughter plants per mother plant increased by 11-29%, depending on CO_2 treatment. Furthermore, whole plant dry mass, daughter leaf number, stolon number, and stolon fresh mass also increased with the increase in light intensity from 250 to 500 µmol m^{-2} s^{-1}. Plant growth (dry mass) increased in both mother and daughter plants. Specifically for daughter plants (the final propagation product), greater light intensity improved daughter dry mass, leaf area, and leaf mass area by 64%, 45%, and 15%, respectively. However, the cost of doubling the light is significant (see next section), and the cost-benefit should be carefully analyzed.

As expected, based on published studies (Hidaka et al. 2013, Stadler 2017, Xu and Hernández 2020, Lee et al. 2020, Choi, Moon, and Kang 2016), increased light intensity improved propagation yield (daughter plants per mother plant). Increasing light intensity has overall shown a positive impact on strawberry propagation rate and vegetative growth in general. For example, Zheng, He, and Ji (2019) reported that the number of stolons and daughter plants increased under daily light integrals from 8.6 to 11.5 mol m^{-2} d^{-1}, but no increase was observed above 11.5 mol m^{-2} d^{-1}. Furthermore, Xu and Hernandez (2020) reported a linear increase in daughter plants with increased light intensity (250-450 µmol m^{-2} s^{-1}) with a propagation efficacy to light between 0.3 and 1.9 daughter plants per mole of light (depending on light intensity and harvest time) and with a maximum recorded yield of 50 plants per mother plant over a 21-week propagation cycle.

Even though the increase in light enhanced daughter plant production, the increase was lower than expected. For example, Xu and Hernández (2020) grew mother plants 'Albion' under increasing light intensity treatments from 250 to 450 µmol m^{-2} s^{-1} under similar environmental conditions to the present study. In Xu and Hernández (2020), an increase in light intensity (250 to 450) produced a 47% increase in the number of daughter plants that developed over a 12-week growth period. Based on Xu and Hernández's (2020) linear predictive model, an increase from 250 to 500 µmol m^{-2} s^{-1} should have produced a 59% increase in daughter plants. However, in the present experiment, the number of daughter plants increased by 11-29%, depending on CO_2 treatment. The difference in the magnitude of this effect could be due to Xu and Hernández (2020) having a longer growth period (12 weeks compared to our 10 weeks) or could be due to the differences in cultivar 'Albion' vs. 'Monterey' (both long-day cultivars). Another plausible explanation is the difference in light diffusion within the canopy. In Xu and Hernández (2020), light penetrated deeper into the canopy due to the use of fluorescent lights as top lighting (providing diffuse light), whereas in the present study, LED fixtures with a narrow beam angle were used at the top of the canopy. Furthermore, Xu Hernández (2020) had fewer mother plants per growing area (5.23 compared to 7.18 plants m-2), which likely allowed greater light penetration into the canopy. Generally, greater light penetration into the canopy allows more light capture at the bottom of the canopy, where many newly developing daughter plants are located. It is plausible that the difference in daughter plant yield is related to Xu Hernández

(2020) having greater light capture at the bottom of the canopy, which may have stimulated more daughter plant development than in the present experiment.

Hypothesis 1: CO_2 enrichment increases plant growth in strawberry and therefore will increase the size and number of daughter plants

Although there is an abundance of literature on CO_2 enrichment on strawberry fruiting plants, no studies are available reporting the impact of CO_2 on strawberry mother plant yield. In the present study, strawberry plants positively responded to CO_2 enrichment. For example, the number of daughter plants increased linearly with CO_2 enrichment from 33%-50%, depending on light intensity. Therefore, CO_2 enrichment can be considered a good strategy for increasing propagation yield since the cost of CO_2 in controlled environments with low room air exchange (0.01-0.1 h^{-1}) is very low (see next section).

Despite the increase in the number of daughter plants per mother plant, this response was not reflected in daughter plant dry mass, mother plant dry mass, and only for whole plant dry mass under low light intensity. This result was unexpected since, based on well-understood CO_2 responses in other horticultural plants, plant growth (dry mass) increases with increased CO_2 due to the greater photosynthetic rate caused by the greater rate of CO_2 diffusion into the stomatal cavity under higher CO_2 concentrations. This is well documented in photosynthetic studies (Keutgen et al. 1997; Balasooriya et al. 2018; Tagawa et al. 2022; Jun et al. 2017; Balasooriya et al. 2018) as well as in fruiting strawberry studies (Itani, Yoshida, and Fujime 1998; Oda 1997; Keutgen, Chen, and Lenz 1997; Jun, Jung, and Imai 2017; Mochizuki et al. 2013; Desjardins et al. 1988; Tagawa et al. 2022). With the results of the current experiment, we do not have evidence that this is the case for strawberry mother plants. A possible explanation for the lack of increase in dry mass is the low light intensity in the canopy, as discussed before, reducing the benefits of CO_2 enrichment. Another potential explanation is that the additional photo-assimilates generated by increased CO_2 enrichment were translocated to the roots, which were not measured in the present study.

A more plausible explanation for the linear increase in the number of daughter plants with increased CO_2 enrichment is the impact of CO_2 on plant development or plant structure, specifically the development of new stolons (primary, secondary, and tertiary) leading to the development of more daughter plants. In the present study, CO_2 linearly increased the number of stolons per plant. Furthermore, the increase in light intensity did not affect the number of stolons,

indicating that new stolon development is not a photosynthetic-based response but rather a developmental response influenced by CO_2 concentration. Research on other plant species under elevated CO_2 has shown altered branching patterns, increased node numbers, increased branches per node, and reduced apical dominance (Pritchard et al., 1999), which supports this idea. Therefore, the current study is the first to report strawberry mother plant responses to elevated CO_2, demonstrating that CO_2 enrichment increases propagation yield by promoting new stolon development.

Hypothesis 3: The combination of high CO_2 concentration and high light intensity will promote faster growth and development than other treatment combinations.

CO_2 enrichment did not perform better under increased light intensity. Plant research has shown a synergistic effect of CO_2 concentration and light intensity due to the chemical requirements of photosynthesis. According to the FvCB biochemical model of C3 photosynthesis, the photosynthetic rate can be limited by either carboxylation or regeneration of ribulose-1,5-bisphosphate (RuBP) (Farquhar, Caemmerer, and Berry 1980; Kanno et al. 2022). When CO_2 concentration is inadequate, carboxylation of RuBP becomes limiting, and when CO_2 is elevated and light intensity is insufficient, regeneration of RuBP can become limiting. Due to this dynamic between RuBP regeneration and carboxylation, we hypothesized that the high light intensity treatment should have larger gains in photosynthesis and growth associated with the increase in CO_2 concentration. On the other hand, the lower light intensity treatment should have a slower rate of RuBP regeneration and would therefore not be as strongly affected by CO_2 elevation. A possible explanation could be due to acclimation to higher CO_2 levels. When plants are first introduced to elevated CO_2 concentration, photosynthesis usually initially increases, but in the long term, photosynthesis may decline or stay the same (Long, Baker, and Raines 1993; Keutgen, Chen, and Lenz 1997). However, different responses to CO_2 are observed under different light levels, nutrient concentrations, and other environmental conditions (Kirschbaum 1994), so the long-term effect of CO_2 concentration on strawberry mother plants is still unknown. Another plausible explanation is the limited light reaching the stolon canopy, a unique morphological characteristic of strawberry mother plants. The canopy of the mother plants (excluding stolons and daughters) is mostly exposed to top lighting, leaving little light to penetrate to the daughter plants. This limitation prevents any synergistic benefits between light and CO_2 enrichment.

Costs of Lighting and CO_2 Supplementation

As shown in Figure 12, although the high light intensity treatment produced more daughter plants, the cost of energy required per daughter plant was greater than the low light intensity treatment. Even as the values seem to stabilize near $0.00 dollars, the ultimate cost per daughter plant was $0.06 under high light intensity, compared to $0.03 under low light intensity. The total cost per square meter of growing area over the 70-day growth period was low, at $44.25 and $22.12 under high and low light intensity, respectively. In a separate study, X. Xu and Hernández 2020 performed similar calculations for strawberry propagation with a different LED fixture and propagation system. X. Xu and Hernández 2020 calculated an efficiency of $0.029 to $0.036 per daughter plant (cv. 'Albion') between 1 and 12 weeks of growth, with different light intensity treatments (250, 350, and 450 μmol m^{-2} s^{-1}). These results are reasonably similar to our efficiency under low light intensity. Although the cost of electricity is typically the largest ongoing cost of indoor propagation systems, the costs calculated for strawberry propagation in the present manuscript and also in X. Xu and Hernández 2020 are low, when divided between the daughter plants.

The economics calculation of the cost of CO_2 yielded a cost of $4.84 m−2 for the 70-day growing cycle. On average under the 1200 μmol m^{-2} s^{-1} CO_2 concentration there were 83.4 daughter plants per mother, and therefore 598.812 daughter plants per square meter of growing area. Distributed between these daughters, the cost of enriching CO_2 to 1200 μmol mol^{-1} would be $0.0081 per daughter plant. This value is very insignificant, especially considering how we were generous with some assumptions we made within the calculation (such as photosynthetic rate, Pn, and air exchange rate, E), and it is more likely that the cost of CO_2 was overestimated rather than underestimated. This calculation demonstrates that even when a high CO_2 concentration is applied, the cost of CO_2 per daughter plant is less than one cent.

The lighting cost per daughter plant under high light intensity ($0.06) was more than seven times greater than that of high CO_2 supplementation ($0.0081) (500 μmol m^{-2} s^{-1} light compared to 1200 μmol mol^{-1} CO_2). Furthermore, the initial cost of fixtures drives the cost of electrical lighting even higher, further supporting our conclusion that CO_2 enrichment is more economical than increasing the light intensity for CE strawberry propagation.

However, it is possible that the CO_2 and lighting costs may be offset by a higher selling price of daughter plants from controlled environments. Further work into economic analyses could open new opportunities for specialty strawberry cultivars. In the conventional outdoor propagation system, any cultivar which produces few daughter plants per mother is difficult to propagate and is therefore unlikely to be sold regardless of its potential fruit quality and quantity. Holmes (2024) describes a personal communication with D. Thomas and M. Nelson, stating that "any cultivar not producing at least 741,000 daughter plants per hectare is not likely to be grown". Even if a strawberry cultivar produces a high fruit yield, or high fruit quality, its inefficient propagation prevents it from being made available to fruit growers. As continued developments are made for CO_2 concentration, lighting, and other methods for increasing daughter plant production, further economic analyses must explore the minimum yield requirements for mother plants to make a profit in CE agriculture. It is possible that strawberry cultivars with poor propagative rate could be especially benefitted by controlled environment propagation and might sell for an increased price as a specialty cultivar.

Ultimately, although CO_2 supplementation and electrical lighting are added costs for strawberry propagation, the daughter plants which are produced may be of higher quality (low risk of disease), they have a relatively low cost per daughter (less than $0.01 even under a combination of high CO_2 and high light intensity), and CE production opens up new opportunities.

Future Work

Intracanopy lighting is another promising research area that could improve daughter plant growth. As observed in the present research, the light intensity at the bottom of the canopy was less than 10 µmol m^{-2} s^{-1}, below the photosynthetic compensation point of most plants. By supplying intracanopy light, the daughter plants may photosynthesize at a greater rate and may grow more vigorously as a result. Furthermore, due to the large amount of leaf area within the canopy of daughter plants, intracanopy light has the potential to increase whole-plant light capture and photosynthetic rate.

Conclusions

This research aimed to elucidate the effects of CO_2 concentration and light intensity on propagation rates of the long-day strawberry cultivar Monterey. Our data support hypothesis 1, that CO_2 enrichment increases daughter number and some plant size metrics. Our data also support hypothesis 2, that greater light intensity increases the number of daughter plants and whole-plant growth. However, in hypothesis 3 we expected the positive effect of CO_2 to be greater under the higher light intensity, but the opposite was found: for a few variables, CO_2 only significantly improved growth under the low light intensity treatment. In addition to further research on CO_2 enrichment and supplemental light intensity, we recommend future research to be done on light quality, light fixture placement (intracanopy lighting), nutrient management, and daughter plant manipulation with hormone treatments.

FIGURES

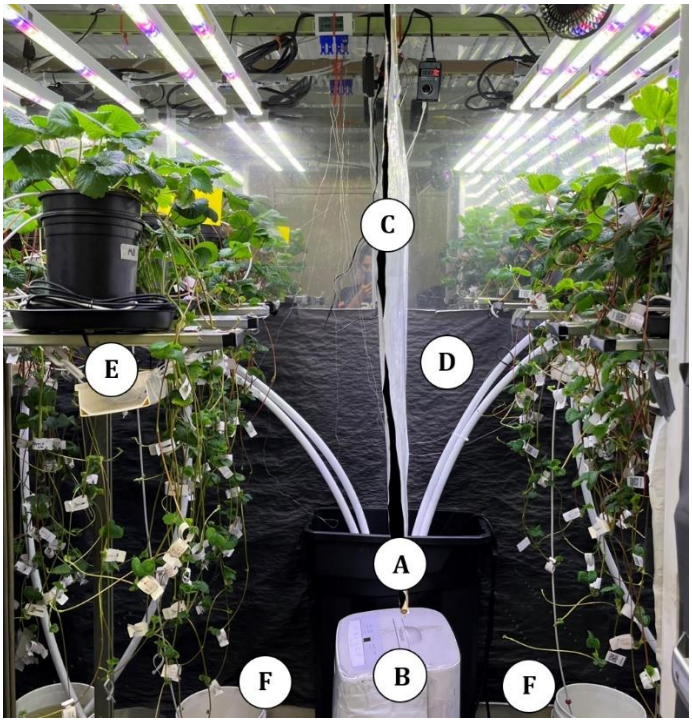

Figure 1: A view of one chamber, shown from the door (replication 1, day 42). Nutrient solution reservoir (A), dehumidifier (B) and humidifier (not visible) are on the floor, in the center of the chamber. Pandafilm (C) is hanging from the ceiling in the center of the growth chamber. The black paper (D) covering the walls is visible behind the daughter plants. Hanging below the benches there are leachate drainage gutters (E), and drainage tubes are visible on the far side of the chamber, away from the door. Leachate collection buckets (F) are located on the floor, below the daughter plant canopy.

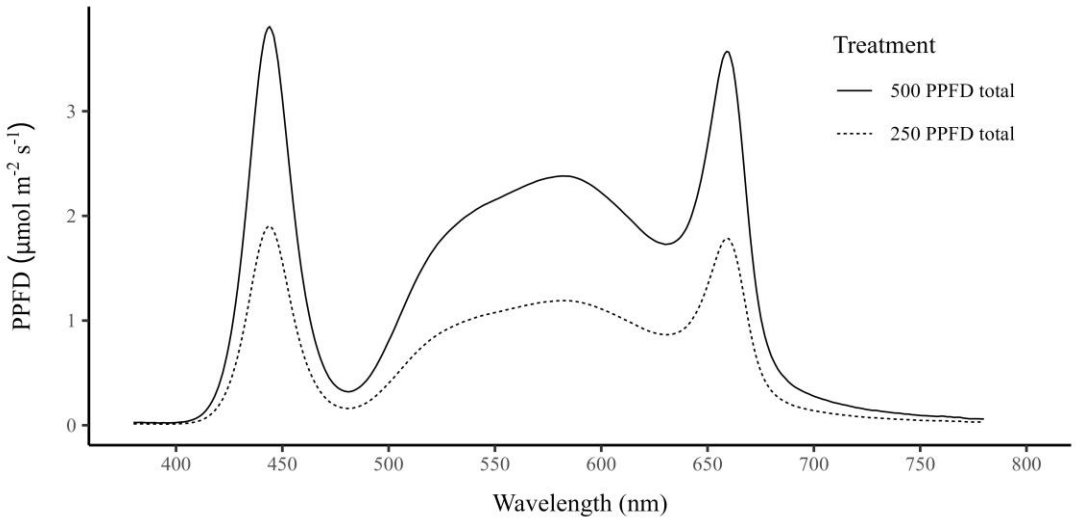

Figure 2: Spectral graph of high and low intensity light treatments under the light fixtures, which visually appear white and have a blue:green:red ratio of 25B:38G:37R (BRV ARIZE Lynk; General Electric Current, Boston, MA, USA) Photosynthetic Photon Flux (PPFD) shown on y-axis, and wavelengths in nanometers (nm) are shown on the x-axis.

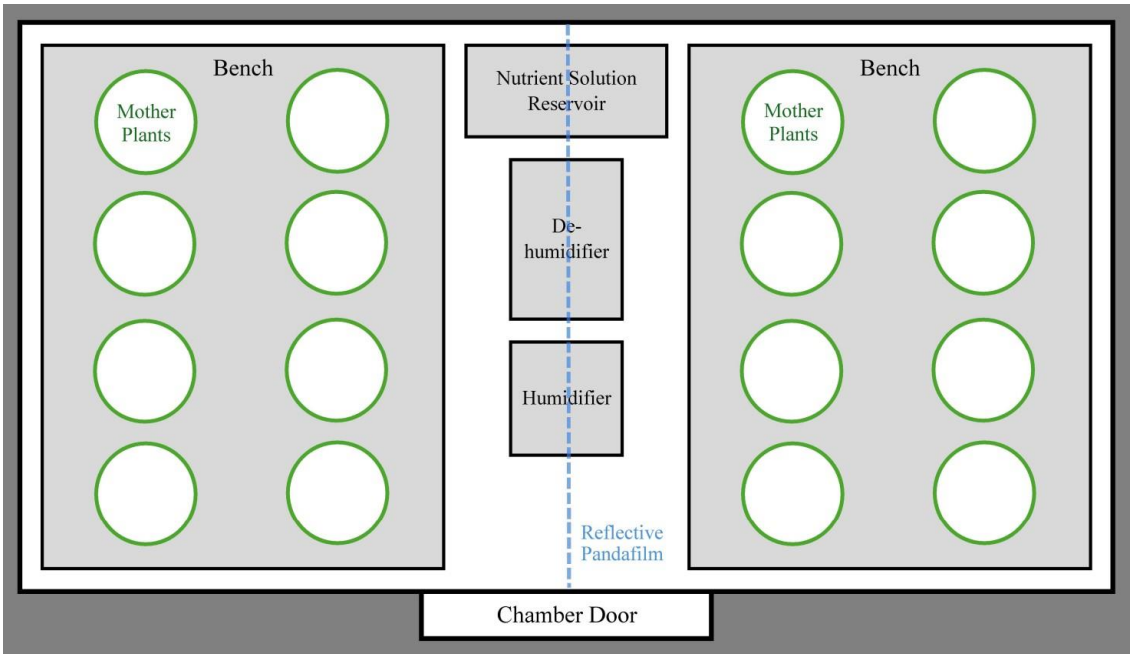

Figure 3: Chamber layout, shown from above. This layout is identical between all three chambers. Nutrient solution reservoir, dehumidifier, and humidifier are located on the floor in the center of the chamber, and the sheet of reflective polyethylene film (Pandafilm, Jiangsu Leader Greenhouse Equipment Co., CN) is located directly above it, hanging from the ceiling in the center of the growth chamber. Each circle represents a mother plant, with a total of 16 mother plants per chamber. A different light intensity treatment was applied to each bench, with one bench receiving 500 µmol m^{-2} s^{-1} and the other bench receiving 250 µmol m^{-2} s^{-1}. CO_2 tanks were stored outside the chambers (not shown in the above figure) and pumped into both sides of the chamber at equal rates, through horizontal vents in the walls.

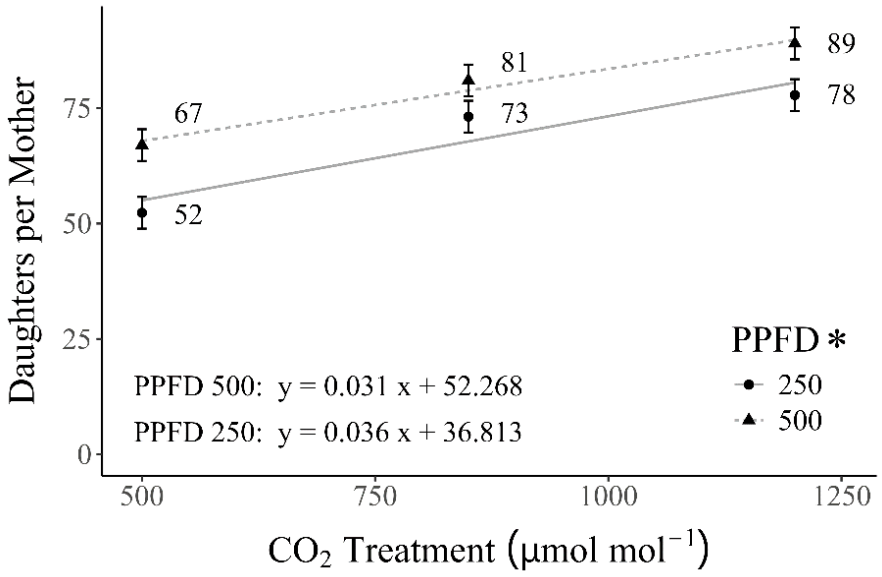

Figure 4: The average number of daughter plants per mother plant (average +/- SE). The mean values are labeled and for each light treatment the linear model equation. The presence of a line represents a significant linear response to CO_2 increase ($p = 0.00217$). The asterisk (*) represents a significant difference between the two light treatments ($p = 0.05065$). No interactions were found between CO_2 and light intensity ($p = 0.72$).

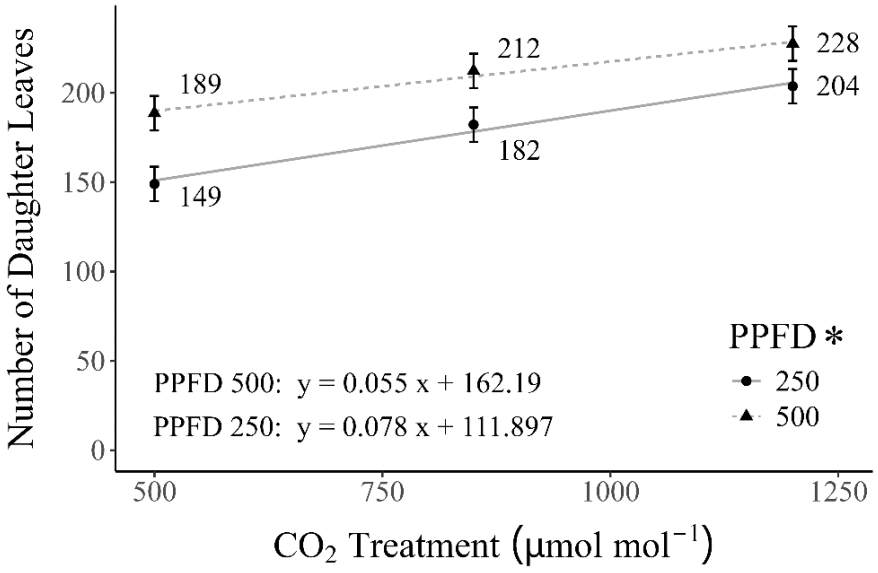

Figure 5: The average number of daughter plant leaves per mother plant (average +/- SE). The mean values are labeled and for each light treatment the linear model equations for each treatment are shown. The p value below the equations indicates the significance of CO_2 concentration. The p value below the legend represents the significant effect of light intensity. The presence of a line represents a significant linear response to CO_2 increase ($p = 0.03089$). The asterisk (*) represents a significant difference between the two light treatments ($p = 0.03987$). No interactions were found between CO_2 and light intensity ($p = 0.55$).

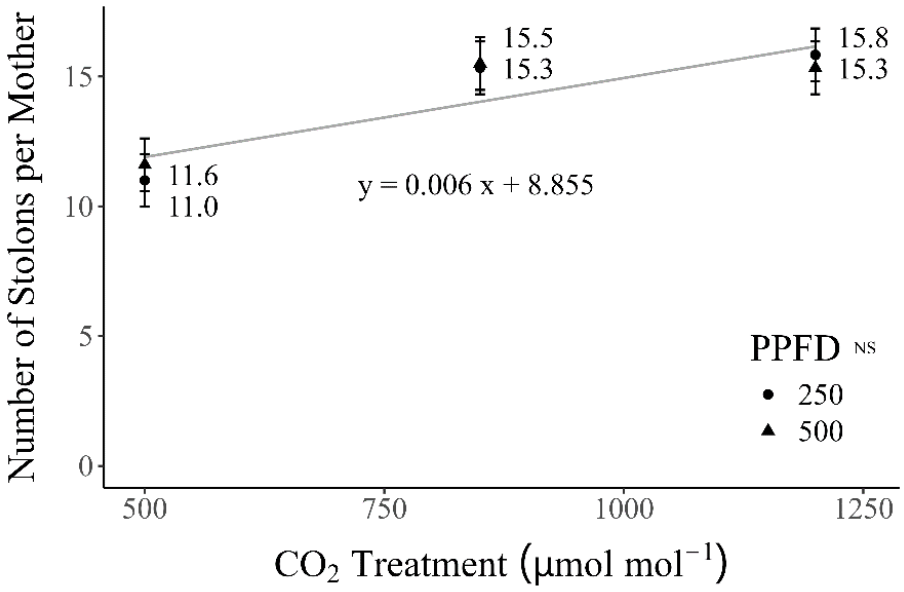

Figure 6: The average number of stolons per mother plant, plotted with standard error bars. The mean values are labeled and for each light treatment the linear model equation with its p value is labeled. The presence of a line represents a significant linear response to CO_2 increase ($p = 0.0321$). The "NS" below the legend indicates no significant effect of light intensity. NS represents no significant differences between light treatments ($p = 0.68$). No interactions were found between CO_2 and light intensity ($p = 0.46$).

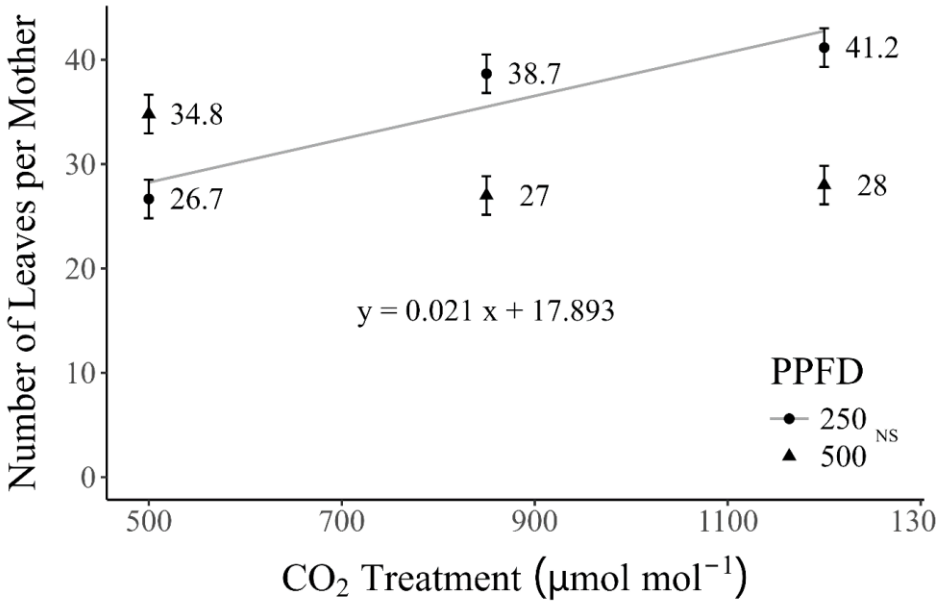

Figure 7: The average number of leaves per mother plant (average +/- SE). All non-destructively harvested plants and the three outlier mother plants were excluded from the linear regression model. The mean values are labeled and for each light treatment the linear model equation for the low light treatment is labeled. The presence of a line represents a significant linear response to CO_2 increase for the 250 PPFD light treatment ($p = 0.009$). NS represents no significant differences between light treatments ($p = 0.08$). An interaction was found between CO_2 and light intensity ($p = 0.013943$).

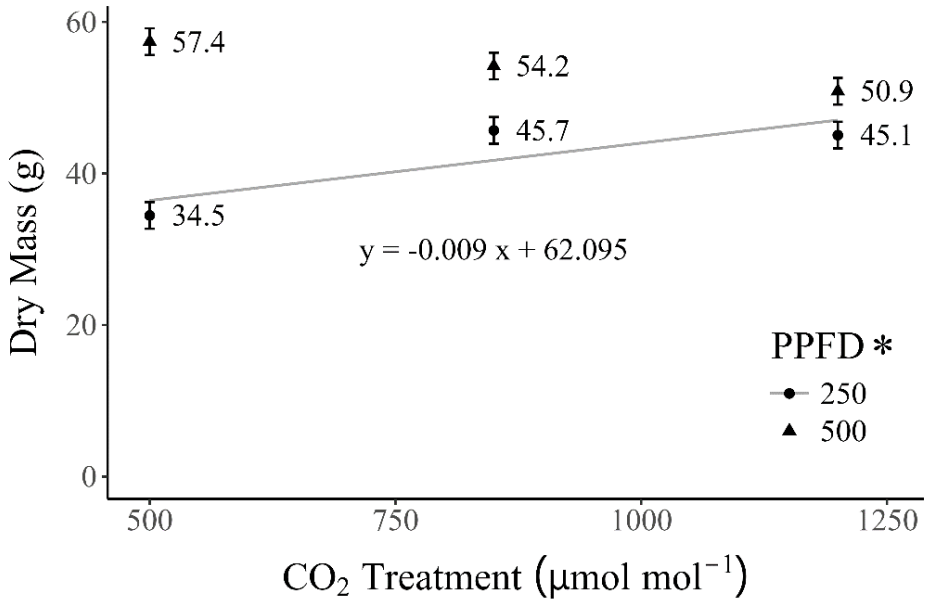

Figure 8: The summed dry mass of mother plant leaves, petioles and stems, and all the daughter plants attached to that mother plant, plotted with standard error bars. All non-destructively harvested plants and the three outlier mother plants were excluded from the linear regression model. The mean values are labeled and for each light treatment the linear model equation for the low light treatment is labeled. The presence of a line represents a significant linear response to CO_2 increase for the 250 PPFD light treatment ($p = 0.0155$). The asterisk (*) represents a significant difference between the two light treatments ($p < 0.0001$). An interaction was found between CO_2 and light intensity ($p = 0.00935$).

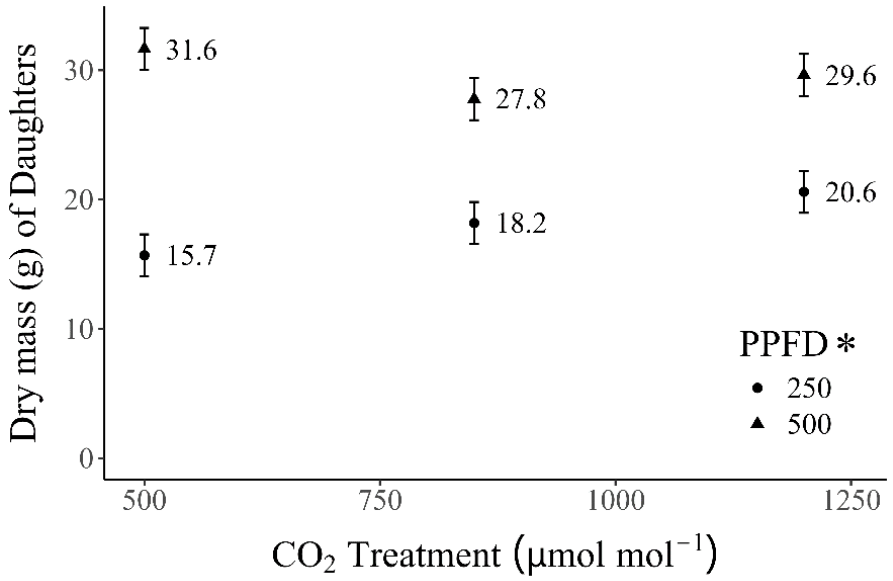

Figure 9: The dry mass of all daughters per mother (average +/- SE). The p value below the legend represents the significant effect of light intensity. The asterisk (*) represents a significant difference between the two light treatments ($p < 0.0001$). No interactions were found between CO_2 and light intensity ($p = 0.10$).

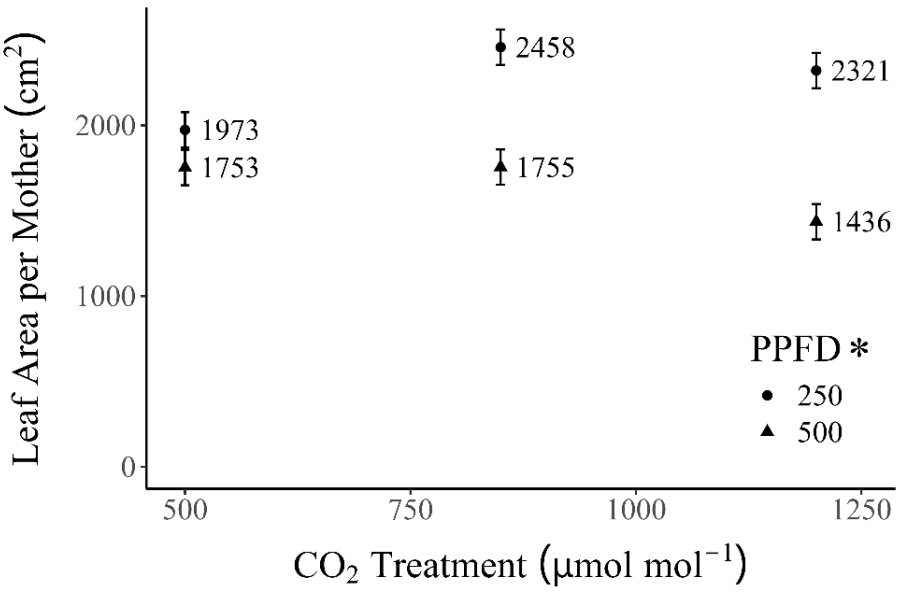

Figure 10: The leaf area per mother plant (average +/- SE). The asterisk (*) represents a significant difference between the two light treatments (p = 0.00127). No interactions were found between CO_2 and light intensity (p = 0.14).

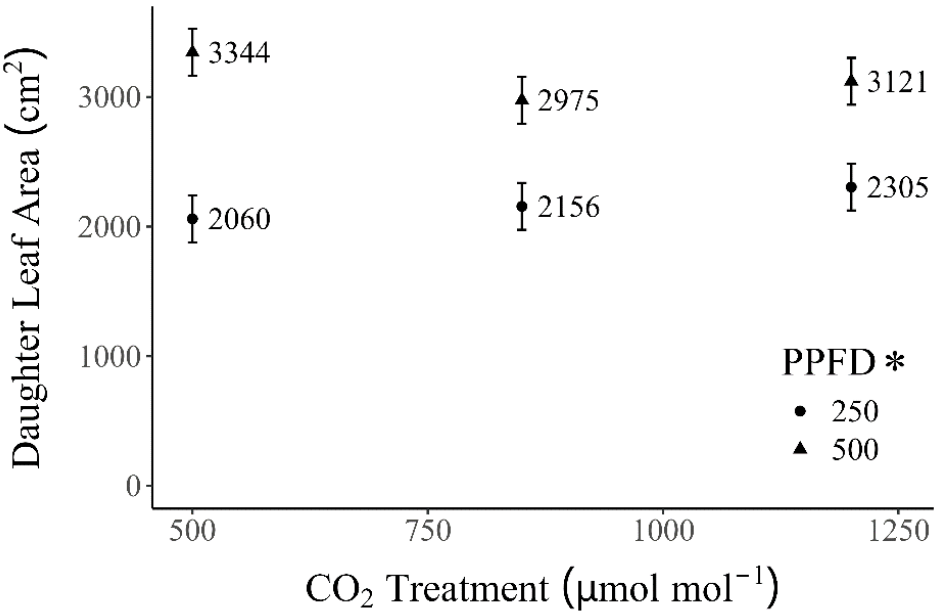

Figure 11: The leaf area of all daughters per mother plant (average +/- SE). The asterisk (*) represents a significant difference between the two light treatments ($p = 0.000198$). No interactions were found between CO_2 and light intensity ($p = 0.30$).

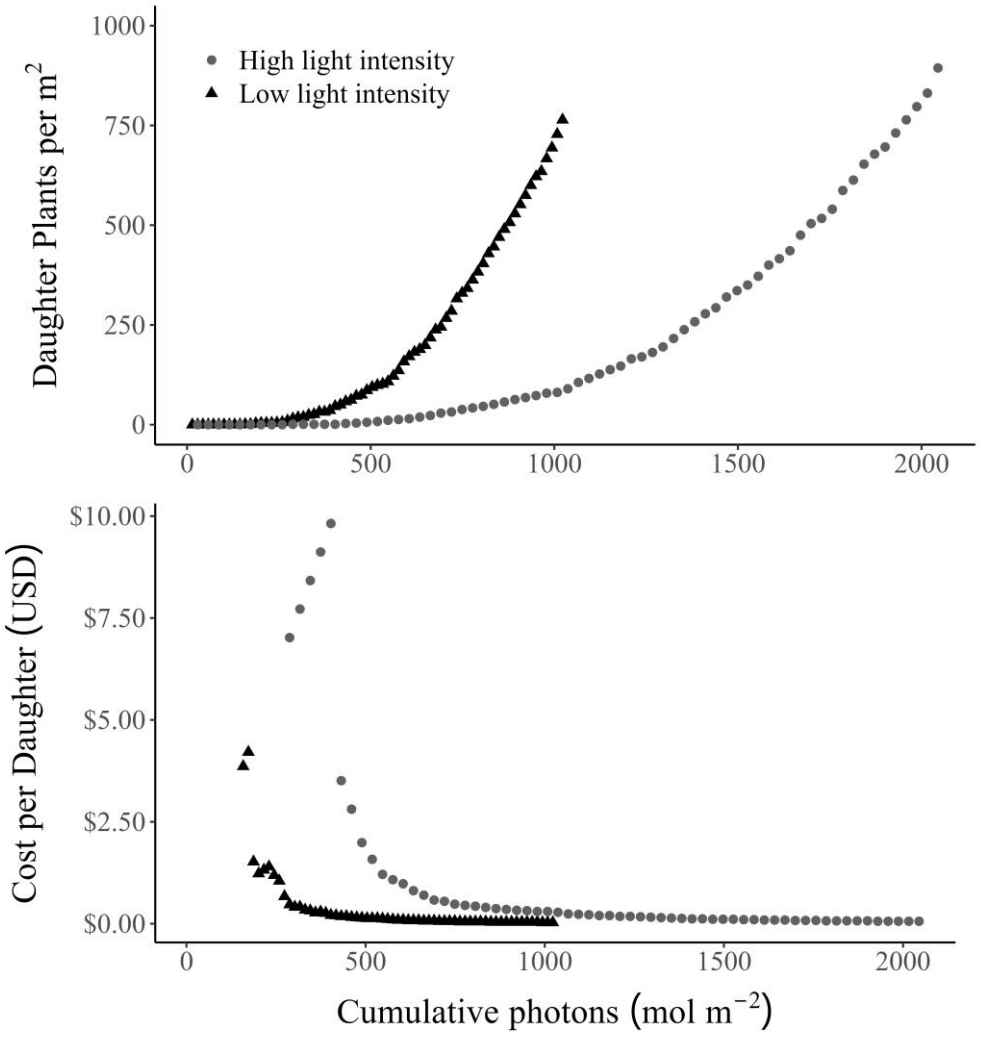

Figure 12: Data from replication 2, plotting cumulative photons against daughter plants per m^2 and cost per daughter plant (where one mole of photon was calculated to cost \$0.0219479). The low light treatment minimum cost is \$0.03, and the high light treatment minimum cost is \$0.06.

Table 1: Growing conditions during the experiment presented with mean ± standard deviation.

This was a split plot design, with CO_2 as the main treatment and light intensity as the split.

Variable	500 µmol mol^{-1}		850 µmol mol^{-1}		1200 µmol mol^{-1}	
	Day	Night	Day	Night	Day	Night
CO_2 Setpoint, µmol mol^{-1}	500	500	850	500	1200	500
Actual CO_2, µmol mol^{-1}	508 ± 36.7	478 ± 27.1	846 ± 51.2	492 ± 41.9	1188 ± 76.4	509 ± 145
Air Temp, °C	26.3 ± 0.47	25.9 ± 0.51	26.2 ± 0.28	26.1 ± 0.2	26.0 ± 0.21	26.0 ± 0.11
Crown Temp, °C	25.1 ± 0.86	24.6 ± 0.80	25.2 ± 0.71	24.9 ± 0.64	25.0 ± 0.64	24.6 ± 0.72
Relative Humidity, %	67.8 ± 5.50	58.8 ± 6.9	72.1 ± 6.86	61.2 ± 5.63	70.5 ± 4.69	59.3 ± 5.61
Irrigation pH, replication 1	5.49 ± 0.31		5.36 ± 0.37		5.31 ± 0.43	
Irrigation EC, µS cm^{-1}, replication 1	1.09 ± 0.09		1.10 ± 0.13		1.15 ± 0.55	
Irrigation pH, replication 2	4.55 ± 0.21		4.7 ± 0.52		4.53 ± 0.45	
Irrigation EC, µS cm^{-1}, replication 2	1.24 ± 0.18		1.27 ± 0.22		1.23 ± 0.2	
Light intensity	500 or 250 µmol m^{-2} s^{-1} depending on plant placement on the left or right side of the chamber. Side selection was randomized between treatments and repetitions.					
Photoperiod	16 hours					
Daily Light Integral	28.8 or 14.4 mol m^{-2} d^{-1}					

Table 2: Symbols, descriptions, values, and units of the inputs to the economics calculations.

Symbol	Description	Value	Unit
A	Total growing area	1	m^2
B	Usage of CO_2 per day	0.11929	\$ kg^{-1}
C	Cost of energy for light	\$0.14	\$ kWh^{-1}
CC	Cost of CO_2	\$0.58	\$ d^{-1} m^{-2}
C_{in}	CO_2 concentration inside the chamber	0.0005-0.0012	mol mol^{-1}
C_{out}	CO_2 concentration outside the chamber	0.0004	mol mol^{-1}
D	Growing days per cycle	70	days
E	Fixture power consumption	32	W
E_a	Air exchange rate	0.10	exchanges h^{-1}
EM	Emission rate of light fixture	70	µmol s^{-1}
K_m	Volume to mass conversion for CO_2 at 26°C	1.7976	kg CO_2 m^{-3}
MF	Maintenance factor	0.90	—
P	Photoperiod	16	h
P_n	Net photosyntheyic rate per LAI	0.007312	kg m^{-2} h^{-1}
TCC	Total cost of CO_2 per production cycle (70 d)	\$4.84	\$ m^{-2}
UF	Utilization factor	0.90	—
V	Volume of growing facility	1	m^3

Table 3: Mother plant measurements, with means and standard deviations. Significance values are given for the model with model effects of repetition, CO_2, light intensity, and the interaction between CO_2 and light intensity. No interactions between CO_2 and light were found for the parameters where no interaction is listed. All analyses have n = 6 except for CO_2 500/light 500, where n = 5, and for CO_2 850/light 500, where n = 4.

Light Treatment	**500 µmol mol^{-1} CO_2**	**850 µmol mol^{-1} CO_2**	**1200 µmol mol^{-1} CO_2**
	Mother plant height (cm)		
250 PPFD	23.1 ± 2.4	23.6 ± 2.89	23.1 ± 1.8
500 PPFD	20.8 ± 2.51	19.5 ± 2.48	21.2 ± 4.62
Significance	CO_2 Regression: p = 0.9372, Light: p = 0.0158, CO_2 × Light: p = 0.9372		
	Leaf fresh mass (g)		
250 PPFD	50.6 ± 14.6	67.3 ± 11.3	60.8 ± 18.7
500 PPFD	56.4 ± 17.4	55 ± 15.3	42.7 ± 14.6
Significance	CO_2 Regression: p = 0.8266, Light: p = 0.0941, CO_2 × Light: p = 0.0929		
	Leaf dry mass (g)		
250 PPFD	18.8 ± 5.88	27.5 ± 6.44	24.5 ± 8.1
500 PPFD	25.8 ± 8.11	26.4 ± 8.16	21.2 ± 7.56
Significance	CO_2 Regression: p = 0.7211, Light: p = 0.9942, CO_2 × Light: p = 0.1037		
	Number of dead leaves per mother plant		
250 PPFD	4.5 ± 2.88	6 ± 2	6.67 ± 3.39
500 PPFD	5.4 ± 2.3	5.25 ± 4.03	5.67 ± 3.33
Significance	CO_2 Regression: p = 0.3586, Light: p = 0.8676, CO_2 × Light: p = 0.3731		
	Number of crowns per mother plant		
250 PPFD	3.5 ± 1.38	5.67 ± 1.21	5.83 ± 1.47
500 PPFD	5.6 ± 2.51	4.75 ± 1.71	4.5 ± 1.87
Significance	CO_2 Regression: p = 0.2759, Light: p = 0.7360, CO_2 × Light: p = 0.0200		
	Average crown diameter per mother plant (cm)		
250 PPFD	30.4 ± 4.67	37.3 ± 4.1	36.2 ± 5.12
500 PPFD	38.6 ± 5.38	39.7 ± 2.77	35.7 ± 9.07
Significance	CO_2 Regression: p = 0.4533, Light: p = 0.1539, CO_2 × Light: p = 0.0807		
	Fresh mass of crowns/stems and petioles (g)		
250 PPFD	38.5 ± 13	56.9 ± 12	55.4 ± 17.5
500 PPFD	47.9 ± 16.4	43.7 ± 14.1	37.7 ± 17.8
Significance	CO_2 Regression: p = 0.5572, Light: p = 0.1390, CO_2 × Light: p = 0.0487		
	Cumulative number of flowers that grew throughout the experiment		
250 PPFD	10 ± 4.29	12.8 ± 7.47	15 ± 7.4
500 PPFD	12.6 ± 6.35	4.75 ± 4.19	8 ± 3.52
Significance	CO_2 Regression: p = 0.7011, Light: p = 0.0163, CO_2 × Light: p = 0.0515		

Table 4: Daughter plant measurements, with means and standard deviations. Significance values are given for the model with model effects of repetition, CO_2, light intensity, and the interaction between CO_2 and light intensity. No interactions between CO_2 and light were found. All analyses have n = 6 except for CO_2 500/light 500, where n = 5, and for CO_2 850/light 500, where n = 4.

Light Treatment	**500 µmol mol^{-1} CO_2**	**850 µmol mol^{-1} CO_2**	**1200 µmol mol^{-1} CO_2**
	Total number of primary daughter plants per mother		
250 PPFD	24.5 ± 5.13	35.7 ± 14.8	38.3 ± 8.45
500 PPFD	29.2 ± 6.91	37 ± 4.97	35.7 ± 15.3
Significance	CO_2 Regression: p = 0.0331, Light: p = 0.7804, CO_2 × Light: p = 0.3533		
	Total number of secondary daughter plants per mother		
250 PPFD	26.8 ± 9.62	34.5 ± 8.98	36 ± 8.83
500 PPFD	30.6 ± 7.64	39.5 ± 15.9	45.7 ± 11.1
Significance	CO_2 Regression: p = 0.0077, Light: p = 0.0629, CO_2 × Light: p = 0.5445		
	Total number of tertiary daughter plants per mother		
250 PPFD	1 ± 1.1	3 ± 2.28	3.5 ± 4.28
500 PPFD	7.2 ± 5.72	4.5 ± 3.7	7.5 ± 6.16
Significance	CO_2 Regression: p = 0.3958, Light: p = 0.0123, CO_2 × Light: p = 0.5910		
	Total fresh mass of all daughters per mother		
250 PPFD	99.9 ± 45.8	106 ± 46.1	126 ± 29.9
500 PPFD	140 ± 45.9	139 ± 75.8	154 ± 28.5
Significance	CO_2 Regression: p = 0.2506, Light: p = 0.0031, CO_2 × Light: p = 0.4856		
	Total fresh mass of all primary daughters per mother		
250 PPFD	69.5 ± 30.1	72.4 ± 32.9	85.2 ± 22.3
500 PPFD	92.1 ± 47.2	94.7 ± 44.3	93.5 ± 28.2
Significance	CO_2 Regression: p = 0.6041, Light: p = 0.0256, CO_2 × Light: p = 0.3322		
	Total fresh mass of all secondary daughters per mother		
250 PPFD	29.8 ± 16.9	31.5 ± 14.7	37.4 ± 9
500 PPFD	41.4 ± 6.71	41 ± 29.2	55.3 ± 10.2
Significance	CO_2 Regression: p = 0.0821, Light: p = 0.0038, CO_2 × Light: p = 0.6729		
	Total fresh mass of all tertiary daughters per mother		
250 PPFD	0.603 ± 0.679	2.22 ± 1.99	3.65 ± 4.65
500 PPFD	6.49 ± 5.96	3.27 ± 3.02	5.24 ± 4.14
Significance	CO_2 Regression: p = 0.5392, Light: p = 0.0366, CO_2 × Light: p = 0.2074		
	Total dry mass of all primary daughters per mother		
250 PPFD	11.2 ± 3.64	12.7 ± 4.93	14.4 ± 3.51
500 PPFD	19.6 ± 11.2	19.9 ± 7.4	18.9 ± 6
Significance	CO_2 Regression: p = 0.7287, Light: p = 0.0003, CO_2 × Light: p = 0.2343		
	Total dry mass of all secondary daughters per mother		
250 PPFD	4.39 ± 2.33	5.19 ± 2.07	5.67 ± 1.38
500 PPFD	7.68 ± 0.976	7.32 ± 4.39	9.79 ± 2.56

Table 4 (continued)

Significance	CO_2 Regression: p = 0.0784, Light: p = 0.0001, CO_2 × Light: p = 0.7385		
	Total dry mass of all tertiary daughters per mother		
250 PPFD	0.0846 ± 0.0945	0.315 ± 0.228	0.528 ± 0.674
500 PPFD	4.38 ± 7.05	0.558 ± 0.498	0.91 ± 0.704
Significance	CO_2 Regression: p = 0.2296, Light: p = 0.0949, CO_2 × Light: p = 0.1185		
	Total leaf number of all primary daughters per mother		
250 PPFD	75.8 ± 22.5	94.5 ± 40.7	108 ± 30.6
500 PPFD	92 ± 32.3	108 ± 23.3	101 ± 44.5
Significance	CO_2 Regression: p = 0.163, Light: p = 0.440, CO_2 × Light: p = 0.297		
	Total leaf number of all secondary daughters per mother		
250 PPFD	71 ± 32.6	80.8 ± 25	87.8 ± 21.5
500 PPFD	79.8 ± 15.9	92.5 ± 45.8	110 ± 27.2
Significance	CO_2 Regression: p = 0.0410, Light: p = 0.0642, CO_2 × Light: p = 0.6165		
	Total leaf number of all tertiary daughters per mother		
250 PPFD	2.17 ± 2.48	6.83 ± 5.23	7.83 ± 8.08
500 PPFD	16.8 ± 14.5	11.2 ± 9.57	15.7 ± 12.3
Significance	CO_2 Regression: p = 0.5322, Light: p = 0.0097, CO_2 × Light: p = 0.4213		
	Total leaf area (cm^2) of all primary daughters per mother		
250 PPFD	1438 ± 650	1479 ± 568	1585 ± 433
500 PPFD	2178 ± 1321	2070 ± 1039	1870 ± 591
Significance	CO_2 Regression: p = 0.5380, Light: p = 0.0061, CO_2 × Light: p = 0.2271		
	Total leaf area (cm^2) of all secondary daughters per mother		
250 PPFD	611 ± 334	631 ± 245	661 ± 118
500 PPFD	1008 ± 121	832 ± 618	1137 ± 280
Significance	CO_2 Regression: p = 0.5136, Light: p = 0.0003, CO_2 × Light: p = 0.8246		
	Total leaf area (cm^2) of all tertiary daughters per mother		
250 PPFD	11.3 ± 13.1	25.7 ± 41.7	59.1 ± 73.3
500 PPFD	159 ± 153	73.3 ± 71.2	112 ± 94
Significance	CO_2 Regression: p = 0.935, Light: p = 0.014, CO_2 × Light: p = 0.215		

Table 5: Stolon measurements, with means and standard deviations. Significance values are given for the model with model effects of repetition, CO_2, light intensity, and the interaction between CO_2 and light intensity. No interactions between CO_2 and light were found for the parameters where no interaction is listed. All analyses have n = 6 except for CO_2 500/light 500, where n = 5, and for CO_2 850/light 500, where n = 4.

Light Treatment	**500 µmol mol^{-1} CO_2**	**850 µmol mol^{-1} CO_2**	**1200 µmol mol^{-1} CO_2**
	Number of stolons per mother plant		
250 PPFD	11 ± 5.87	15.3 ± 7.61	15.8 ± 4.49
500 PPFD	11.6 ± 3.51	15.5 ± 6.24	15.3 ± 6.77
Significance	CO_2 Regression: p = 0.0321, Light: p = 0.6756, CO_2 × Light: p = 0.4569		
	Number of secondary stolons per mother plant		
250 PPFD	9 ± 1.26	13 ± 5.73	13.7 ± 5.96
500 PPFD	13.2 ± 5.81	16.8 ± 7.14	14.8 ± 4.79
Significance	CO_2 Regression: p = 0.0262, Light: p = 0.0868, CO_2 × Light: p = 0.4927		
	Number of tertiary stolons per mother plant		
250 PPFD	0.5 ± 0.548	1.67 ± 1.21	1.33 ± 1.37
500 PPFD	3.2 ± 1.92	2.25 ± 1.89	3.67 ± 3.44
Significance	CO_2 Regression: p = 0.3720, Light: p = 0.0096, CO_2 × Light: p = 0.9039		
	Length of longest stolon (cm)		
250 PPFD	155 ± 18.5	180 ± 22.8	165 ± 35.4
500 PPFD	158 ± 34	174 ± 26.6	179 ± 29.2
Significance	CO_2 Regression: p = 0.192, Light: p = 0.786, CO_2 × Light: p = 0.640		
	Stolon fresh mass (g)		
250 PPFD	40.4 ± 6.95	59.3 ± 33.2	89.3 ± 13.5
500 PPFD	78.1 ± 14.2	73.4 ± 13.2	85.8 ± 3.53
Significance	CO_2 Regression: p = 0.0029, Light: p = 0.0324, CO_2 × Light: p = 0.0224		

Table 6, Supplemental: This table shows the nutrient analysis measurements from the NCSU Phytotron solution.

Nutrient Name	**Formula**	**mg L^{-1}**
Nitrate nitrogen	NO_3	4.60
Ammonium nitrogen	NH_4	79.2
Phosphorus	P	7.78
Potassium	K	121
Calcium	Ca	45.7
Magnesium	Mg	9.54
Sulfur	S	11.2
Chlorine	Cl	0.83
Iron (chelated)	Fe	3.56
Boron	B	0.19
Manganese	Mn	0.05
Copper	Cu	0.09
Zinc	Zn	0.21
Sodium	Na	11.0
Aluminum	Al	0.00
Hardness	$CaCO_3$	153
SAR	unitless	0.39

REFERENCES

Acock, B. and M. Acock (1989). “Calculating air leakage rates in controlled-environment chambers containing plants”. In: *Agronomy Journal* 81.4, pp. 619–623.

Aldrich, R. A. and J. W. Bartok (1994). *NRAES-33 Greenhouse Engineering*. Ithaca, NY, USA: NRAES-Natural Resources, Agriculture, and Engineering Service.

Allen Jr, L. H. et al. (2011). “Elevated CO_2 increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum”. In: *Journal of Plant Physiology* 168.16, pp. 1909–1918.

Baggio, J. S. et al. (2021). “Outbreak of leaf spot and fruit rot in Florida strawberry caused by Neopestalotiopsis spp.” In: *Plant Disease* 105.2, pp. 305–315.

Balasooriya, H. N. et al. (2018). “Interaction of elevated carbon dioxide and temperature on strawberry (*Fragaria* × *ananassa*) growth and fruit yield”. In: *International Journal of Agricultural and Biosystems Engineering* 12.9, pp. 279–287.

Burgess, P. and B. Huang (2014). “Growth and physiological responses of creeping bentgrass (*Agrostis stolonifera*) to elevated carbon dioxide concentrations”. In: *Horticulture Research* 1.

Cao, J. and H. Ruan (2015). “Responses of the submerged macrophyte *Vallisneria natans* to elevated CO_2 and temperature”. In: *Aquatic Biology* 23.2, pp. 119–127.

Choi, H. G., B. Y. Moon, and N. J. Kang (2016). “Correlation between strawberry (*Fragaria* ×*ananassa* Duch.) productivity and photosynthesis-related parameters under various growth conditions”. In: *Frontiers in Plant Science* 7, p. 1607.

Choi, H. G. and H. J. Jeong (2020). “Comparison of chlorophyll fluorescence and photosynthesis of two strawberry cultivars in response to relative humidity”. In: *Horticultural Science and Technology* 38.1, pp. 66–77.

Desjardins, Y. et al. (Sept. 1988). “Effect Of CO_2 Enrichment And High Photosynthetic Photon Flux On The Development Of Autotrophy And Growth Of Tissue-Cultured Strawberry,

Raspberry And Asparagus Plants". In: *Acta Horticulturae* 230, pp. 45–54. issn: 0567-7572, 2406-6168. doi: 10.17660/ActaHortic.1988.230.3.

Despommier, D. (2011). "The vertical farm: controlled environment agriculture carried out in tall buildings would create greater food safety and security for large urban populations". In: *Journal fu¨r Verbraucherschutz und Lebensmittelsicherheit* 6, pp. 233–236.

Durner, E. F., J. Barden, et al. (1984). "Photoperiod and temperature effects on flower and runner development in day-neutral, Junebearing, and everbearing strawberries". In: *Journal of the American Society for Horticultural Science* 109.3, pp. 396–400.

Durner, E. F., E. B. Poling, and J. L. Maas (Jan. 2002). "Recent Advances in Strawberry Plug Transplant Technology". In: *HortTechnology* 12.4. Publisher: American Society for Horticultural Science Section: HortTechnology, pp. 545–550. issn: 1943-7714, 1063-0198. doi: 10.21273/HORTTECH.12.4.545.

Eamus, D. (1991). "The interaction of rising CO_2 and temperatures with water use efficiency". In: *Plant, Cell & Environment* 14.8, pp. 843–852.

Farquhar, G. D., S. v. von Caemmerer, and J. A. Berry (1980). "A biochemical model of photosynthetic CO 2 assimilation in leaves of C 3 species". In: *planta* 149, pp. 78–90.

Fennimore, S. A., & Boyd, N. S. (2018). Sustainable weed control in strawberry. In *Weed Control* (pp. 383-403). CRC Press.

Fox, J. and S. Weisberg (2019). *An R Companion to Applied Regression*. Third. Thousand Oaks CA: Sage. url: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Frantz, J. M. (2011). "Elevating carbon dioxide in a commercial greenhouse reduced overall fuel carbon consumption and production cost when used in combination with cool temperatures for lettuce production". In: *HortTechnology* 21.5, pp. 647–651.

Guiamba, H. D. S. S. et al. (2022). "Enhancement of photosynthesis efficiency and yield of strawberry (*Fragaria ×ananassa* Duch.) plants via LED systems". In: *Frontiers in Plant Science* 13, p. 918038.

Heide, O. M., J. A. Stavang, and A. Sønsteby (Jan. 2013). “Physiology and genetics of flowering in cultivated and wild strawberries – a review”. In: *The Journal of Horticultural Science and Biotechnology* 88.1, pp. 1–18. issn: 1462-0316. doi: 10.1080/14620316.2013.11512930.

Hidaka, K. et al. (2013). “Effect of supplemental lighting from different light sources on growth and yield of strawberry”. In: *Environmental Control in Biology* 51.1, pp. 41–47.

Hoffmann, M. (Aug. 2020). “An Overview of the Strawberry Nursery Industry in North America”. In: ASHS. url: https://ashs.confex.com/ashs/2020/meetingapp.cgi/Paper/33736 (visited on 04/08/2024).

Holmes, G. J., Mansouripour, S. M., & Hewavitharana, S. S. (2020). Strawberries at the crossroads: Management of soilborne diseases in California without methyl bromide. *Phytopathology*, 110(5), 956-968.

Holmes, G. J. (2024). “The California Strawberry Industry: Current Trends and Future Prospects”. In: *International Journal of Fruit Science* 24.1, pp. 115–129.

Huber, B. M., F. J. Louws, and R. Hernández (Mar. 2021). “Impact of Different Daily Light Integrals and Carbon Dioxide Concentrations on the Growth, Morphology, and Production Efficiency of Tomato Seedlings”. In: *Frontiers in Plant Science* 12. Publisher: Frontiers. issn: 1664-462X. doi: 10.3389/fpls.2021.615853.

Itani, Y., Y. Yoshida, and Y. Fujime (1998). “Effects of CO_2 enrichment on growth, yield and fruit quality of strawberry grown with rockwool”. In: *Environment Control in Biology* 34.3, pp. 125–129.

JMP Note 575394 (2022). url: https://community.jmp.com/t5/JMP-Knowledge-Base/ (visited on 05/28/2024).

Jun, H., H. Jung, and K. Imai (July 2017). “Gas exchange characteristics of a leading cultivar of Korean strawberry (*Fragaria ×ananassa*, ‘Sulhyang’)”. In: *Scientia Horticulturae* 221, pp. 10–15. issn: 0304-4238. doi: 10.1016/j.scienta.2017.04.009.

Kanno, K. et al. (2022). “Leaf photosynthesis characteristics of seven Japanese strawberry cultivars grown in a greenhouse”. In: *The Horticulture Journal* 91.1, pp. 8–15.

Keutgen, N., K. Chen, and F. Lenz (Jan. 1997). “Responses of strawberry leaf photosynthesis, chlorophyll fluorescence and macronutrient contents to elevated CO_2”. In: *Journal of Plant Physiology* 150.4, pp. 395–400. issn: 0176-1617. doi: 10.1016/S0176-1617(97)80088-0.

Kimball, B. A., K. Kobayashi, and M. Bindi (Jan. 2002). “Responses of Agricultural Crops to Free-Air CO_2 Enrichment”. In: *Advances in Agronomy*. Ed. by D. L. Sparks. Vol. 77. Advances in Agronomy. Academic Press, pp. 293–368. doi: 10.1016/S0065-2113(02)77017-X.

Kirschbaum, M. U. (Jan. 2011). “Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO_2 Enrichment Studies”. In: *Plant Physiology* 155.1, pp. 117–124. issn: 0032-0889. doi: 10.1104/pp.110.166819.

Kirschbaum, M. (1994). “The sensitivity of C3 photosynthesis to increasing CO_2 concentration: a theoretical analysis of its dependence on temperature and background CO_2 concentration”. In: *Plant, Cell & Environment* 17.6, pp. 747–754.

Kroggel, M. and C. Kubota (Apr. 2017). “Controlled environment strategies for tipburn management in greenhouse strawberry production”. In: *Acta Horticulturae* 1156, pp. 529– 536. issn: 0567-7572, 2406-6168. doi: 10.17660/ActaHortic.2017.1156.78. (Visited on 04/08/2024).

Kubota, C. et al. (2016). “Does supplemental lighting make sense for my crop?–empirical evaluations”. In: *VIII International Symposium on Light in Horticulture 1134*, pp. 403–412.

Lee, G.-B. et al. (2020). “Effect of low-light intensity on growth, yield and quality of strawberries”. In: *Journal of Environmental Science International* 29.2, pp. 167–175.

Leibar-Porcel, E. and I. C. Dodd (2023). “Role of Plant Hormones in Plant Response to Elevated CO_2 Concentrations: Above-and Below-ground Interactions”. In: *Plant Hormones and Climate Change*. Springer, pp. 55–74.

Li, R. et al. (2024). “Auxin mediated elevated CO_2-induced stolon growth and soluble sugar accumulation in creeping bentgrass”. In: *Environmental and Experimental Botany* 217, p. 105567.

Li, X. et al. (Jan. 2020). “Physiological and molecular basis of promoting leaf growth in strawberry (Fragaria ×ananassa Duch.) by CO_2 enrichment”. In: *Biotechnology & Biotechnological Equipment* 34.1, pp. 905–917. issn: 1310-2818. doi: 10.1080/13102818.2020.1811766.

Long, S., N. Baker, and C. Raines (1993). “Analysing the responses of photosynthetic CO_2 assimilation to long-term elevation of atmospheric CO_2 concentration”. In: *CO_2 and biosphere*, pp. 33–46.

Meier, M. and J. Fuhrer (1997). “Effect of elevated CO_2 on orchard grass and red clover grown in mixture at two levels of nitrogen or water supply”. In: *Environmental and Experimental Botany* 38.3, pp. 251–262.

Mochizuki, M. J. et al. (Feb. 2010). “Carbon Dioxide Enrichment May Increase Yield of Field-grown Red Raspberry under High Tunnels”. en. In: *HortTechnology* 20.1. Publisher:

American Society for Horticultural Science Section: HortTechnology, pp. 213–219. issn: 1943-7714, 1063-0198. doi: 10.21273/HORTTECH.20.1.213.

Mochizuki, Y. et al. (2013). “Analysis of a High-yielding Strawberry (*Fragaria ×ananassa* Duch.) Cultivar ‘Benihoppe’ with Focus on Dry Matter Production and Leaf Photosynthetic Rate”. en. In: *Journal of the Japanese Society for Horticultural Science* 82.1, pp. 22–29. issn: 1882-3351, 1882-336X. doi: 10.2503/jjshs1.82.22.

Muramoto, J., Baird, G., Koike, S. T., Bolda, M. P., Klonsky, K., Zavatta, M., & Shennan, C. (2014, July). Integrated rotation systems for soilborne disease, weed and fertility

management in strawberry/vegetable production. In *VIII International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation* 1044 (pp. 269-274).

Neri, D. et al. (Nov. 2012). "Strawberry production in forced and protected culture in Europe as a response to climate change". In: *Canadian Journal of Plant Science* 92.6. Publisher: NRC Research Press, pp. 1021–1036. issn: 0008-4220.

Oda, Y. (Sept. 1997). "Effects Of Light Intensity, CO_2 Concentration And Leaf Temperature On Gas Exchange Of Strawberry Plants - Feasibility Studies On CO_2 Enrichment In Japanese Conditions". In: *Acta Horticulturae* 439, pp. 563–574. issn: 0567-7572, 24066168. doi: 10.17660/ActaHortic.1997.439.95.

Ohyama, K. and T. Kozai (1998). "Estimating electric energy consumption and its cost in a transplant production factory with artificial lighting: A case study." In: *Journal of Society of High Technology in Agriculture* 10.2, pp. 96–107.

Pan, T. et al. (Feb. 2019). "Interaction of Supplementary Light and CO_2 Enrichment Improves Growth, Photosynthesis, Yield, and Quality of Tomato in Autumn through Spring Greenhouse Production". In: *HortScience* 54.2. pp. 246–252. issn: 0018-5345, 2327-9834. doi: 10.21273/HORTSCI13709-18.

Paulus, A. O. (1990). "Fungal diseases of strawberry". In: *HortScience* 25.8, pp. 885–889.

Pritchard, S. G., Rogers, H. H., Prior, S. A., & Peterson, C. M. (1999). Elevated CO_2 and plant structure: a review. *Global Change Biology*, 5(7), 807-837.

R Core Team (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. url: https://www.R-project.org/.

Ryle, G. et al. (1992). "Influence of elevated CO_2 and temperature on the photosynthesis and respiration of white clover dependent on N_2 fixation". In: *Annals of Botany* 70.3, pp. 213–220.

Sachs, T. (2002). “Developmental processes and the evolution of plant clonality”. In: *Ecology and Evolutionary Biology of Clonal Plants: Proceedings of Clone-2000. An International Workshop held in Obergurgl, Austria, 20–25 August 2000*. Springer, pp. 263–278.

Samtani, J. B. et al. (Feb. 2019). “The Status and Future of the Strawberry Industry in the United States”. In: *HortTechnology* 29.1. Publisher: American Society for Horticultural Science Section: HortTechnology, pp. 11–24. issn: 1943-7714, 1063-0198. doi: 10.21273/HORTTECH04135-18.

SAS Institute Inc. (2023). *JMP® Statistical Discovery*. Cary, NC. url: https://www.jmp. com/.

Shahbandeh, M. (2023). *U.S. total strawberry production 2022*. https://www.statista.com/statistics/193288/us-total-strawberry-production-since-2000/. Accessed 2024-04-08.

Shi, X., R. Hernández, and M. Hoffmann (Dec. 2021a). “Impact of Nitrate and Ammonium Ratios on Flowering and Asexual Reproduction in the Everbearing Strawberry Cultivar *Fragaria ×ananassa* Albion”. In: *Horticulturae* 7.12. Number: 12 Publisher: Multidisciplinary Digital Publishing Institute, p. 571. issn: 2311-7524. doi: 10.3390/horticulturae7120571.

Shi, X., R. Hernández, and M. Hoffmann (June 2021b). “Timing of Stolon Removal Alters Daughter Plant Production and Quality in the Ever-bearing Strawberry ‘Albion’”. en. In: *HortScience* 56.6. Publisher: American Society for Horticultural Science Section: HortScience, pp. 650–656. issn: 0018-5345, 2327-9834. doi: 10.21273/HORTSCI15624-20.

Shur, B. A. (2024). “The Characterization and Development of Soilless Substrate Systems for Enhanced Mother Plant Production of Strawberries in a Precision Indoor Propagation (PIP) Environment.” Masters thesis. Raleigh, NC: North Carolina State University.

Sønsteby, A. and O. M. Heide (Jan. 2007). “Long-day control of flowering in everbearing strawberries”. In: *The Journal of Horticultural Science and Biotechnology* 82.6, pp. 875–884. issn: 1462-0316. doi: 10.1080/14620316.2007.11512321.

Stadler, C. (2017). Effect of light intensity on yield of winter grown strawberries in Iceland (2017). Vol. 7. *Deutsche Gartenbauwissenschaftliche Gesellschaft Proceedings*, pp. 1–5.

Stanley, D. and K. Hammond (1998). "Hydroponic Strawberries Avoid Soil Pests". In: *USDA ARS AgResearch Magazine*, pp. 10–11.

Sun, P., Mantri, N., Lou, H., Hu, Y., Sun, D., Zhu, Y., ... & Lu, H. (2012). Effects of elevated CO_2 and temperature on yield and fruit quality of strawberry (*Fragaria ×ananassa* Duch.) at two levels of nitrogen application. *PloS one*, 7(7), e41000.

Tabatabaei, S., L. Fatemi, and E. Fallahi (2006). "Effect of ammonium: nitrate ratio on yield, calcium concentration, and photosynthesis rate in strawberry". In: *Journal of Plant Nutrition* 29.7, pp. 1273–1285.

Tagawa, A. et al. (2022). "Effects of CO_2 enrichment on yield, photosynthetic rate, translocation and distribution of photoassimilates in strawberry 'Sagahonoka'". In: *Agronomy* 12.2, p. 473.

Vatistas, C., D. D. Avgoustaki, and T. Bartzanas (2022). "A systematic literature review on controlled-environment agriculture: How vertical farms and greenhouses can influence the sustainability and footprint of urban microclimate with local food production". In: *Atmosphere* 13.8, p. 1258.

Wada, Y., T. Soeno, and Y. Inaba (2010). "Effects of light and temperature on photosynthetic enhancement by high CO_2 concentration of strawberry cultivar Tochiotome leaves under forcing or half-forcing culture". In: *Japanese Journal of Crop Science* 79.2, pp. 192–197. doi: 10.1626/jcs.79.192.

Wang, A. et al. (Oct. 2022). "CO_2 enrichment in greenhouse production: Towards a sustainable approach". In: *Frontiers in Plant Science* 13. doi: 10.3389/fpls.2022.1029901.

Wickham, H. et al. (2023). *dplyr: A Grammar of Data Manipulation*. R package version

1.1.3. url: https://CRAN.R-project.org/package=dplyr.

Wu, F., Z. Guan, and A. J. Whidden (Dec. 2018). *An Overview of the US and Mexico Strawberry Industries*. url: https://edis.ifas.ufl.edu/publication/FE971 (visited on 04/08/2024).

Xu, Q. et al. (2018). "Enhanced stolon growth and metabolic adjustment in creeping bentgrass with elevated CO_2 concentration". In: *Environmental and experimental botany* 155, pp. 87–97.

Xu, X. and R. Hernández (Jan. 2020). "The Effect of Light Intensity on Vegetative Propagation Efficacy, Growth, and Morphology of "Albion" Strawberry Plants in a Precision Indoor Propagation System". In: *Applied Sciences* 10.3, p. 1044. issn: 2076-3417. doi: 10.3390/app10031044.

Yanagi, T., K. Okamoto, and S. Takita (1996). "Effect of blue and red light intensity on photosynthetic rate of strawberry leaves". In: *International Symposium on Plant Production in Closed Ecosystems 440*, pp. 371–376.

Yoshida, H. et al. (2012). "Effects of light quality and light period on flowering of everbearing strawberry in a closed plant production system". In: *VII International Symposium on Light in Horticultural Systems 956*, pp. 107–112.

Zhang, Y. et al. (2020). "CFD analysis for evaluating and optimizing spatial distribution of CO_2 concentration in a strawberry greenhouse under different CO_2 enrichment methods". In: *Computers and Electronics in Agriculture* 179, p. 105811.

Zheng, J., D. He, and F. Ji (2019). "Effects of light intensity and photoperiod on runner plant propagation of hydroponic strawberry transplants under LED lighting". In: *International Journal of Agricultural and Biological Engineering* 12.6, pp. 26–31.

Zuniga, A. I., Wang, N. Y., & Peres, N. A. (2023). Heat treatment as a possible means to reduce Botrytis inoculum on strawberry transplants. *Plant Health Progress*, 24(3), 345-352.

CHAPTER 2

Rooting Efficacy of Different Size Strawberry (*Fragaria ×ananassa* cv. Monterey) Daughter Plants in a Controlled Environment

Abstract

Strawberry propagation relies on the asexual production of daughter plants from mother plants. It has been recently demonstrated that controlled environment techniques can yield up to 100 daughter plants per mother in 9 weeks. However, these daughter plants are of different sizes, and it is unclear whether the smallest daughter plants are capable of rooting and growing to an adequate size for commercialization. The objective of this study was to compare the rooting success rate of strawberry (Fragaria ×ananassa Duch., ‘cv. Monterey) daughter plants, categorized as large (L), medium (M), small (SM), or very small (VSM). The categories were based on their number of root nodules (L, 22-54; M, 14-20; SM, 7-12; VSM, 1-6) and crown diameter (3.5-6.7 or 6.7-13.3 mm) at planting. All daughters were transplanted into a completely enclosed growth chamber and conditions were maintained at setpoints of 25 °C, 80 µmol m^{-2} s^{-1} photosynthetic photon flux density, and ≥90% relative humidity, with a 18-hour photoperiod, for 28 days. At 14 days after transplant, 100% of the evaluated daughters had successfully developed roots. When all daughters were destructively harvested, 98% of them had well-developed roots. Furthermore, the relative growth rate during the rooting process decreased with the increase of initial plant size—smaller daughter plants had higher relative growth rate during the 28-day rooting period. This research shows that daughter plants of all sizes can be rooted successfully in controlled environment rooting chamber, allowing nurseries to use all plants produced by the stolon including small daughter plants sizes as low as 0.883 ± 0.515 g fresh mass, 4.75 ± 0.797 mm crown diameter.

Introduction

Conventional strawberry (*Fragaria ×ananassa*) propagation is a multi-year, multi-step process, currently facing several challenges including labor cost and availability, fumigation constraints and regulations, and the risk of disease. In addition to these challenges, a limiting factor for efficiency is the production of daughter plants of adequate size and quality. Strawberry mother plants produce long stolons with many daughter plants, where daughters develop young leaves and small root buds called "peg roots" (Xu and Hernández 2020, Shi et al. 2021). In conventional strawberry nurseries, these daughter plants are allowed to develop roots in the field and are later dug up and sold with the roots attached, called "bare root" plants. This method is the most common strawberry propagation method in the United States, however bare-root propagation has many challenges (Hoffmann 2020). These open-field nurseries are at high risk of disease transmission, they require annual fumigation, and they need regular maintenance (such as flower pruning and irrigation management) throughout the season (Poling 2008, Maas 2012, Hoffmann 2020, Holmes 2024). Furthermore, it takes years to propagate enough strawberry plants to sell. In California alone, the nursery system produces 1.5 billion plants annually (Holmes 2024). This high number of plants demanded by fruit growers takes three to five years to produce, and therefore the costs of this propagation system are compounded by time. Due to these mounting challenges, nurseries are seeking additional methods for propagating strawberry daughter plants.

Using controlled environment (CE) systems to propagate strawberries has potential to mitigate some of the abovementioned challenges. Controlled environment systems have several benefits, including control of the environmental conditions and reduction of pathogens. Research in CE using day neutral 'Albion' showed that mother plants can produce up to 56-100 daughters

in 63 days (Shi et al 2021). In greenhouse experiments using other cultivars, mother plants have been reported to produce up to 84 daughter plants (Bish et al, 2001).

There are many questions open about CE strawberry propagation, especially in one key area: we don't know how many of these daughter plants are usable for rooting and sale. In greenhouses and CE systems daughter plants are grown vertically and they develop a range of daughter plant sizes, becoming progressively smaller the farther they are from the mother plant. For example, Hokanson and Takeda 2001 weighed 'Chandler' daughters from different positions on the stolon and found that the first-position daughters (closest to the mothers) weighed 672 grams per 100 daughters, compared to the fifth-position daughters which weighed less than 100 grams per 100 daughters. In addition to fresh mass, these daughters typically differ in their leaf number, leaf area, and other size metrics, such as crown diameter. Xu and Hernández 2020 classified the different daughter plant sizes based on crown diameter and showed that ≤10% of daughter plants were in the large or extra-large categories, about 60% to 66% of daughter plants were small and medium categories, and 20% to 26% were in the "premature bud" category. Additionally, Shi et al. 2021 described the relative size of daughter plants by calculating the percentage of plants in the lower 25%, middle 50%, or upper 25% for metrics like crown diameter, leaf number, number of roots, and dry weight, and used these metrics to compare between treatments of harvest interval (7-day, 21-day, and 63-day treatments).

Limited research on daughter plant size has already been conducted, but these studies vary in scope and results. Some reports (Takeda and Newell 2007, Hokanson and Takeda 2002, E. Bish et al. 1996) have concluded that smaller daughter plants could have a similar rooting success rate to 'standard' sized plug plants. For instance, Hokanson and Takeda 2002 categorized daughter plant size by fresh mass, transplanted them into plasticulture beds, and

found that plug plants greater than 1 gram in size had a 96% rate of successful rooting in two weeks, and that 1-gram daughters had an 87% rate. Takeda and Newell 2007 concluded that daughters greater than 1 gram in size were suitable for plasticulture. Kozai et al. 2019 also found that young daughters on recently developed stolons, with only one unfolded bract (small leaflike structure) could develop root systems in controlled environment conditions. This research indicates that daughters seem to have a similar rate of successful rooting, regardless of initial size.

Research has shown that younger leaves have higher net photosynthetic and growth rates compared to older leaves (Bielczynski et al. 2017). Therefore, it is plausible that smaller daughter plants (with younger leaves) will exhibit a higher relative growth rate during the rooting process than larger daughter plants under the same conditions. The objective of this experiment is to evaluate the rooting capability of various sizes of strawberry (‘Monterey’) daughter plants. Additionally, we aim to determine whether daughter plant size affects their growth and development during rooting. The two hypotheses are: 1) All daughter plants will have the capability to root, regardless of size. 2) Smaller daughter plants will exhibit an increased relative growth rate during rooting.

Materials and Methods

Mother Plant Conditions

‘Monterey’ was selected for this study due to its widespread popularity with fruit growers (Holmes 2024) and the fact that it is a long-day cultivar and produces flowers in propagation fields. As a long day cultivar, it produces flowers/fruits at the same time as it produces daughter plants, and the practice of removing flowers to promote stolon development is costly for conventional nurseries (Hoffmann 2020, Shi, et al 2021).

The original stock plants were sourced from Lassen Canyon Nursery (Macdoel, CA) and grown at North Carolina State University in a heated plastic hoop house from October 11, 2021, to March 2022. These stock plants had no visible pests, diseases, or deficiencies by the time they started producing stolons and daughter plants in mid-February (Figure 14A). Stolon and daughter plant development was tracked for two weeks prior to daughter plant harvest, and on March 8th, 2022, daughters were harvested from stock plants.

Experiment Initiation

On the day of experiment initiation, stolons were harvested from the stock mother plants and data was collected (Figure 14B), and each daughter was planted. One of these measurements was "position", indicating the position of the daughter plant on the stolon, relative to the other daughters on the stolon. Morphological data was collected (see Table 7), including crown diameter (mm) and number of visible peg roots, where any round bump-like protrusion on the crown was considered a peg root, regardless of its color or length. All peg roots did not exceed 5 mm. Number of leaves longer than 1 cm and length of the longest leaf (including petiole) were measured. SPAD chlorophyll content (Model SPAD-502; Spectrum Technologies, Plainfield, IL, USA) was measured from the apical leaflet of the largest leaf on each daughter plant. After measurement, daughters were cut from their stolons (with 1 cm of stolon still attached to each daughter plant) and were weighed with an electronic scale for fresh mass (g) (Ohaus Corporation, Pine Brook, New Jersey, USA). Based on these measurements, 48 daughters were selected for this trial, and were organized into four treatment categories: large (L), medium (M), small (SM), and very small (VSM). This trial was not replicated in time. Treatments were separated by root number and crown diameter, where L had 22-54 roots and 6.7-13.3 mm crown

diameter, M had 14-20 roots and 6.7-13.3 mm crown diameter, SM had 7-12 roots and 3.5-6.7 mm crown diameter, and VSM had 1-6 roots and 3.5-6.7 mm crown diameter.

Daughters were planted into 50-cell seedling trays with 70-mL wells (Figure 13). These seedling trays were prepared for the experiment to allow for easy access to the roots: Each well had a single cut down one side, and the cut was taped closed. This cut made it easy for researchers to periodically remove the tape and gently open the well, for non-destructive root assessment throughout the experiment. After assessment, the well was taped shut again and returned to its original position within the growth chamber. Substrate was composed of 50% coconut coir, 50% perlite by volume, and daughters were planted with peg roots about 5 mm below the substrate surface.

Experiment Conditions

Four daughter plants were spaced evenly in the center of each seedling tray, to avoid shading, with one daughter plant of each treatment per tray. A total of 12 trays were used (12 repetitions), and all trays were placed within the same growth chamber. Each tray was placed under its own tunable light emitting diode (LED) fixture (325 Pro HV LED; LumiGrow, Emeryville, CA, USA), and all fixtures were set to provide 80 µmol m^{-2} s^{-1} at leaf level, with an 18-hour photoperiod. Between the light fixtures and the plants there was a transparent acrylic sheet to minimize temperature effects. To ensure all daughter plants within each tray received approximately the same intensity of light (Table 8), individual wells were raised to the height of the tallest plant in each tray. For example, if the tallest plant within the tray was 5 cm taller than the smallest plant, the smallest plant was raised by 5 centimeters. As these plants grew taller, plant height was manually adjusted.

The chamber was maintained at setpoints of 24 °C and 99% relative humidity until day 26, when the relative humidity setpoint was lowered to 90%. The humidity setpoint was lowered once transplants were able to take up adequate water through their roots. These environmental conditions were controlled with a CR1000 data logger (CR1000; Campbell Scientific Inc., Logan, UT, USA), and high humidity was maintained with deionized water and ultrasonic foggers.

Starting six days after the experiment was initiated, every other day daughters were watered with nutrient solution containing 49.5 mg L^{-1} N, 23.2 mg L^{-1} P, 72.6 mg L^{-1} K, 101 mg L^{-1} Ca, 29.6 mg L^{-1} Mg, 57.2 mg L^{-1} S, 53.4 mg L^{-1} Cl, and micronutrients (Table 11). The plants were removed one by one for watering. Using a 10 mL plastic dosing syringe, we applied a known volume of water to the substrate of each plant. Five minutes after watering leachate was collected, but if no leachate was observed, plants were given another dose of water. This process was repeated until leachate began dripping from the bottom of the seedling well.

Fourteen days after the experiment was initiated, 50% of the daughters were evaluated for daughter plant height and for whether any roots developed (yes/no binary rating). The daughters chosen for this evaluation were from the reps on one half of the growth chamber (trays 1, 2, 3, 7, 8, and 9). Root assessment involved removing one seedling tray well and gently opening it from the split side (Figure 14D). This gentle method allowed us to see if roots were touching the wall of each seedling tray well, while also minimizing disruption of roots during root assessment.

Destructive Measurements

At 28 days after experiment initiation, final harvest data was collected, and daughters were destructively harvested. These measurements included shoot height (mm), number of

leaves larger than 1 cm, length of longest leaf, crown diameter (averaged from two measurements), and SPAD chlorophyll contents (averaged from two measurements) measured from both the oldest and youngest leaves. We gently pulled the plants and their roots and substrate from the seedling trays, and roots were visually evaluated using a rating scale from 1-3, where 1 denoted plants which grew some roots, but those roots were not holding the substrate together, 2 denoted plants which grew enough roots throughout the substrate to hold the mass of substrate together in a single clump, and 3 denoted plants which grew vigorous roots, which layered upon each other in the substrate (Figure 16). For all the plants, the substrate was washed away, root length (cm) was measured, and root and shoot fresh mass (g) were measured separately. We took pictures of each treatment block for later comparison. Root and shoot tissue of each daughter plant were separately placed in paper bags and left in a drying oven (VWR-1685; Avantor Inc. PA, USA) for three days, before being weighed using an electric scale (MS104TS; Mettler Toledo, Greifensee, CH).

Statistical Analyses

All statistical analyses were conducted using R version 4.2.1 (R Core Team 2021). The data comprised four treatments with twelve repetitions each, however, the final measurements excluded three plants: the two that died during the experiment, and the one that did not produce any roots. Data was cleaned and prepared using the tidyverse package (Wickham et al. 2019).

To check for normality, linear regression models were fitted with the lm() function in base R, with various combinations of dependent and independent variables. Residual plots were generated to assess the goodness-of-fit, and these plots seemed to confirm the assumption of normality (R Core Team 2021).

To evaluate the assumption of homogeneity of variances across different treatment groups, both Levene's Test and Bartlett's Test were used, with functions leveneTest() from the car package and bartlett.test() from base R (Fox and Weisberg 2019, R Core Team 2021). We evaluated the significance of the results, where the significance level was set at $\alpha = 0.05$. Numerous variables were statistically significant in one or both tests, indicating that the assumption of homogeneity of variances was not met for multiple variables (including initial root number, root and shoot dry mass, and others). A logarithmic transformation was applied to these variables using the log() function from base R, and the normality and homogeneity tests were repeated on the transformed variables. Many of these log transformed variables still generated significant values, indicating that even after transformation there remained statistically significant differences in the variances between treatment groups.

Therefore, to accommodate the limitations of our dataset, we applied the Welch's ANOVA test using base R function: oneway.test(variablename ~ treatment, data = dataset, var.equal = FALSE) (R Core Team 2021). This test is generally recommended as an alternative to Classic ANOVA when the dataset does not assume homogeneity of variances. We set the significance level at $\alpha = 0.05$.

In our analysis of relative growth rate data, we also applied a linear model using the base R function lm(), with the only model effect being the variable of interest, listed on the x-axis of the pertinent plots (R Core Team 2021). Treatment was not used as a model effect for these linear models.

To compare overall rates of rooting, survival, and success, we created contingency tables for each of these metrics, then applied Fisher's Exact Test. This test was used to detect if there

are statistically significant differences in rooting, survival, and success between treatments. To perform this test we used the function fisher.test() from base R (R Core Team 2021).

Results and Discussion

As shown in Table 9, the survival rate was 100% in all treatments except the large treatment, where two plants died. This could be due to desiccation as a result of too much transpiration—the large plants started out with more leaves and greater leaf area, which likely caused their whole-plant transpiration rate to be greater than the other treatments. It is possible that even in this high humidity environment they were unable to retain adequate water for survival, however this is unlikely because the VPD was calculated as 0.02 ± 0.06 kPa. This could also be an issue resulting from their morphology—in our previous preliminary work, we found that large daughter plants tended to stick poorly in substrate, where their large leaves make them more likely to tip over during transport/handling of the tray, causing them to have poor crown-to-substrate contact. This is due to the heavy weight of the leaves, causing the plant to shift after transplant, even when handled gently.

Between all treatments the rooting rate was also greater than expected, where 100% of the surviving plants rooted, except for one plant in the very small treatment. Although this plant survived and seemed visually healthy for the full duration of the 28-day experiment, it did not develop any roots longer than 5 mm. The success rate combined the survival rate and rooting rate into one “success” value, and these results indicate that the medium and small treatments may have a greater overall success rate compared to the large and very small treatments. However, when we applied the Fisher’s Exact test to detect significant differences between treatments, we

found that the significance of survival, rooting, and success rate was not significant. whether this effect might be significant when greater sample sizes are used.

Table 9: Survival rate is the number of plants per treatment by the end of the 28-day experiment. Rooting rate indicates the number of plants that produced roots longer than 5 mm by the end of the 28 days. Success rate combines both survival rate and rooting rate into a single value, where "success" is granted to any plant that has produced roots longer than 5 mm and that has not died.

Root Growth

Root development was measured in three ways: by a binary "rooting success" metric, by visual root rating, and by quantitative measurement. We expected that the most roots would develop in the largest treatment, and that the fewest roots (or no roots) would develop in the very small treatment. We found that in general, larger plants developed more roots than smaller plants, but smaller plants had much more rooting success and overall root mass than expected.

Rooting Rate

"Rooting rate" was a binary measurement of whether the plants developed any roots longer than 5 mm. We expected some plants to develop no roots, but almost 100% of surviving plants developed roots that proliferated through the soil volume. Even the smallest daughter plants developed several inches of roots that extended through the substrate, far surpassing the 5 mm threshold we initially specified. This rooting rate of 45/46 (or 97.83% between all treatments) was far greater than expected from previous preliminary experiments. This

experiment maintained higher relative humidity than our preliminary work, and we believe that is why rooting success rate was so high. Rooting success is described in Table 9.

Visual Assessment: Root Rating

The visual root rating indicated how thoroughly the roots had proliferated through the substrate, from what was visible on the surface of the "root ball". Examples of each rating are shown in Figure 16, and the distribution of plants from each treatment assigned to each rating are shown in Figure 17. As illustrated in Figure 17, plants with very strong root development (rating 3) tended to be large and medium plants, and very small plants tended to have roots that were more loosely holding the substrate (rating 1).

Although root rating is a visual categorical metric, we can also average these ratings to indicate what the 'average' root rating of each treatment was (Figure 15). The average root rating measurements were almost evenly spaced between treatments: large (2.5), medium (2.33), small (1.88), and very small (1.27).

These root rating results indicate that plants which started at a large or medium size filled their root zone with roots more quickly than the small and very small plants and were possibly ready to be transplanted into larger pots or trays sooner than the small and very small plants. This is also consistent with E. Bish et al. 1996, who found that daughters at a later developmental stage (i.e. plants that were initially larger) were ready to be transplanted sooner. Plants with root rating of 2-3 are considered salable (pers comm. Hoffman); therefore, plants which are large, medium, or small may be salable within 28 days of transplant, and that very small plants may need slightly more time to establish a stronger root ball before they can be sold.

Quantitative Root Measurements

The quantitative measurements of root development included length of longest root (cm), fresh biomass of roots (belowground biomass, g), dry biomass of roots (g), and the percent of plant fresh mass that was allocated to the roots (belowground biomass, %).

The root dry mass was significantly different between treatments ($p = 0.0009$) and was greatest for the large and medium treatments, compared to the small and very small treatments. This trend was also similar for the fresh mass ($p = 0.0011$), where the fresh mass of the large and medium treatments (2.14 and 1.92 g) seemed greater than the fresh mass of the small and very small treatments (0.69 and 0.53 g). The percentage of the plant mass that is made up of the roots (belowground biomass, %) is also significantly different ($p = 0.0183$). As shown in Table 10, the total biomass is made up of a greater percentage of roots for the large (21%) and medium (19%) treatments than for the small (16%) and very small (13%) treatments.

Although Welch's ANOVA only tests for differences between group means and does not indicate the nature of these differences, we observe that generally the medium and large treatments seem to have significantly more root development than the small and very small treatments. This indicates that root development is significantly impacted by initial crown diameter and fresh mass. This could be explained in part by stored carbohydrates, where plants with larger crown diameters have a larger crown mass overall, which will provide a larger store of energy for the daughter plants to allocate to root development.

Overall Growth

We hypothesized that growth rate would vary between daughter plants of different initial size. Generally, the larger two treatments (large and medium) remained larger than the small and

very small treatments at the end of the 28-day growth period (Table 10). The large and medium treatments produced similar results for final shoot height, leaf number, and other final measurements, despite having significantly different fresh mass and leaf number at the start of the experiment. For the smaller treatments, a greater proportion of the mass was made up by the shoots, and a lesser proportion was made up by the roots. We calculated the percentage of the plant fresh mass that was made up of the aboveground portion of the plant, there were significant treatment differences ($p = 0.0183$) (Table 10). We also recorded the position of the daughter on the stolon, and whether the daughter came from the primary stolon or from a branched stolon coming off the primary stolon. In theory, position could affect the photoassimilates the daughter received from the mother, and thus the long-term performance of daughters, however we saw no effect of position on growth or size by the end of the 28-day rooting period. However, the relative growth rate of fresh mass decreased with the increase of the plant's initial size (initial plant fresh mass and initial crown diameter) (Figure 18).

Application in Industry

The key finding of this research was that most daughter plants are able to root, even the very small daughter plants. However, even though most daughter plants can root successfully, categorizing them and growing them separately based on these categories is probably ideal to prevent the large plants from shading the smaller plants. In industry applications, two categories are probably enough to adequately separate large and small daughters. This is the current standard practice in Europe, where daughters are split into two categories based on size (per comm. Hoffmann 2024). We recommend that industry members select daughter plant categories based on a metric that is easy and quick to measure, like leaf number or fresh mass. For example,

there is no need to measure crown diameter because it takes much more time and effort, and our correlation analysis showed that it is not a better predictor of daughter plant growth than the other metrics (data not shown).

This finding could broaden the scope of which daughter plants are salable. Production of daughter plants is somewhat dependent on cultivar, but also dependent on how the grower defines the minimum size for sale. In personal communications with growers, the authors learned that different growers use different methods to decide which plants are salable, including by looking at the number of roots and the overall fresh mass of the daughter plant. However, this research indicates that almost all daughter plants (93.75%) may survive and produce roots and may be sold after rooting for only ~30 days. Furthermore, when mother plants are grown in a greenhouse or controlled environment, they tend to produce a greater number of daughter plants than in the field (Bish, et al, 2001; Shi, et al 2021). Based on previous research that demonstrated mother plants can grow 56-100 daughter plants, if we assume that 93.75% of those daughter plants were salable, this would be an improvement over the field production by as little as 87% (30 vs 56) to as much as 900% (10 vs 100). This increase in mother plant efficiency indicates that controlled environments may be a more efficient approach for strawberry propagation, compared to conventional field methods.

As shown in Figure 7, even after rooting there is a difference in the size of the plug plants—therefore we must discuss how the size of the plug plants affects ultimate fruit production. To do this, we must separately discuss early yield (produced in late winter and early spring) separately from late yield (produced in late spring through fall).

Rice (1986) suggests that early yield is highly dependent on the number of flowers which have initiated prior to planting, where fruit weight may be more dependent on the carbohydrates

stored in the plant. According to this theory, we may expect larger plug plants to have greater early yields. This is consistent with data reported by Bish (1996), who found that larger plugs had increased early production, and theorized that this was due to larger plug plants having more carbohydrate reserves. Takeda (2001) also found that although the size of small daughters (1 compared to 5 g) had no effect on the initial bloom date or crown development, the larger daughters produced slightly more fruit early. However, this early fruiting effect seems cultivar dependent. Rice (1986) found that larger initial plant size correlated positively with early yield of the early cultivar 'Cruz', but not early yield of the late-season cultivar 'Sequoia'. In the same study, the somewhat-early cultivar 'Douglas' had a greater number of fruits from plants of larger daughters, however smaller plants produced larger berries, and initial plant size did not correlate with total fruit weight. This result with 'Douglas' indicates that initial tip size affected flower number but did not affect the plant's ability to yield fruit biomass. Overall, these findings from Bish (1996), Takeda (2001), and 'Cruz' in Rice (1986) indicate that initial plant size affects early season fruit yield, but the findings from 'Douglas' and 'Sequoia' in Rice (1986) suggest that this effect is highly cultivar dependent.

In the same publication, Rice (1986) also theorized that late yield is most dependent on the photosynthetic capacity and growth rate of the plant, which seems to be uniform despite initial size differences. Rice (1986) found that late yield was unaffected for all initial sizes regardless of cultivar, between 'Cruz', 'Douglas', and 'Sequoia'. Bish (2001) also found that size differences between strawberry plants at transplanting did not affect yields of 'Sweet Charlie'. And, as previously described, 'Douglas' produced the same fruit mass regardless of initial size, even though the initially smaller plants produced fewer fruits. Overall, it seems that regardless of cultivar, late-season fruit mass is not affected by initial tip size. In several cultivars, it seems that

propagating small daughters may not affect overall yield, and therefore smaller cuttings are a viable option for growers that use these cultivars.

Economics

Overall, propagating and rooting strawberry plants in protected environments like greenhouses and controlled environments will introduce additional costs. However, one of the biggest limiting factors for strawberry nurseries is the number of daughter plants produced per mother, and protected environment propagation has the potential to double or triple the number of daughter plants that could be produced by a single mother plant. It takes 3 to 5 years for conventional nurseries to produce enough daughter plants for fruit growers. However, this method could greatly reduce the time required, by increasing the efficiency of rooted plug plants that can be produced by one mother plant.

Conclusion

Conventional open-field strawberry propagation has many risks and costs, but recent research indicates that controlled environments (greenhouse or controlled environment systems) could help reduce these challenges. The research presented above indicates that most daughters, even very small daughters, can be rooted successfully as tray plants. The implication of this finding is that mother plants grown in controlled environments could be 2 to 4 times more productive than they are in conventional field systems, where controlled environments can yield as many as 100 daughter plants, compared to the 10 to 30 daughters per mother produced in the field. Future work must continue to investigate the economics of sheltered environment

strawberry propagation. Future work also must be done to determine methods which prepare strawberry plug plants to be transplanted into the field, such as vernalization techniques.

FIGURES

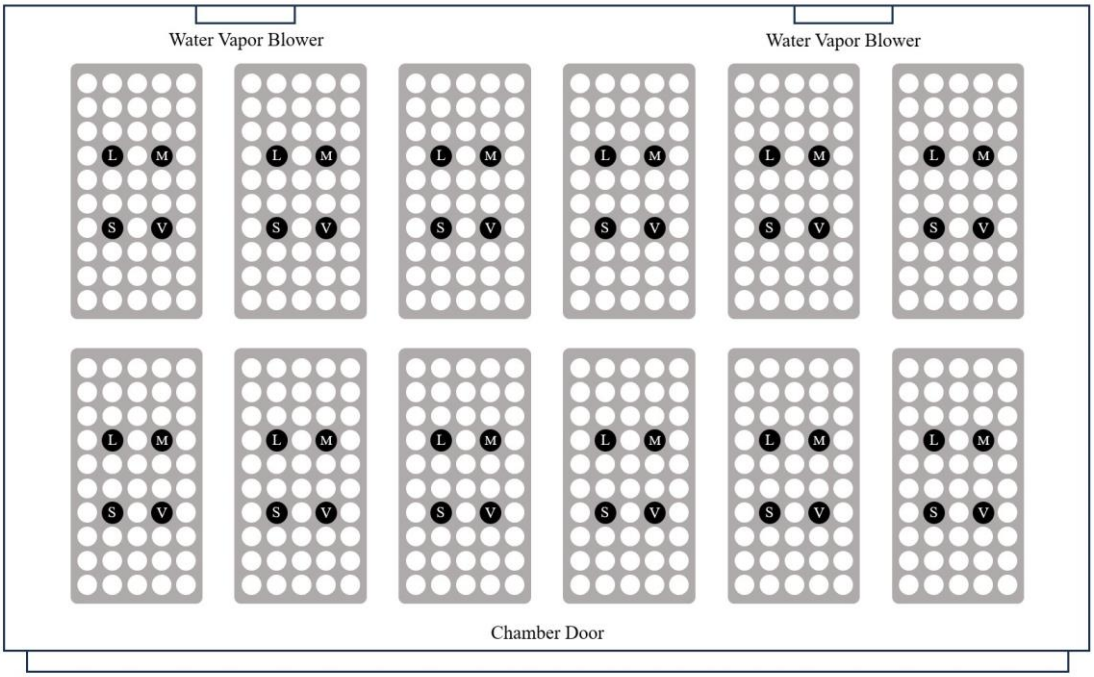

Figure 13: Chamber layout, showing door placement and water vapor blower placement relative to all trays.

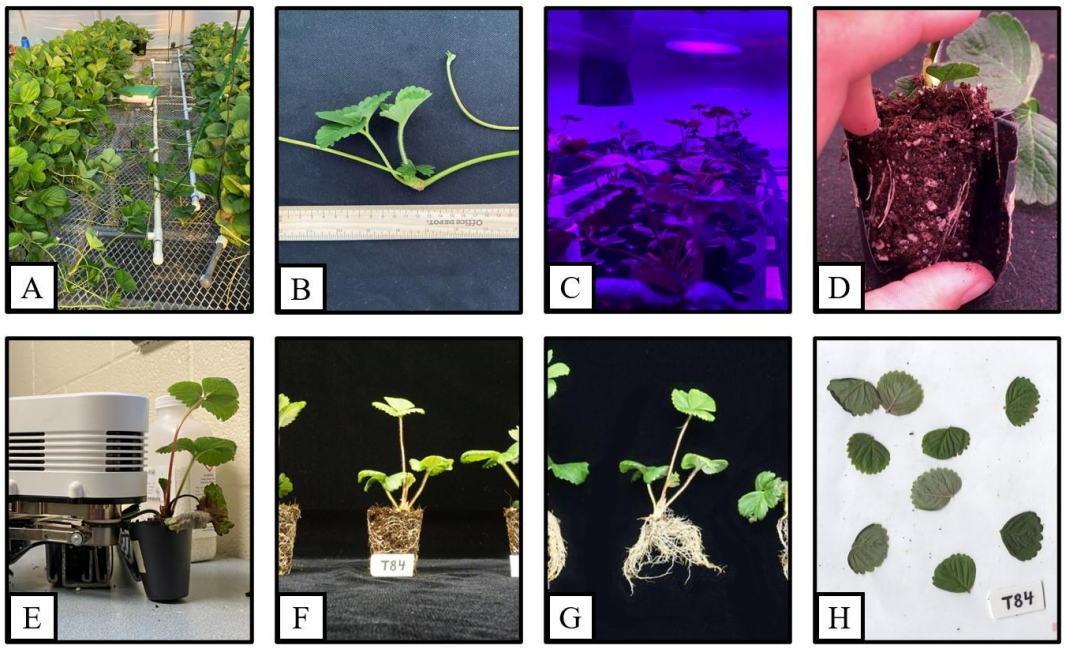

Figure 14: The progression of the experiment, in sequential order from A to H. Part A: Daughter plants growing in the greenhouse. Part B: Initial evaluation of daughter plant size and assignment of treatments. Part C: Plants placed into the chamber. Part D: On day 14, plants were evaluated for presence of roots. Part E: On day 22, photosynthetic measurements were taken on a sample of plants (data not shown). Part F: Plants were evaluated for root development and nondestructive measurements. Part G: Roots were washed, additional pictures were taken, and plants were destructively harvested. Part H: Leaf scans were collected so that leaf area may be evaluated in the future, after harvest by image processing with ImageJ software.

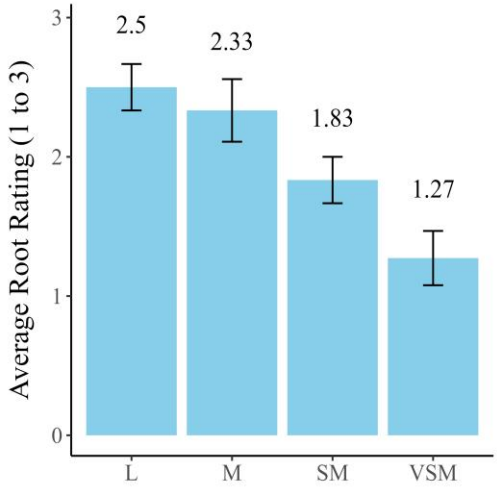

Figure 15: Average root rating on day 28, plotted by treatment. These values are unitless mean values based on the visual root rating assessment.

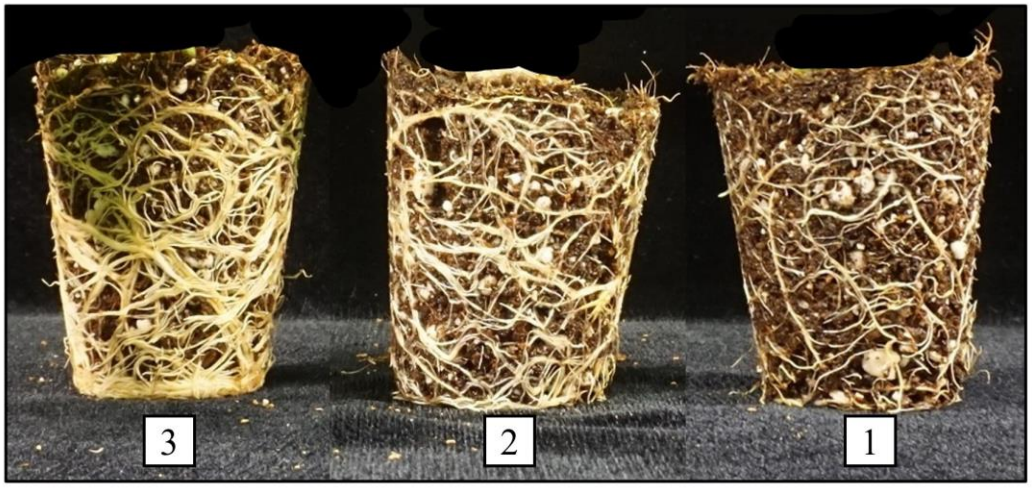

Figure 16: Examples of each root rating, where 1 denoted plants which grew some roots, but those roots were not holding the substrate together, 2 denoted plants which grew enough roots throughout the substrate to hold the mass of substrate together in a single clump, and 3 denoted plants which grew vigorous roots, which layered upon each other in the substrate.

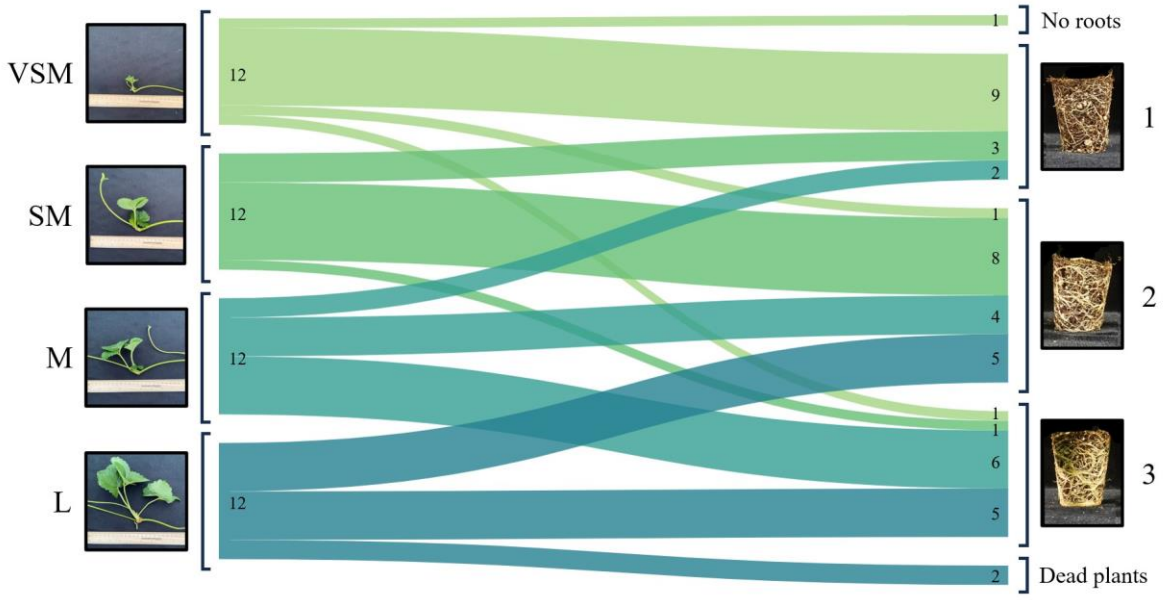

Figure 17: Sankey plot demonstrating the distribution of plants from each treatment varied at day 28 when root rating (1 to 3) was evaluated.

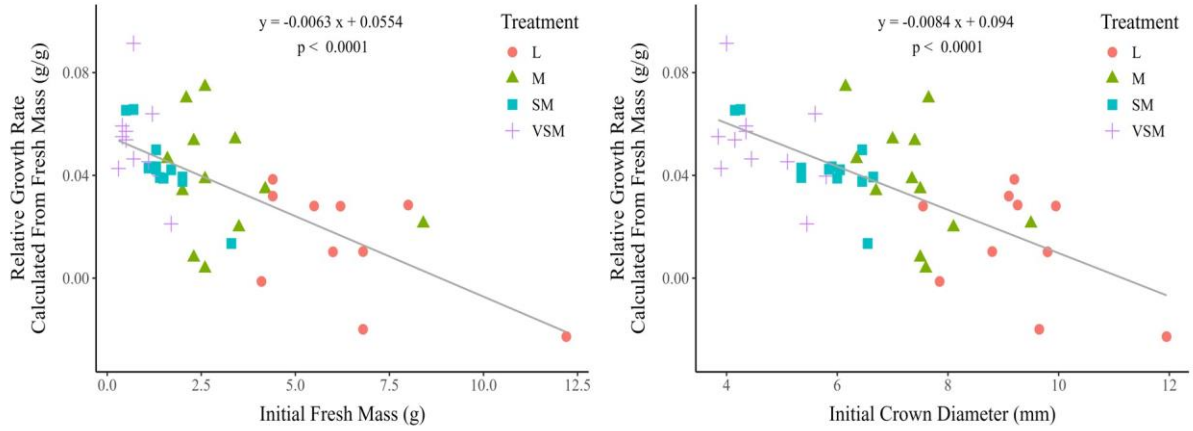

Figure 18: Relative growth rate, plotted against initial fresh mass and crown diameter. These plots show how relative growth rate at day 28 can be extrapolated from the fresh mass before transplant.

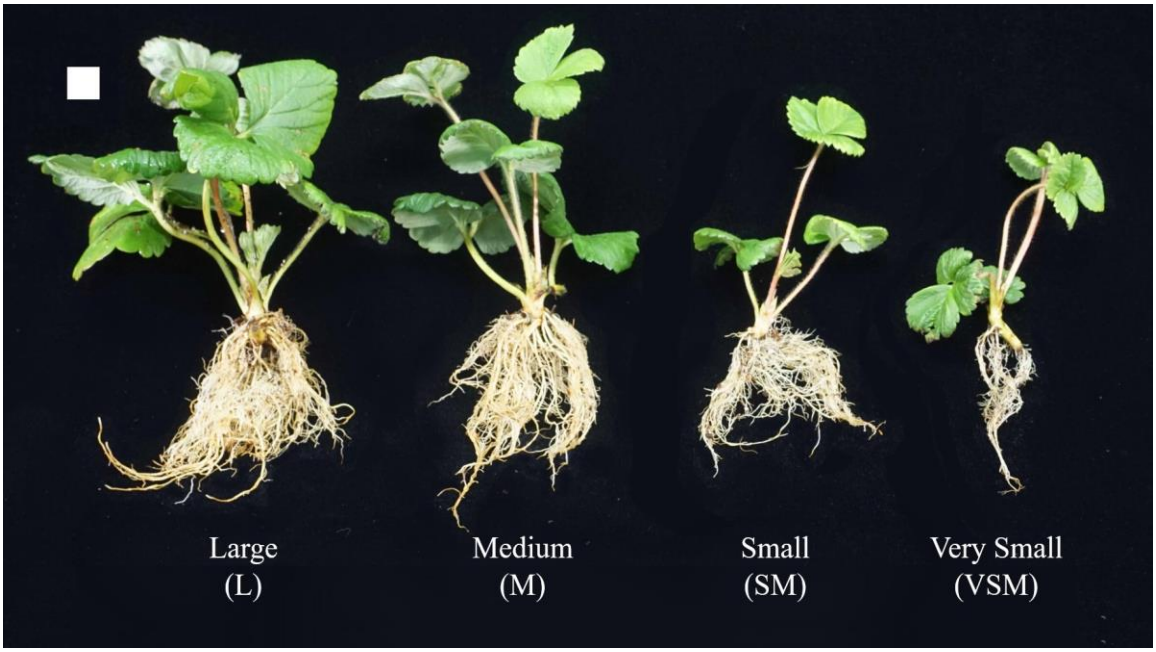

Figure 19: An example image of all the plants from one tray, showing a clear difference in root mass between treatments. This picture was taken after the roots were washed, but before the destructive measurements were collected. The white square in the upper left corner is a 1 cm size reference. This image was edited only to remove dust from the black background, the plants themselves were not manipulated to create this image.

Table 7: Initial size measurements, collected on the day of experiment initiation. The sample size for each treatment is n = 12.

Dependent Variable	Large	Medium	Small	Very Small
Root Number	33.6 ± 10.3	16.9 ± 2.27	9.08 ± 2.11	3.17 ± 1.59
Leaf Number	3.58 ± 0.67	3.08 ± 0.67	2.17 ± 0.58	1.83 ± 0.39
Length of longest leaf (cm)	13.5 ± 1.44	9.88 ± 2.43	6.19 ± 1.54	3.71 ± 1.40
Fresh Mass (g)	6.35 ± 2.17	3.13 ± 1.81	1.51 ± 0.72	0.88 ± 0.51
Crown Diameter (mm)	9.36 ± 1.11	7.4 ± 0.87	5.75 ± 0.84	4.75 ± 0.80
SPAD (unitless)	36.9 ± 3.78	36.5 ± 3.82	33.7 ± 2.94	30.6 ± 5.35

Table 8: Measured values (average +/- SD) for environmental conditions.

Measurement	**Value**
Temperature (°C)	24.2 ± 1.30
CO_2 Concentration (µmol mol^{-1})	470 ± 73.0
Relative Humidity (%) for the first 26 days	99.3 ± 2.27%
Relative Humidity (%) for the last 2 days	90.4 ± 4.79%
Photoperiod (hours)	18
Light intensity (µmol m^{-2} s^{-1})	80.4 ± 0.95
Light spectrum (%)	70.0 ± 1.03 red, 30.0 ± 1.03 blue
Light spectrum (µmol m^{-2} s^{-1})	56.2 ± 0.97 red, 24.1 ± 0.92 blue
pH	5.8 ± 0.5
EC (µS cm^{-1})	2.0 ± 0.4

Table 9: Survival rate is the number of plants per treatment by the end of the 28-day experiment. Rooting rate indicates the number of plants that produced roots longer than 5 mm by the end of the 28 days. Success rate combines both survival rate and rooting rate into a single value, where "success" is granted to any plant that has produced roots longer than 5 mm and that has not died.

Treatment	**Survival Rate**		**Rooting Rate**		**Success Rate**	
Large	10/12	83.3%	10/10	100%	10/12	83.3%
Medium	12/12	100%	12/12	100%	12/12	100%
Small	12/12	100%	12/12	100%	12/12	100%
Very Small	12/12	100%	11/12	91.7%	11/12	91.7%
Significance	P = 0.2340		P = 1.0000		P = 0.6004	

Table 10: Final measurements collected on day 28 from the surviving daughter plants, excluding the two dead plants and the unrooted plant. Therefore, the sample size for each treatment is n = 12, except for the large (L) treatment where n = 10, and the very small (VSM) treatment where n = 11.

Dependent Variable	Large	Medium	Small	Very Small	Significance
Shoot Height (mm)	108 ± 13.6	105 ± 25.2	92.7 ± 17.5	86.8 ± 30.0	p = 0.0707
Total Number of Leaves	5.00 ± 1.05	5.17 ± 1.19	4.42 ± 0.52	4.09 ± 0.94	p = 0.0784
Length of Longest Leaf (mm)	129 ± 14.9	134 ± 18.3	113 ± 19.4	101 ± 24.0	p = 0.0038
Average Crown Diameter (mm)	9.73 ± 1.80	9.44 ± 2.02	7.78 ± 0.775	6.99 ± 1.65	p = 0.0033
Average SPAD of Old Leaves	36.5 ± 3.95	35.7 ± 2.67	34.5 ± 2.76	32.7 ± 2.10	p = 0.0188
Average SPAD of Young Leaves	24.3 ± 4.18	28.4 ± 4.58	25.2 ± 4.06	22.8 ± 2.91	p = 0.0186
Average SPAD	30.4 ± 2.81	32.1 ± 2.83	29.8 ± 2.20	27.7 ± 2.05	p = 0.0036
Length of Longest Root (cm)	11.8 ± 1.5	10.9 ± 1.60	12.3 ± 1.91	11.4 ± 1.65	p = 0.3145
Aboveground Biomass (g)	7.50 ± 3.05	7.50 ± 4.23	3.77 ± 0.81	2.87 ± 1.71	p = 0.0009
Belowground Biomass (g)	2.14 ± 1.19	1.92 ± 1.24	0.69 ± 0.19	0.53 ± 0.55	p = 0.0011
Total Biomass (g)	9.65 ± 4.22	9.42 ± 5.36	4.46 ± 0.85	3.41 ± 2.23	p = 0.0009
Aboveground Biomass (%)	0.79 ± 0.05	0.81 ± 0.06	0.84 ± 0.05	0.87 ± 0.06	p = 0.0183
Belowground Biomass (%)	0.21 ± 0.05	0.19 ± 0.06	0.16 ± 0.05	0.13 ± 0.06	p = 0.0183
Shoot Dry Mass (g)	1.66 ± 0.69	1.68 ± 0.94	0.73 ± 0.14	0.57 ± 0.44	p = 0.0005
Root Dry Mass (g)	0.35 ± 0.18	0.32 ± 0.20	0.13 ± 0.03	0.09 ± 0.09	p = 0.0009
Total Dry Mass (g)	2.01 ± 0.87	2.01 ± 1.12	0.85 ± 0.16	0.66 ± 0.53	p = 0.0005
Leaf Mass Ratio, LMR (kg/kg)	0.83 ± 0.03	0.85 ± 0.04	0.85 ± 0.03	0.87 ± 0.04	p = 0.14
Percent Dry Mass	0.21 ± 0.03	0.21 ± 0.03	0.19 ± 0.01	0.19 ± 0.02	p = 0.057

Table 11, Supplemental: Nutrient solution test results from the North Carolina Department of Agriculture.

Nutrient Name	**Formula**	**mg L^{-1}**
Nitrate nitrogen	NO_3	49.51
Phosphorus	P	23.22
Potassium	K	72.61
Calcium	Ca	100.99
Magnesium	Mg	29.58
Sulfur	S	57.17
Chlorine	Cl	53.35
Iron (chelated)	Fe	10
Boron	B	0.18
Manganese	Mn	0.31
Copper	Cu	0.25
Zinc	Zn	0.17
Sodium	Na	0.19
Molybdenum	Mo	0.03

REFERENCES

Barclay Poling, E. and J. Maas (1998). “Strawberry plug transplant technology”. In: *XXV International Horticultural Congress, Part 3: Culture Techniques with Special Emphasis on Environmental Implications, 513*, pp. 393–402.

Bielczynski, L. W. et al. (2017). “Leaf and plant age affects photosynthetic performance and photoprotective capacity”. In: *Plant physiology* 175.4, pp. 1634–1648.

Bish, E. et al. (1996). “Development of containerized strawberry transplants for Florida’s winter production system”. In: *III International Strawberry Symposium 439*, pp. 461–468.

Bish, E. B., D. J. Cantliffe, and C. K. Chandler (2001). “A system for producing large quantities of greenhouse-grown strawberry plantlets for plug production.” Publisher: *American Society for Horticultural Science, HortTechnology*, 11(4), 636-638. doi: 10.21273/HORTTECH.11.4.636.

Durner, E. F., E. B. Poling, and J. L. Maas (Jan. 2002). “Recent Advances in Strawberry Plug Transplant Technology”. In: HortTechnology 12.4. Publisher: *American Society for Horticultural Science, HortTechnology*, pp. 545–550. issn: 1943-7714, 1063-0198. doi: 10.21273/HORTTECH.12.4.545.

Fox, J. and S. Weisberg (2019). *An R Companion to Applied Regression*. Third. Thousand Oaks CA: Sage. url: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Hoffmann, M. (Aug. 2020). “An Overview of the Strawberry Nursery Industry in North America”. In: ASHS. url: https://ashs.confex.com/ashs/2020/meetingapp.cgi/Paper/33736 (visited on 04/08/2024).

Hoffmann, M. et al. (2024). “Southern Regional Strawberry Plasticulture Production Guide, 2nd Edition”. In: *NC State Extension Publications*.

Hokanson, S. and F. Takeda (2002). “Strawberry fruit and plug plant production in the greenhouse”. In: *XXVI International Horticultural Congress: Berry Crop Breeding, Production and Utilization for a New Century 626*, pp. 283–285.

Holmes, G. J. (2024). “The California Strawberry Industry: Current Trends and Future Prospects”. In: *International Journal of Fruit Science* 24.1, pp. 115–129.

Keutgen, N., K. Chen, and F. Lenz (Jan. 1997). “Responses of strawberry leaf photosynthesis, chlorophyll fluorescence and macronutrient contents to elevated CO_2”. In: *Journal of Plant Physiology* 150.4, pp. 395–400. issn: 0176-1617. doi: 10.1016/S0176-1617(97)80088-0.

Kozai, T., G. Niu, and M. Takagaki (2019). “Plant factory: an indoor vertical farming system for efficient quality food production”. In: Academic press, pp. 260–266.

Maas, J. L. (2012). “Strawberry diseases and pests-progress and problems”. In *VII International Strawberry Symposium* 1049 (pp. 133-142).

Poling, E. B. (2008). “Anthracnose on strawberry: Its etiology, epidemiology, and pathology, together with management strategies for strawberry nurseries: Introduction to the workshop”. *HortScience*, 43(1), 59-65.

R Core Team (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. url: https://www.R-project.org/.

Rice Jr, R. and N. Duna (1986). “The effect of initial plant size on yield components of winter-planted strawberries in coastal Lebanon”. In: *Journal of Horticultural Science* 61.2, pp. 201–203.

Shi, X., R. Hernández, and M. Hoffmann (June 2021). “Timing of Stolon Removal Alters Daughter Plant Production and Quality in the Ever-bearing Strawberry ‘Albion’”. In: *HortScience* 56.6. Publisher: American Society for Horticultural Science Section: HortScience, pp. 650–656. issn: 0018-5345, 2327-9834. doi: 10.21273/HORTSCI15624-20.

Takeda, F. and S. Hokanson (2001). “Effects of transplant conditioning on ‘Chandler’strawberry performance in a winter greenhouse production system”. In: *Strawberry research*, pp. 132–135.

Takeda, F. and M. Newell (2007). “Effects of runner tip size and plugging date on fall flowering in short-day strawberry (*Fragaria* ×*ananassa* Duch.) cultivars”. In: *International journal of fruit science* 6.4, pp. 103–117.

Wickham, H. et al. (2019). “Welcome to the tidyverse”. In: *Journal of Open Source Software* 4.43, p. 1686. doi: 10.21105/joss.01686.

Xu, X. and R. Hernández (Jan. 2020). “The Effect of Light Intensity on Vegetative Propagation Efficacy, Growth, and Morphology of “Albion” Strawberry Plants in a Precision Indoor Propagation System”. In: *Applied Sciences* 10.3, p. 1044. issn: 2076-3417. doi: 10.3390/app10031044.