AN EVALUATION OF NOVEL CONTROLLED ENVIRONMENT PROPAGATED STRAWBERRY PLUG PLANTS

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ABSTRACT

The commercial production of strawberries faces many challenges to farm profitability and viability, including fumigation bans, plant availability, and the spread of soil-borne pathogens. **F**ield propagated **b**are-**r**oot **p**lants (**Fbrp**) are the most commonly used planting material, but the production and use of Fbrp has been criticized for being unsustainable, unable to meet grower demands, and a common way for soil-borne pathogens to enter fields. As a potential solution, controlled environment-based propagation systems have been proposed as an alternative to field-based propagation systems, but there is little research on how this novel strawberry plant propagation method may affect plant phenotype, yield, and fruit quality.

This thesis investigates the differences in plant performance between the industry standard and the novel propagation method. Strawberry cultivars Albion, Cabrillo, and Monterey were propagated either via standard field procedure as bare-root plantlets or via controlled environment as plug plants and were subsequently measured through a regionally standard field trial to compare potential phenotypic differences surrounding vegetative, harvest, and fruit chemical properties.

Results indicated that **C**ontrolled **E**nvironment **A**griculture propagated **p**lug **p**lants (**CEApp**) were overall more vigorous than Fbrp. During the vegetative analysis, CEApp were found to have significantly higher quantities of branch crowns and overall higher plant dry mass. In the harvest analysis, CEApp also had higher quantities of fruit, overall mass of fruits, and average individual fruit mass. There were no significant differences between CEApp and Fbrp in the quantity of runners produced, average largest fruit, brix (ºBx), titratable acidity (TA), ºBx:TA ratio, or pH.

This research has provided validation that the novel system is a viable alternative to field-based propagation systems. Growers can opt for CEApp to decrease the risk of soil-borne pathogens entering their production systems and increase planting date flexibility, while also increasing their yield and fruit quality.

BIOGRAPHICAL SKETCH

Ava Forystek was born in Columbus, Ohio. She spent her childhood wandering the Franklin Park Conservatory and planting tulip bulbs in the yard. Ava received her undergraduate degree in Sustainable Plant Systems with a specialization in Horticulture from The Ohio State University. Throughout her undergraduate degree she worked at Strader's Garden Center and completed internships with Peking University, Corteva Agriscience, and USDA ARS. She also helped reinstate the Epsilon chapter of Pi Alpha Xi at OSU. Post graduation, Ava pursued a Master's degree in Horticultural Biology at Cornell University with Dr. Courtney Weber. During her Master's she served on the Society of Horticulture and the Student Association of the Geneva Experiment Station executive boards, and completed an internship with Plant Sciences Inc.

To my companions, flora and fauna.

To my variegated monstera, you have been in my life for longer than I have been a horticulturalist and have served as a living metaphor of how my understanding and love of plants has grown. Every time you unfurl a new leaf I'm still in awe, thank you for always inspiring me.

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PREFACE

Strawberry Biology

Strawberry (*Fragaria x ananassa*) is a high value specialty fruit crop consumed around the world. The fruits are known for their sweet and aromatic flavor, bright red color, and biochemical compounds associated with human health. Strawberry fruits are produced for fresh consumption, frozen products, and value-added processing.

Strawberry is a member of the Rosaceae family and is within the genus *Fragaria*. It is a low growing herbaceous plant with a creeping growth habit. They are perennial plants that are hardy in USDA zones 3a-7b. The crop is grown in both annual and perennial production systems and is cultivated from temperate to subtropical regions. The modern domesticated strawberry plant is a hybridization between two species within *Fragaria*; *F. virginiana* from North America and *F. chiloensis* from South America. The initial hybridization occurred in Europe during the 18th century, making the modern strawberry one of the most recently domesticated crops (Fan and Whitaker, 2023).

The strawberry plant has a short primary stem from which it creates a crown that leaves, runners, and flower laterals emerge from. Plants frequently produce stolons, also referred to as runners, as a method of asexual reproduction. Modern commercially grown strawberry plants produce hermaphroditic flowers which are capable of self-pollination as well as cross-pollination. Following pollination, the fruit develops from the receptacle of the flower. The strawberry fruit is botanically classified as an accessory fruit, while the true fruits are the small achenes on the surface of the strawberry. Despite the botanical classification, horticulturally and culinarily the strawberry fruit itself is referred to as a berry. The fleshy receptacle and achenes are the only edible portions of the plant.

Two types of strawberry plants, short-day and day-neutral, are used for commercial production and there are many different cultivars associated with both types. Short-day strawberries are facultative short-day plants that initiate flower buds when they receive less than 14 hours of daylight, which is in the fall or early spring in northern temperate regions. They may also initiate buds during long days when temperatures are below 60ºF (Durner et al., 2002). Because of this, short-day strawberries often ripen in June in the northern hemisphere and are referred to as June-bearing strawberries. Day-neutral strawberries produce flower buds throughout the growing season when temperatures are moderate and are sometimes referred to as “everbearing” because of this trait. Strawberry crops of both types are established at different times in different regions, allowing for fresh fruit to be available throughout the year. The overall peak volume of fruit in the Northern hemisphere occurs from February through June (Samanti et al., 2019).

Strawberry Fruit Nutritional Qualities

In comparison to other culinarily relevant fruits, strawberries have moderate sugar levels and contain many nutrients that are thought to be beneficial to human health (Miller et al., 2019). Sugar content in fruit is often reported as soluble solids content (SCC) as soluble sugars account for 80-90% of the total SCC content and are therefore highly correlated with each other (Menzel, 2022). In strawberries, the SSC value typically ranges between 6 to 12% by weight in fresh fruits (Akšić et al., 2019; Kallio et al., 2000; Pistón et al., 2017). Strawberry fruits also have high levels of ascorbic acid, also known as vitamin C (Sapei and Hwa, 2014). Having a balance of acids and sugars is not only important for human nutrition but is also essential for flavor quality. Previous studies have found that consumers prefer the flavor of strawberries when the ratio of sugar content to acid content exceeds 1.0 (Kubota and Kroggel, 2019). Strawberries also contain folate and are one of the richest plant sources of the micronutrient (Basu et al., 2013). To a lesser extent strawberries also are a dietary source of thiamin, riboflavin, niacin, pyridoxine, vitamin K, and vitamin A (Giampieri et al., 2012). Strawberries may also contain significant levels of dietary minerals such as manganese, iodine, selenium, calcium, iron, phosphorus, potassium, sodium, zinc, and copper depending on soil composition (Miller et al., 2019).

Another nutritional quality of strawberries is their relatively high phytochemical content. Phytochemicals are biologically active organic compounds found in plants that may be beneficial for human health. Phytochemicals found in strawberries include polyphenolic compounds such as flavonoids, phenolic acids, and tannins (Warner, 2021). Polyphenolic compounds are secondary metabolites produced by plants, typically as a defense mechanism, with antioxidant properties which can be utilized for the prevention and treatment of various human diseases (Hano, Christophe, and Tungmunnithum, 2020). Some studies have even seen a positive effect on memory and overall brain health with the consumption of strawberries, likely because of their polyphenols (Agarwal et al., 2019). Anthocyanins are a class of polyphenolic compounds that are thought to be important for human health, and fresh strawberry fruits have been found to contain up to 800 mg/kg anthocyanins (Blesso, 2019). Anthocyanins, like those found in strawberries, may help prevent inflammation and protect the body against diabetes, cancer, and heart diseases (Mattioli et al., 2020). Over 25 different anthocyanins have been described in strawberries (Lopes da Silva et al., 2007). Anthocyanin levels are highest in darker colored fruits, typically when the fruits are at full maturation. Ellagitannins are also abundant in strawberry fruits. It is known that ellagitannins have an apoptotic effect and can induce cell cycle arrest of cancer cells, prevent cardiovascular diseases, and scavenge oxidative free radicals (Li et al., 2020). Research into ellagitannins in strawberry fruits is fairly new, but it has been reported that fresh strawberry fruits contain 25 to 59 mg/100g ellagitannins (Li et al., 2020).

Strawberry fruits also have a very diverse and complex volatile compound profile. Significant volatile compounds in strawberries include esters, terpenes, and furans. Thirty-one volatile compounds found in strawberries have been found to be positively associated with flavor intensity (Schwieterman et al., 2014). Volatile compounds play a key role in the perception of foods and are capable of enhancing the sweet flavor of a fruit (Baldwin et al., 1998).

It is important to note that the chemical composition and nutritional quality of strawberries can vary between fruits. Firstly, different cultivars will inherently have different phenotypic qualities from each other which will cause variation in nutrient levels, phytochemical profile, sugar content, and other measures. Postharvest practices also have a strong impact on the nutritional quality of the fruits. Poor postharvest management can cause premature breakdown of many compounds in the fruits which will reduce the overall nutrient content (Maraei and Elsawy 2017).

Strawberry Global Relevance and Economics

The strawberry industry is a significant contributor to the global economy. In 2021 the value of strawberries produced in the United States was valued at $3.422 billion USD (USDA Noncitrus Fruits and Nuts, 2022), and the global fresh strawberry market was estimated to be $22.9 billion USD (FAO, 2021). As of 2019, the United States is the second largest producer of strawberry fruit by volume. China is the largest global producer. (FAO, 2021). Within the United

States, California and Florida are the top strawberry producing states with California producing over 91% of the entire crop (AGMRC, 2023). The United States is the second largest international exporter of strawberries, exporting a value of $467.8 million USD in 2013 (Wu et al., 2018). Since 2020, production acreage has increased in the US by about 13% with the most acreage increases in Florida and California (USDA NASS, 2022).

American consumption of strawberries has been steadily increasing. In 1980 the average American consumed 2 pounds per year, and by 2013 this had increased to 8 pounds per year (USDA ERS, 2014). Most of the revenue generated from fresh strawberry production comes from fresh fruit sales through direct markets, pick your own farms, produce stands, and direct sales to wholesalers (Samtani et al., 2019). The remainder of the revenue is generated from frozen strawberry sales or processed strawberry products (Simpson, 2018).

Strawberry Cropping Systems

There are many different cropping systems that may be used for strawberry production. Typically, production methods fall within two categories: field production and protected culture production. Annual hill production (AHP) is currently the most widely used system for commercial production, which involves plastic mulching and fumigants to control pathogens. Drip irrigation is used within rows to maintain soil moisture and to promote efficient water usage. Both short-day and day-neutral strawberries may be used in this system and are replanted yearly, most commonly as bare-root plantlets. Perennial matted row (PMR) systems involve short-day strawberry varieties and utilize perennial production, typically for three to five seasons (Weber, 2021). In PMR systems, strawberry plants, typically as bare-root plantlets, are started on bare or mulched ground in regularly spaced rows. Runners are allowed to develop into plants which eventually fill in the row and produce fruit in the following season. This system helps control weeds, retain topsoil, and allows for ample light penetration (Klodd et al., 2021).

Protected culture production of strawberries involves the use of tunnels or greenhouses and can be used in conjunction with AHP or soilless production systems. Because of disease incidence, harsh weather, or short local seasons, growers may decide to utilize protected culture to produce a high-quality final product. Low tunnels involve fitting approximately 4 ft tall plastic or metal hoops over rows and then covering them with a thin polyethylene sheeting with holes for ventilation. Low tunnel covered rows have been observed to have 27% higher yields and 15% more marketable fruits than uncovered rows in New York production systems (Pritts and McDermott, 2017). When utilizing low tunnels, marketable yield of day-neutral strawberries has been observed to be 313% higher compared to strawberries grown in open beds in the Mid-Atlantic region (Lewers et al., 2017). Greenhouse production of strawberries is a relatively new practice in the United States, but interest is quickly growing as the year-round demand for quality strawberries increases (Garcia and Kubota, 2017). Hydroponic systems are commonly used in greenhouse production systems, allowing for precise control of the rootzone. Producing strawberries via controlled environment agriculture (CEA) is a promising, relatively new production style as it allows growers to have a high level of control over all inputs into the plant and enables the production of extremely high quality fruits. One downside of CEA strawberry production is the potential risk of insect mediated damage as plants must stay in the greenhouse, which is an optimal environment for many pest insects, for extended periods of time. The longer a plant remains in a CEA operation, the higher the risk of infestation (Levine and Mattson, 2022). CEA production systems are also more expensive to run as they require more specialized equipment, specialized labor, and energy inputs than other strawberry production systems.

Organic production of strawberries is a practice that avoids the application of synthetic products such as conventional fertilizers and pesticides. The goal of organic agriculture is to produce a commercially viable crop while maintaining soil quality and minimizing environmental impact. In northeastern US growing systems, organic production of strawberries often utilizes a one to two year crop cycle, hand weeding, the use of natural pesticides, and the use of natural fertilizers (Carroll and Pritts, 2022). Since organically produced strawberries cannot be treated with conventional treatments, strict and precise postharvest handling is essential to avoid excessive postharvest loss.

Commercial Strawberry Production

In 2021 the United States produced 1.4 million tons of strawberries (USDA Noncitrus Fruits and Nuts, 2022). The majority of commercial growing systems are located in California and utilize AHP type systems (California Strawberry Commission; Poling, 2015). In order to promote water use efficiency and to decrease disease incidence, drip irrigation is used. Despite being perennial plants, strawberry plants are only kept in the system for one growing season before being removed and replaced in the following year. AHP systems utilize plastic mulches or row covers and soil fumigation treatments prior to planting to decrease soil-borne disease incidence. The combination of methyl bromide (MB) and chloropicrin is commonly used because of their wide pest control spectrum when used in combination and overall efficacy (Holmes et al., 2020). The type of strawberry plant (short-day or day-neutral), cultivar, and planting material used in the system varies by location. Most growers use bare-root plants in their systems, but the prevalence of plug plant use has been steadily increasing (Samtani et al., 2019).

Commercial Strawberry Propagation - Nurseries

In order to support the strawberry fruit production industry, commercial strawberry nurseries produce strawberry plants for growers. California is the largest producer of strawberry plants internationally, producing over 1.5 billion plants annually, primarily as bare-root plantlets (Holmes, 2024). Most international strawberry industries are dependent on foundation stock and mother plants produced in California (Holmes, 2024). High elevation (1295 m) and low elevation (~ sea level) nurseries provide distinct climatic environments, allowing for the production of strawberry plants for different markets and purposes (Smith, 2022). High elevation nurseries produce 80% of the strawberry plants while low elevation nurseries produce the remaining 20%. Plants produced from low elevation nurseries are typically used for summer plantings in California high elevation nurseries after an extended cold treatment or are shipped out for international production (Holmes, 2024). Strawberry plants from high elevation nurseries are primarily sold in California and Mexico, while 10% are shipped to Florida (Holmes, 2024). High elevation nurseries allow for chilling prior to harvest, which is associated with higher levels of plant vigor and fruit production (Strand, 2008).

Producing strawberry plants in a commercial propagation system is an intensive multistep process. Several weeks prior to planting the soil is fumigated with a combination of MB and chloropicrin, or other fumigants as MB has largely been phased out due to environmental concerns, which are applied under a plastic tarp to prevent local contamination. The tarp is removed after about 5 days (NCSU NSF Center for Integrated Pest Management, 1999). After fumigation, growers typically start propagation fields with bare-root “mother plants” that are transplanted into flat field soil. Drip irrigation lines are often run in the rows and in between the rows to encourage runner development. Large spaces are left between rows of mother plants to allow for daughter plant formation from runners (Holmes, 2024). Once the daughter plants have matured and begin to go dormant, a trommel digger is used to dig the daughter plants, excess soil is removed from their roots, and they are put in sorting bins before leaving the field (Thomas, 2019). After leaving the field, the daughter plant material is hand sorted based on plant quality in a trim shed (Thomas, 2019). Depending on the region they are produced in, plantlets are either used immediately for production fields or are trimmed, packaged, and kept in cold storage as bare-root plantlets until transplanted (NCSU NSF Center for Integrated Pest Management, 1999).

Challenges in the Production of Strawberries

Despite having well established fruit production and nursery systems, there are many challenges that threaten commercial strawberry production. Challenges vary by region, but one of the most widespread challenges is a lack of available labor. Strawberry fruits are harvested by hand, which is incredibly labor intensive (Martin, 2017). Finding adequate labor during the harvest season is difficult because of issues including, but not limited to, immigration policies and the increasing availability of jobs with higher economic opportunities (Guan et al., 2016). Another challenge in the production of strawberries is the limited availability of quality strawberry planting material in various regions. Bare-root strawberry plants are the most frequently available type of planting material, but are not as vigorous as other types of strawberry plantlets such as fresh dug plantlets or plug plants (Orde and Sideman, 2023). Because of production timing, fresh dug bare-root plants are not commercially available until the early fall, preventing summer plantings in colder regions such as the northeastern US (Weber, 2021). Currently, there is not a large-scale commercial strawberry plug plant industry, making plug plants a far less accessible option (Orde and Sideman, 2023). This makes it difficult for local growers in the region to compete with fruit shipped in from larger growers on the West Coast.

Pathogen incidence is another relevant challenge in the production of strawberries. Strawberries are susceptible to many different pathogens which can lead to reductions or complete loss of yield. Some of the most prevalent pathogens observed in field systems include *Colletotrichum spp.*, *Botrytis cinerea*, *Xanthomonas fragariae*, *X. arboricola* pv. *fragariae*, *Fusarium oxysporum* f. sp. *fragariae*, and *Macrophomina phaseolina* (Bozbuga et al., 2023; Pastrana et al., 2023). Many of these pathogens are soil-borne and have been controlled through soil fumigants. MB is no longer permissible for use as a soil fumigant in fields for fruit production and has been replaced with chloropicrin and 1,3-dichloropropene (Samtani et al., 2019) which are less effective for disease control. In perennial systems it has actually been observed that fumigation is correlated with increased disease incidence over time (Wing et al., 1995). When incorrectly handled, soil fumigants have been known to cause adverse effects in humans, putting farm laborers and local residents at risk (Nagami and Suenaga, 2022). MB exposure has resulted in neurotoxicity and death and chloropicrin exposure is known to cause dyspnea, eye pain, sore throat, and headaches (Committee on Acute Exposure Guideline Levels, 2012; Nagami and Suenaga, 2022). Because of this, fumigants have been subjected to regulatory restrictions, making the adequate control of soil-borne pathogens increasingly difficult (Chellemi, 2014).

New York State Strawberry Industry

In the United States, New York State is the 8th largest producer of strawberries, producing 1800 tons valued at $8.5 million USD from 1700 acres of land annually (McDermott, 2024). The most common growing system in the region is a perennial matted row utilizing short-day cultivars (Pritts et al., 1998). Growers often can maintain a planting for three to five years before observing a noticeable decline in harvest. Commonly used cultivars in the region include Earliglow, Honeoye, and Jewel (Orde and Sideman, 2023). Spring planting is typical for this system. Strawberry plantings are most commonly established with dormant bare-root plants due to seasonal availability (Pritts et al., 1998). The majority of the crop is sold from late May to early July. Day-neutral strawberries are growing in popularity throughout the state which extends the season to as late as November (Orde and Sideman, 2023).

New York State has a primarily local strawberry market with 95% of the strawberries produced being sold at local farm stands, farmer's markets, and U-pick operations (McDermott, 2024). The remaining 5% is sold for value added processing (McDermott, 2024). The most common strawberry pests and diseases in the region include botrytis fruit rot, verticillium wilt, red stele, leather rot, tarnished plant bugs, strawberry bud weevil, spittlebugs, spider mites, and slugs (Orde and Sideman, 2023).

Overview

Strawberries are a high value specialty crop known for their sweet flavor and nutritional qualities. The United States is a leader in the strawberry industry, producing the second most strawberries by total yield internationally, as well as being relied upon internationally for the production of planting material. There are many challenges including labor shortages, inadequate plant availability, and disease incidence that threaten the future of the strawberry industry. In order to support the strawberry industry, it is critical to understand the industry itself as well as its challenges to perform targeted research where it is most needed.

Preface References

Agarwal, P., Holland, T. M., Wang, Y., Bennett, D. A., & Morris, M. C. (2019). Association of strawberries and anthocyanin intake with Alzheimer's dementia risk. *Nutrients*, 11(12), 3060. https://doi.org/10.3390/nu11123060

Agricultural Marketing Resource Center. (2023). *AGMRC*. https://www.agmrc.org/media/cms/AgMRC_OctDec_2021_Report_CA9351CFB5844.pdf

Akšić, M. F., Tosti, T., Sredojević, M., Milivojević, J., Meland, M., & Natić, M. (2019). Comparison of sugar profile between leaves and fruits of blueberry and strawberry cultivars grown in organic and integrated production systems. *Plants*, 8(7), 205. https://doi.org/10.3390/plants8070205

Baldwin, E. A., Scott, J. W., Einstein, M. A., Malundo, T. M., Carr, B. T., Shewfelt, R. L., & Tandon, K. S. (1998). Relationship between sensory and instrumental analysis for tomato flavor. *Journal of the American Society for Horticultural Science*, 123(5), 906–915. https://doi.org/10.21273/jashs.123.5.906

Basu, A., Nguyen, A., Betts, N. M., & Lyons, T. J. (2013). Strawberry as a functional food: An evidence-based review. *Critical Reviews in Food Science and Nutrition*, 54(6), 790–806. https://doi.org/10.1080/10408398.2011.608174

Blesso, C. N. (2019). Dietary anthocyanins and human health. *Nutrients*, 11(9), 2107. https://doi.org/10.3390/nu11092107

Bozbuga, R., Uluisik, S., Aridici Kara, P., Yuceer, S., Gunacti, H., Gok Guler, P., Ince, E., Nilufer Yildiz, H., & Tetik, O. (2023). Pests, diseases, nematodes, and weeds management on strawberries. *IntechOpen Recent Studies on Strawberries*. https://doi.org/10.5772/intechopen.103925

California Strawberry Commission. (n.d.). https://www.calstrawberry.com/

Carroll, J., & Pritts, M. (2022). Organic production and IPM guide for strawberries. *New York State Integrated Pest Management Program*. https://ecommons.cornell.edu/server/api/core/bitstreams/50d20e86-8467-45dc-ac74-9937bbb4d7dc/content

Chellemi, D. O. (2014). Plant Health Management: Soil Fumigation. *Encyclopedia of Agriculture and Food Systems*, 456–459. https://doi.org/10.1016/b978-0-444-52512-3.00250-3

Committee on Acute Exposure Guideline Levels. (2012). Methyl bromide: Acute exposure guideline levels. *Acute exposure guideline levels for selected airborne chemicals*: Volume 12. https://www.ncbi.nlm.nih.gov/books/NBK201456/

da Silva, F. L., Escribano-Bailón, M. T., Pérez Alonso, J. J., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (2007). Anthocyanin pigments in strawberry. *Food Science and Technology*, 40(2), 374–382. https://doi.org/10.1016/j.lwt.2005.09.018

Durner, E. F., Poling, E. B., & Maas, J. L. (2002). Recent advances in strawberry plug transplant technology. *HortTechnology*, 12(4), 545–550. https://doi.org/10.21273/horttech.12.4.545

Eames-Sheavly, M. (2003). Strawberries. https://cceoneida.com/resources/guide-to-growing-strawberries

Fan, Z., & Whitaker, V. M. (2023). Genomic signatures of strawberry domestication and diversification. *The Plant Cell*, 36(5), 1622–1636. https://doi.org/10.1093/plcell/koad314

Faostat. (2021). Value of strawberry agricultural production. http://www.fao.org/faostat/en/#data/QV

Faostat. (2018). Food and Agriculture Organization of the United Nations 2015 crops. http://www.fao.org/faostat/en/#data/QC

Garcia, K., & Kubota, C. (2017). Physiology of strawberry plants under controlled environment: Diurnal change in leaf net photosynthetic rate. *Acta Horticulturae*, (1156), 445–452. https://doi.org/10.17660/actahortic.2017.1156.66

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, 28(1), 9–19. https://doi.org/10.1016/j.nut.2011.08.009

Guan, Z., Wu, F., & Whidden, A. (2016). Top challenges facing the Florida strawberry industry: Insights from a comprehensive industry survey. *EDIS*, 2016(2), 3. https://doi.org/10.32473/edis-fe972-2015

Hano, C., & Tungmunnithum, D. (2020). Plant polyphenols, more than just simple natural antioxidants: Oxidative stress, aging and age-related diseases. *Medicines*, 7(5), 26. https://doi.org/10.3390/medicines7050026

Holmes, G. J. (2024). The California strawberry industry: Current trends and future prospects. *International Journal of Fruit Science*, 24(1), 115–129. https://doi.org/10.1080/15538362.2024.2342900

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. https://doi.org/10.1094/phyto-11-19-0406-ia

Huang, W., Zhang, H., Liu, W., & Li, C. (2012, January 27). Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *Journal of Zhejiang University*, https://link.springer.com/article/10.1631/jzus.B1100137

Kallio, H., Hakala, M., Pelkkikangas, A. M., & Lapveteläinen, A. (2000). Sugars and acids of strawberry varieties. *European Food Research and Technology*, 212(1), 81–85. https://doi.org/10.1007/s002170000244

Klodd, A., Hoover, E., & Tepe, E. (2021). Planting a new strawberry field. *UMN Extension*. https://extension.umn.edu/strawberry-farming/planting-new-strawberry-field

Kubota, C., & Kroggel, M. (2019). Fruit quality. *Controlled environment berry production information.* https://u.osu.edu/indoorberry/fruit-quality/

Lewers, K. S., Fleisher, D. H., & Daughtry, C. S. (2017). Low tunnels as a strawberry breeding tool and season-extending production system. *International Journal of Fruit Science*, 17(3), 233–258. https://doi.org/10.1080/15538362.2017.1305941

Maraei, R. W., & Elsawy, K. M. (2017). Chemical quality and nutrient composition of strawberry fruits treated by γ-irradiation. *Journal of Radiation Research and Applied Sciences*, 10(1), 80–87. https://doi.org/10.1016/j.jrras.2016.12.004

Martin, P. (2017). Trump and U.S. immigration policy. *California Agriculture*, 71(1), 15–17. https://doi.org/10.3733/ca.2017a0006

Mattioli, R., Francioso, A., Mosca, L., & Silva, P. (2020). Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. *Molecules*, 25(17), 3809. https://doi.org/10.3390/molecules25173809

Mattson, N., & Levine, C. (2022). CEA strawberry operations. *Produce Grower*. www.producegrower.com/article/cea-strawberry--operations/

McDermott, L. (2024). Strawberries. *Cornell Cooperative Extension Eastern New York Commercial Horticulture*. https://enych.cce.cornell.edu/crop.php?id=33

Menzel, C. M. (2022). Effect of temperature on soluble solids content in strawberry in Queensland, Australia. *Horticulturae*, 8(5), 367. https://doi.org/10.3390/horticulturae8050367

Miller, K., Feucht, W., & Schmid, M. (2019). Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. *Nutrients*, 11(7), 1510. https://doi.org/10.3390/nu11071510

Mitcham, E. J. (n.d.). Strawberry, Recommendations for maintaining postharvest quality. *Postharvest Research and Extension Center*. https://postharvest.ucdavis.edu/produce-facts-sheets/strawberry

Nagami, H., & Suenaga, T. (2022). Health effects caused by soil fumigant chloropicrin, reduction of exposure to chloropicrin, and alternative technology of soil fumigants. *Journal of UOEH*, 44(4), 395–404. https://doi.org/10.7888/juoeh.44.395

NCSU NSF Center for Integrated Pest Management. (1999). Crop profile for strawberries in California. https://ucanr.edu/datastoreFiles/391-501.pdf

Noncitrus Fruits and Nuts - 2022 summary (2023). https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/zk51wx21m/k356bk214/ncit0523.pdf

Orde, K. and Sideman, B., (Eds.). (2023) Strawberry production guide for the Northeast, Midwest, and Eastern Canada (2nd ed.). *University of New Hampshire Cooperative Extension*. https://scholars.unh.edu/extension/1670/

Pastrana, A. M., Borrero, C., Pérez, A. G., & Avilés, M. (2023). Soilborne pathogens affect strawberry fruit flavor and quality. *Plant Science*, 326, 111533. https://doi.org/10.1016/j.plantsci.2022.111533

Pistón, F., Pérez, A. G., Sanz, C., & Refoyo, A. (2017). Relationship between sugar content and °brix as influenced by cultivar and ripening stages of strawberry. *Acta Horticulturae*, (1156), 491–496. https://doi.org/10.17660/actahortic.2017.1156.72

Poling, B., Krewer, G., & Smith, J. P. (2005). Southeast regional strawberry plasticulture production guide. *NC State Extension*. https://smallfruits.org/files/2019/06/2005culturalguidepart1bs1.pdf

Pritts, M. P., Handley, D., & Walker, C. (1998). Strawberry production guide: For the Northeast, Midwest, and Eastern Canada. *Northeast Regional Agricultural Engineering Service, Cooperative Extension*.

Pritts, M., & McDermott, L. (2017). Protected culture for strawberries using low tunnels. http://www.hort.cornell.edu/fruit/pdfs/low-tunnel-strawberries.pdf

Samtani, J. B., Rom, C. R., Friedrich, H., Fennimore, S. A., Finn, C. E., Petran, A., Wallace, R. W., Pritts, M. P., Fernandez, G., Chase, C. A., Kubota, C., & Bergefurd, B. (2019). The status and future of the strawberry industry in the United States, *HortTechnology*, *29*(1), 11-24. https://doi.org/10.21273/horttech04135-18

Sapei, L., & Hwa, L. (2014). Study on the kinetics of vitamin C degradation in fresh strawberry juices. *Procedia Chemistry*, 9, 62–68. https://doi.org/10.1016/j.proche.2014.05.008

Schwieterman, M. L., Colquhoun, T. A., Jaworski, E. A., Bartoshuk, L. M., Gilbert, J. L., Tieman, D. M., Odabasi, A. Z., Moskowitz, H. R., Folta, K. M., Klee, H. J., Sims, C. A., Whitaker, V. M., & Clark, D. G. (2014). Strawberry flavor: Diverse chemical compositions, a seasonal influence, and effects on sensory perception. *PLoS ONE*, 9(2). https://doi.org/10.1371/journal.pone.0088446

Simpson, D. (2018). The economic importance of Strawberry Crops. *Compendium of Plant Genomes*, 1–7. https://doi.org/10.1007/978-3-319-76020-9_1

Smith, J. (2022). Siskiyou County Department of Agriculture. *Siskiyou County 2022 Annual Crop & Livestock Report*. https://www.co.siskiyou.ca.us/agriculture

Strand, L. (2008). Integrated pest management for Strawberries. *University of California, Statewide Integrated Pest Management Program.* https://ipm.ucanr.edu/ipmproject/ads/manual_strawberry.html

Thomas, D. (2019). California Strawberry Nursery Industry. *Cal Poly AEPS 400*. Retrieved 2024, from https://www.youtube.com/watch?v=JfV54nzIOL4.

U.S. strawberry consumption continues to grow. *USDA ERS*. (2014). http://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=77884

United States Department of Agriculture. *USDA NASS*. (2013). https://www.nass.usda.gov/Charts_and_Maps/Crop_Progress_&_Condition/2013/index.php

Warner, R., Wu, B.-S., MacPherson, S., & Lefsrud, M. (2021). A review of strawberry photobiology and fruit flavonoids in controlled environments. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.611893

Weber, C. A. (2021). Strawberry crown plugs provide flexibility and improved performance in Cold Climate Plasticulture production. *Agronomy*, 11(8), 1635. https://doi.org/10.3390/agronomy11081635

Weber, C. A. (2021). Performance of strawberry varieties developed for perennial matted-row production in annual plasticulture in a cold climate region. *Agronomy*, 11(7), 1407. https://doi.org/10.3390/agronomy11071407

Wing, K. B., Pritts, M. P., & Wilcox, W. F. (1995). Biotic, edaphic, and cultural factors associated with Strawberry Black Root Rot in New York. HortScience *30*(1), 86-90. https://doi.org/10.21273/HORTSCI.30.1.86

Wu, F., Guan, Z., & Whidden, A. (2016). Overview of the US and Mexico strawberry industries. *EDIS*, 2016(1), 4. https://doi.org/10.32473/edis-fe971-2015

CHAPTER 1 - An Evaluation of Novel Controlled Environment Propagated Strawberry Plug Plants on Harvest and Vegetative Attributes

1.1 Abstract

The strawberry industry is reliant on the availability of quality planting material. **F**ield propagated **b**are-**r**oot **p**lants (**Fbrp**) are the most commonly used type of strawberry plant used in commercial production systems, but the production of Fbrp has been criticized for being unsustainable, inadequate, and a common way for soil-borne pathogens to enter previously disease-free fields. **C**ontrolled **E**nvironment **A**griculture propagated **p**lug **p**lants (**CEApp**) are a promising alternative to the current standard of Fbrp for strawberry field production systems, but research into the phenotypic differences and production characteristics of strawberry plants propagated via different methods throughout a growing season is limited.

In order to determine if CEApp is a viable alternative to Fbrp, a field trial evaluating three strawberry cultivars, Albion, Cabrillo, and Monterey, propagated as either CEApp or Fbrp, was performed in the summer of 2023 to look at the effects of CEApp on vegetative and harvest parameters. Vegetative traits such as runner production, branch crown incidence, and plant dry mass were evaluated. Measured harvest parameters included: quantity of fruits harvested, overall mass of fruits harvested, average largest fruit per harvest, and average individual fruit mass over the fruit production period.

Overall, it was found that CEApp performed equally or more vigorously than Fbrp in all measures. In the vegetative analysis CEApp had significantly higher quantities of branch crowns and overall higher plant dry mass. In the harvest analysis, CEApp had higher quantities of fruit, overall mass of fruits, and average individual fruit mass. There was no significant difference

between CEApp and Fbrp in runner production or average largest fruit at each harvest. The resulting conclusion from this study is that CEApp is a viable alternative to Fbrp and that CEApp offers additional benefits besides those inherent to the propagation method.

1.2 Introduction

Strawberries are a widely consumed specialty small fruit crop of high economic importance. As of 2021, the US strawberry farming industry was valued at $3.422B USD (USDA Noncitrus Fruits and Nuts, 2022). The strawberry nursery industry contributes an additional $200M-$1B to the industry (USDA NASS, 2017; USDA ERS, 2018). In order to keep up with consumer demands and to support the strawberry industry, a sustainable and reliable strawberry plant propagation system is of critical importance.

The current standard method of large-scale strawberry plant propagation is complex, costly, and time consuming, which has prompted research into more efficient and sustainable propagation methods. In the current system, virus-indexed mother plants are propagated in methyl-bromide (MB) and chloropicrin treated fields for 2-3 years primarily in CA, NC, and Eastern Canada (Thomas, 2019). Daughter plants, produced from runners from the mother plants, are harvested as unrooted plantlets by hand. They are then either used immediately to sell as fresh dug or plug plants, or are allowed to root in the field, are mechanically dug, and are hand sorted for cold storage for up to eight months prior to shipment to growers. Stored daughter plants are sold as dormant bare-root plantlets (Figure 1.1) to growers across the US and internationally, generally for spring and early summer planting. Plants for winter and early spring production in Florida and the southeastern US are primarily produced in eastern Canada and are transferred directly to production fields after fall digging (Santos et al., 2012; Thomas., 2019).

Because of plant susceptibility and high incidence rates of soil-borne pathogens, the success of field propagation operations is reliant on soil disinfection treatments such as fumigation (Koike et al., 2013).

Figure 1.1. Example of bare-root strawberry plants (BerryCrop Agro LLP., n.d.)

While soil fumigation is often effective, it has many drawbacks. Because of known adverse health effects from compounds used in fumigation, the practice has faced public scrutiny, legal restrictions, and complete bans in certain situations (Chellemi, 2014). Also, fumigation is not always completely effective, which may allow for disease outbreaks and the spread of soil-borne pathogens. If daughter plants come in contact with pathogens they may carry a latent infection, which will then allow them to act as disease vectors when planted in clean fields, potentially leading to significant plant and crop losses. Strawberry propagules are often asymptomatic carriers of disease and will only show symptoms in favorable disease conditions after being planted in a new field, giving way for disease to spread without detection (Pettitt & Pegg, 1994; Marin et al., 2019; Forcelini & Peres, 2018; Oliveira et al., 2017).

Another problem with the current production system is the limited window of availability for quality strawberry plants when establishing new plantings. In cold climate regions, such as New York State, vigorous strawberry planting material such as fresh dug plantlets are not commercially available until early October (Weber, 2021), and dormant field grown bare-root plants are the only type available for spring or early summer planting. The limited window of vigorous plant availability puts local growers at a disadvantage as they have to compete with large scale commercial growers in California at peak season (Orde and Sideman, 2023). If growers in cold climate regions had access to quality strawberry planting material year-round, they could modify their production systems in order to better meet consumer demands and better compete with California growers.

To address issues with the current strawberry plant propagation system, the utilization of a novel controlled environment (CE) based propagation system to produce strawberry plug plants (Figure 1.2) has been proposed. CE systems are not reliant on MB or chloropicrin applications and plants are not exposed to soil-borne pathogens, allowing for a higher level of certainty in the production of clean plantlets (Holmes el al., 2020). While CEApp seems like a promising alternative, research into the impact of propagation method on harvest and vegetative qualities of strawberry plants is limited. Understanding the potential differences in plant performance is important, as growers need to have confidence in the quality of their plants and knowledge of how the crop management and harvest may be impacted. The objective of this study is to quantify and understand the potential differences in phenotypic trait expression between Fbrp and CEApp to determine the potential of CE as an alternative propagation method.

Figure 1.2. Example of a strawberry plug plant (strawberryplants.org, 2022)

While it is known that environment can influence phenotypic expression, there is little research on how varying propagation methods may impact strawberry plant phenotypic expression. Phenotypic differences between plants of the same genotype may be expressed as differences in vegetation, harvest quantity, harvest timing, and fruit quality. In order to determine if controlled environment propagation is a viable alternative to field propagation it is critical to quantify such potential phenotypic differences observed between plants propagated via both methods.

Vegetative traits such as runnering (daughter plant production) and branch crown development impact the manageability and harvestability of strawberry plants (James et al., 2022). These traits are also often correlated with various aspects of fruit quality as energy is allocated to different plant tissues, effectively pulling resources away from fruit development. One important quality related to vegetation that growers look for in a strawberry plant is an open leaf canopy (Yue et al., 2014). If a strawberry plant develops a heavy leaf canopy, harvesting becomes more difficult, disease incidence increases, and fruit quality is negatively impacted (Poling, 2012). The decrease in fruit quality is partially due to the plant allocating energy towards producing vegetative structures such as branch crowns and excessive leafage. The dense canopy also traps moisture and prevents airflow, creating an optimal environment for disease development. Excessive runnering is another negative vegetative trait in strawberry plants grown for fruit production. When strawberry plants produce runners, resources are diverted away from fruit production and fruit growth is stunted (Guthman & Jiménez-Soto, 2021). In many types of strawberry production systems runners also need to be cut back, creating additional labor costs for growers (Guthman & Jiménez-Soto, 2021). Branch crown numbers also have an impact on fruit quality. The effect is cultivar dependent, but it is known that fruit size is significantly reduced in plants with many crowns (McWhirt, 2021).

Differences in traits associated with fruit harvest such as the quantity of fruits harvested, overall mass of fruits harvested (yield), and average individual fruit mass are also important factors to quantify. Because of the high labor requirements and costs surrounding strawberry harvesting, it is important for harvests to be predictable. Strawberries are highly perishable and must be hand harvested. If there is a shortage of labor and fruits are not harvested, fruits will rot in the field, reducing the overall profitability of the growing operation (Guthman and

Jiménez-Soto, 2021). Field worker salaries are often partially determined by how many boxes they can fill, and if fruits are smaller than expected employees may leave to find more profitable work elsewhere, contributing to labor shortage concerns (Guthman, 2016).

In order to quantify the potential differences between Fbrp and CEApp, a variety of phenotypic qualities of 'Albion', 'Cabrillo', and 'Monterey' strawberry plants were recorded throughout a growing season at a field site in Geneva, New York. My research evaluated vegetative traits as well as harvest qualities. I hypothesized that CEApp would perform equally well as or better than Fbrp.

1.3 Materials and Methods

1.3.1 Plant Material and Trial Establishment

Three strawberry cultivars, Albion, Cabrillo, and Monterey, were selected for this study (Table 1.1). CEApp were sent from North Carolina State University for the trial and arrived on 17 May 2023. 'Albion' and 'Monterey' Fbrp were delivered on 17 May 2023 from Indiana Berry in Plymouth, IN and 'Cabrillo' Fbrp were obtained from EZ Grow in Langton, ON on 23 May 2023. Prior to planting, the roots of the Fbrp were soaked in tap water for 24 hours to ensure proper hydration and to assist in breaking dormancy. Because of problems with shipping, the 'Cabrillo' Fbrp were visibly stressed. The highest quality plants in the shipment were sorted out for establishing the plantings. Additionally, extra bare-root plants were potted in growing media and were placed in a cold frame at the Cornell Agritech campus to develop, to act as replacements of similar age in the case that plants died in the field. On 6 July 2023, plants that did not survive transplanting in the field were replaced with plants from the cold frames if available.

Table 1.1. Description of Strawberries Used in the Trial

Cultivar	Breeding Name	Patent Number	Origin	Pedigree	Flowering Type	Year Released
Albion	CN220	USPP16228P3	Univ. of California	Diamante x Cal 94.16-1	Day-neutral	2004
Cabrillo	CN236	US20160227687P1	Univ. of California	Cal 3.149-8 x Cal 5.206-5	Day-neutral	2015
Monterey	CN222	USPP19767P2	Univ. of California	Albion x Cal 97.85-6	Day-neutral	2008

1.3.2 Site Description

In the fall of 2022, prior to the trial establishment, the field was planted with grain rye (*Secale cereale*) as a cover crop. The trial was performed in the summer of 2023 in adjacent plots within the same field at Cornell AgriTech at the New York State Agricultural Experiment station in Geneva, NY, USA (42°52'14.3" N, 77°02'38.6" W) in the USDA hardiness zone 6a (USDA Plant Hardiness Zone Map, 2023). The field consists of Honeoye loam soil (mesic Glossic Hapludalfs) and has a grade of 3%-8% (USDA Web Soil Survey, 2023). The cover crop was mowed, plowed under, and then rotovated prior to bed formation. The site was unfumigated.

1.3.3 Experimental Design

The plots were arranged in rows in a randomized split-plot block design with four replications within a larger strawberry planting. Each row consisted of six plots (three CEApp and three Fbrp plots per row) representing each cultivar (whole plots) and propagation method. Their positions were randomly assigned within rows (Figure 1.3). Each split plot consisted of 15-25 plants depending on plant availability.

Figure 1.3: Layout of the 2023 field trial by plots and rows arranged as a randomized split-plot block design with four replications.

Row 3	Albion Test	Albion Control	Cabrillo Test	Cabrillo Control	Monterey Test	Monterey Control
Row 4	Monterey Test	Monterey Control	Albion Test	Albion Control	Cabrillo Test	Cabrillo Control
Row 5	Cabrillo Test	Cabrillo Control	Monterey Test	Monterey Control	Albion Test	Albion Control
Row 6	Albion Control	Albion Test	Cabrillo Control	Cabrillo Test	Monterey Control	Monterey Test

1.3.4 Crop Management

Rows consisted of raised beds which were 15 cm high and 61 cm wide that were spaced 1.7 m center to center and were covered with 5 ft wide white plastic (Filmtech Corp., Allentown, PA, USA). Irrigation was supplied via a single center 10 mm T-tape drip line with 30 cm emitter spacing (Rivulis Irrigation, San Diego, CA). Within each row, plants were planted in double-offset rows with 30 cm spacing between plants and rows.

Strawberry plants were grown under low tunnels (Dubois Agrinovation, Saint-Remi, Quebec, Canada) in an annual hill plasticulture system with drip irrigation. Plants were planted on 22 May 2023 and 23 May 2023. After planting, flowers were removed from the plants as they appeared for 4 weeks, until 19 June 2023, to allow for adequate vegetative growth to support fruit development. Low tunnels were put up on 27 July 2023. Fruit harvesting began on 18 July 2023 and occurred three times per week to prevent overripening, mitigate loss due to opportunistic pathogens, and reduce pest incidence.

Rows were fertigated via the drip line starting after planting with 20-20-20 fertilizer, (Jack's Fertilizer, JR Peters, Allentown, PA, USA, 20N-20P-20K) on a weekly basis, supplying approximately 5 lb/acre N per week equivalent throughout the growing season. Irrigation was supplied three times per week for an application of 2.5 cm of water per week prior to the fruiting phase. During the fruiting phase irrigation was increased to 5 cm per week. Weeds were removed by hand as needed throughout the season. No other significant pest management strategies were applied to the trial.

1.3.5 Fruit Harvest

Within each plot, fifteen contiguous strawberry plants were flagged for fruit collection to maintain consistency as the number of strawberry plants within each plot varied due to dieback and/or availability of plants. Throughout the production period ripe fruits were harvested by hand every Monday, Wednesday, and Friday as weather permitted. Fruits were harvested once they reached commercial maturity (75%-100% red), which was assessed visually. Fruits that would typically be classified as 'unmarketable', due to pest damage or otherwise, were still harvested as the goal of the study was to analyze the differences between the two propagation methods and total potential yield. In cases of unavoidable conflict, fruits were harvested the next day. Once harvested, the fruits were brought back to the research station to be counted, weighed, and then frozen for chemical analysis after the growing season. The last harvest date was 25 October 2023. Data collected immediately after each harvest included the quantity of fruits harvested per plot, overall mass of fruits harvested per plot, largest fruit per plot, and average individual fruit mass.

1.3.6 Vegetative Structure Collection and Analysis

For runner data collection, five plants were selected from each plot to have their runner counts recorded. Runners were removed using hand pruners periodically throughout the growing season and were counted prior to being discarded.

At the end of the growing season the aboveground section of five plants per plot were cut near the soil line, shaken to remove soil, placed in paper bags, and brought back to the research station to determine dry mass and number of branch crowns for each plant. The strawberry plants were dried in paper bags starting on 17 November 2023 until 28 November 2023 when they were completely dried. After drying, the mass was recorded from each plant while they remained in the bag to avoid loss as they were very fragile. Following dry mass measurements each plant was carefully dissected to count the branch crowns.

1.3.7 Weather Data

Weather data were obtained from the Network for Environmental and Weather Applications (NEWA). Specifically, the Geneva (Agritech North), NY weather station data were used. For historical daily weather averages, weather data from the Geneva (Agritech North), NY weather station data were averaged by day for the 2019-2023 period.

1.3.8 Statistical Analysis

Using R version 4.4.0 (R Core Team 2021) linear mixed model analyses from R packages ‘lme4’ and ‘lmertest’ were used to evaluate the effects of propagation method, cultivar, and the interaction between propagation method and cultivar on runner counts, dry mass, branch crown counts, overall fruit mass harvested (yield), largest fruit harvested, quantity of fruits harvested, and average individual fruit mass over the 2023 production period. The overall total or average values per plot, depending on the measure, from the entire production period were used for the statistical analysis. Dry mass and branch crown count data were only collected once and therefore their analysis was based on a singular end of season measurement. The differences between CEApp and Fbrp within specific cultivars were also analyzed. Row was specified as a random effect. The 'DHARMa' R package was used to map the residuals of the data output from the mixed models to determine if the distribution of the residuals matched the expected distribution. Estimated marginal means were calculated using the 'emmeans' R package. They were calculated by fitting the linear mixed model to the data, extracting the fixed effects of the model, computing predicted values for each factor level or combination of factors, and then averaging over the model predicted random effects using the 'emmeans' package software. Estimated marginal means were used instead of recorded means in order to correct for effects from variation between rows on specific measures. Because estimated marginal means provide a way to interpret the fixed effects of the model while still accounting for the variability between rows, they allow for a better comparison of the relationship between the tested variables. The 'performance' R package was used to calculate Intraclass correlation coefficients (ICC) which were used to quantify the impact of the random effect (variation between rows) on the various measures. ICC quantifies the random effect variance in comparison to the total variance. Adjusted ICC values only account for the random effect variance while unadjusted ICC values account for the random effect variance as well as the sum of the fixed effect variances (Figure 1.4; Nakagawa et al., 2017). For trend graphs, the reported data were averaged by week.

Figure 1.4: Formulas for the adjusted and unadjusted Intraclass Correlation Coefficient (ICC) values

$$ICC_{adj} = \frac{\sigma^2_{RE}}{\sigma^2_{RE} + \sigma^2_{\varepsilon}}$$

$$ICC = \frac{\sigma^2_{RE}}{\sigma^2_{RE} + \sigma^2_{FE} + \sigma^2_{\varepsilon}}$$

σ^2_{RE} is the variance of the random effect (an output of the model fitting)

σ^2_{ε} is the variance of the model residual (an output of the model fitting)

σ^2_{FE} is the sum of the variances explained by each of the fixed effects (an output of the model fitting)

1.4 Vegetative Analysis Results

1.4.1 Runner Quantity and Timing

There was no significant effect on the quantity of runners produced per plant from propagation method, cultivar, or from the interaction between cultivar and propagation method (Table 1.2). There were also no significant differences observed between CEApp and Fbrp within specific cultivars (Table 1.2).

The first runner was observed on 28 June 2023, 37 days after planting (Table 1.2). Runners were observed from both CEApp and Fbrp for each cultivar on this date (Table 1.2).

Table 1.2. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average number of runners produced per strawberry plant throughout the 2023 field trial based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Runners/Plant (#)	SE	p-value	First Runner Date
Propagation Method			p = 0.24	
CEA	9.5	0.501		6/28/23
Field	10.3			6/28/23
Cultivar			p = 0.70	
Albion	9.6 a[1]	0.614		6/28/23
Cabrillo	10.3 a			6/28/23
Monterey	9.9 a			6/28/23
Cultivar by Propagation Method			p = 0.70	
Albion CEA	8.9	0.868	p = 0.27	6/28/23
Albion Field	10.2			6/28/23
Cabrillo CEA	10.3	0.868	p = 1.0	6/28/23
Cabrillo Field	10.3			6/28/23
Monterey CEA	9.3	0.868	p = 0.35	6/28/23
Monterey Field	10.4			6/28/23

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

1.4.2 Branch Crown Quantity

Both propagation method (p = 0.0009) and cultivar (p = 0.0013) significantly affected the estimated marginal average quantity of branch crowns produced per plant with ‘Cabrillo’ producing significantly fewer (6.5) than ‘Monterey’ (8.5) (Table 1.3). The estimated marginal average quantity of branch crowns produced by ‘Albion’ was not significantly different from ‘Cabrillo’ or ‘Monterey’ (Table 1.3). On average, CEApp produced an estimated 1.5 more branch crowns per plant than Fbrp (Table 1.3). The interaction between cultivar and propagation method did not significantly affect branch crown quantities (Table 1.3).

‘Monterey’ CEApp had significantly more branch crowns than ‘Monterey’ Fbrp ($p = 0.0034$) and ‘Cabrillo’ CEApp also had significantly more branch crowns than ‘Cabrillo’ Fbrp ($p = 0.04$) (Table 1.3). ‘Monterey’ CEApp produced an estimated 2.25 more branch crowns on average compared to ‘Monterey’ Fbrp (Table 1.3). ‘Cabrillo’ CEApp produced an estimated average of 1.6 more branch crowns per plant than ‘Cabrillo’ Fbrp (Table 1.3). There was no significant difference in the quantity of branch crowns between ‘Albion’ CEAp and Fbrp (Table 1.3).

Table 1.3. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average number of branch crowns produced per strawberry plant throughout the 2023 field trial based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Branch Crowns/Plant (#)	SE	p-value
Propagation Method			p = 0.0009†*
CEA	8.2	0.307	
Field	6.7		
Cultivar			p = 0.0013†*
Albion	7.4 ab[1]	0.375	
Cabrillo	6.5 a	0.376	
Monterey	8.5 b	0.375	
Cultivar by Propagation Method			p = 0.32†
Albion CEA	7.8	0.531	p = 0.39‡
Albion Field	7.1		
Cabrillo CEA	7.3	0.532	p = 0.04‡*
Cabrillo Field	5.7	0.531	
Monterey CEA	9.6	0.531	p = 0.0034‡*
Monterey Field	7.4		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

1.4.3 Dry Mass

Propagation method ($p = 0.0043$) and cultivar ($p = 0.01$) significantly affected plant dry mass (Table 1.4). On average, CEApp were an estimated 10.0 g heavier than Fbrp at the end of the production period (Table 1.4). 'Cabrillo' and 'Monterey' plants had significantly different dry mass. 'Cabrillo' plants had an estimated marginal average dry mass of 57.8 g, and 'Monterey' plants had an estimated marginal average dry mass of 71.6 g (Table 1.4). 'Albion' plants did not have significantly different dry mass than ‘Cabrillo’ or ‘Monterey’ plants (Table 1.4). The interaction between propagation method and cultivar did not significantly affect dry mass (Table 1.4).

‘Monterey’ CEApp had significantly higher dry mass than ‘Monterey’ Fbrp ($p = 0.0040$) (Table 1.4). On average, ‘Monterey’ CEApp were 18.5 g more massive than ‘Monterey’ Fbrp (Table 1.4). There were no significant differences in dry mass between the propagation methods within ‘Albion’ or ‘Cabrillo’ (Table 1.4).

Table 1.4. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average dry mass (g) at the end of the 2023 production period per strawberry plant based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Dry Mass/Plant (g)	SE	p-value
Propagation Method			p = 0.0043†*
CEA	69.7	5.54	
Field	59.7		
Cultivar			p = 0.01†*
Albion	63.7 ab[1]	5.84	
Cabrillo	57.8 a		
Monterey	71.6 b		
Cultivar by Propagation Method			p = 0.31†
Albion CEA	67.1	6.63	p = 0.29‡
Albion Field	60.3		
Cabrillo CEA	61.1	6.64	p = 0.30‡
Cabrillo Field	54.5	6.63	
Monterey CEA	80.8	6.63	p = 0.0040‡*
Monterey Field	62.3		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

1.4.4 Field Effects on Vegetative Qualities

The random effect of row had varying levels of effect on different vegetative measures. For runner quantity and branch crown incidence, the effect from row could not be computed as the variance within row was estimated by the model to be ≈0 (Table 1.5; Table 1.6). For dry mass, row accounted for an estimated 19.5% of the variation within the data (Table 1.7).

Table 1.5. Intraclass Correlation Coefficient from the random effect of row on strawberry runner quantity throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	†
Adjusted ICC	†
Unadjusted ICC	†

† value could not be computed as the random effect from the variable was determined to be ≈0.

Table 1.6. Intraclass Correlation Coefficient from the random effect of row on the quantity of branch crowns produced/plant throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	†
Adjusted ICC	†
Unadjusted ICC	†

† value could not be computed as the random effect from the variable was determined to be ≈0.

Table 1.7. Intraclass Correlation Coefficient from the random effect of row on strawberry plant dry mass from the 2023 field trial.

Random Effect Variables	Intraclass Correlation Coefficient
Row	19.5%
Adjusted ICC	19.5%
Unadjusted ICC	17.1%

1.5 Fruit Analysis Results

1.5.1 Harvest Timing

The first fruit was harvested on 18 July 2023, 57 days after planting and 29 days following the final flower removal date (Table 1.8). CEApp were first harvested on 18 July 2023 and Fbrp were first harvested 8 days later on 26 July 2023 (Table 1.8). Within ‘Albion’, CEApp were first harvested on 18 July 2023 and Fbrp were first harvested 9 days later on 27 July 2023 (Table 1.8). Within ‘Cabrillo’, CEApp were first harvested on 21 July 2023 and Fbrp were first harvested 5 days later on 26 July 2023 (Table 1.8). Within ‘Monterey’, CEApp were first harvested on 24 July 2023 and Fbrp were first harvested on 31 July 2023 (Table 1.8). Both CEApp and Fbrp of all varieties produced fruit until the last harvest date of 27 October 2023 (Table 1.8).

When looking at differences between propagation methods, the peak harvest date (determined by fruit mass harvested) for both CEApp and Fbrp, was on 4 September 2023 (Table 1.8). When looking at propagation method by cultivar, all treatment combinations had their peak harvest date on 4 September 2023 except for ‘Albion’ CEApp which peaked on 11 September 2023 (Table 1.8).

Table 1.8. Strawberry harvest timing data as day of the year by treatment for the 2023 production period.

Treatment	First Harvest Date	Peak Harvest Date (Highest Mass)	Last Harvest Date
Propagation Method			
CEA	199	247	300
Field	207	247	300
Cultivar			
Albion	199	247	300
Cabrillo	202	247	300
Monterey	205	247	300
Cultivar by Propagation Method			
Albion CEA	199	254	300
Albion Field	208	247	300
Cabrillo CEA	202	247	300
Cabrillo Field	207	247	300
Monterey CEA	205	247	300
Monterey Field	212	247	300

Day 199 is 18 July 2023

Day 202 is 21 July 2023

Day 205 is 24 July 2023

Day 207 is 26 July 2023

Day 208 is 27 July 2023

Day 212 is 31 July 2023

Day 247 is 4 September 2023

Day 254 is 11 September 2023

Day 300 is 27 October 2023

1.5.2 Quantity of Fruits Harvested

Overall, propagation method ($p = 0.02$), and the interaction between cultivar and propagation method ($p = 0.04$) had significant effects on the quantity of fruits harvested (Table 1.9). On average, CEApp produced 7.2 more fruits, or 14.1% more fruits, per plant than Fbrp (Table 1.9). 'Albion' and 'Monterey' plants produced significantly different quantities of fruits

(Table 1.9). ‘Albion’ plants produced an estimated average of 43.3 fruits per plant throughout the production period and ‘Monterey’ plants produced an average of 52.1 fruits per plant (Table 1.9). ‘Cabrillo’ did not produce a significantly different quantity of fruits compared to ‘Albion’ or ‘Monterey’ (Table 1.9). There was also a significant difference in the number of fruits harvested between CEApp and Fbrp ‘Cabrillo’ plants ($p = 0.0026$) (Table 1.9). On average, ‘Cabrillo’ CEApp produced 14.3 more fruits, or 31.3% more fruits, than ‘Cabrillo’ Fbrp throughout the production period (Table 1.9). There was no significant difference in the quantity of fruits harvested between ‘Albion’ or ‘Monterey’ CEApp or Fbrp (Table 1.9).

The average quantity harvested gradually increased from the start of the production period, reached a peak, and then steadily declined for both CEApp and Fbrp (Figure 1.3). CEApp peaked during the week of 18 September 2023 and Fbrp peaked during the week of 11 September 2023 (Figure 1.5). Throughout the production period, CEApp produced more fruits than Fbrp for the majority of the weeks (Figure 1.5). CEApp had a noticeably higher average quantity of fruits harvested compared to Fbrp, most notably in the early season between 17 July 2023 and 28 August 2023 (Figure 1.5). ‘Albion’ CEApp had lower average quantities of fruits produced between 28 August 2023 and 25 September 2023 than ‘Albion’ Fbrp (Figure 1.6).

The cumulative average quantity harvested for both CEApp and Fbrp followed similar trends (Figure 1.7). CEApp consistently had a higher cumulative average quantity harvested than Fbrp (Figure 1.7). The difference between CEApp and Fbrp was noticeable starting from 24 July 2023 and continued throughout the production period but was largest from 2 October 2023 until the end of the production period (Figure 1.7).

Table 1.9. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average quantity of strawberries harvested/plant throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Quantity of Fruits/Plant (#)	SE	p-value
Propagation Method			p = 0.02†*
CEA	33.9	3.71	
Field	29.1		
Cultivar			p = 0.06†
Albion	28.9 a[1]	3.96	
Cabrillo	31.0 ab		
Monterey	34.7 b		
Cultivar by Propagation Method			p = 0.04†*
Albion CEA	28.4	4.62	p = 0.76‡
Albion Field	29.3		
Cabrillo CEA	36.8	4.62	p = 0.0026‡*
Cabrillo Field	25.3		
Monterey CEA	36.7	4.62	p = 0.23‡
Monterey Field	32.7		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of the effect of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 1.5. Effect of propagation method on the average quantity of strawberries harvested (#) per plant throughout the 2023 production period by week.

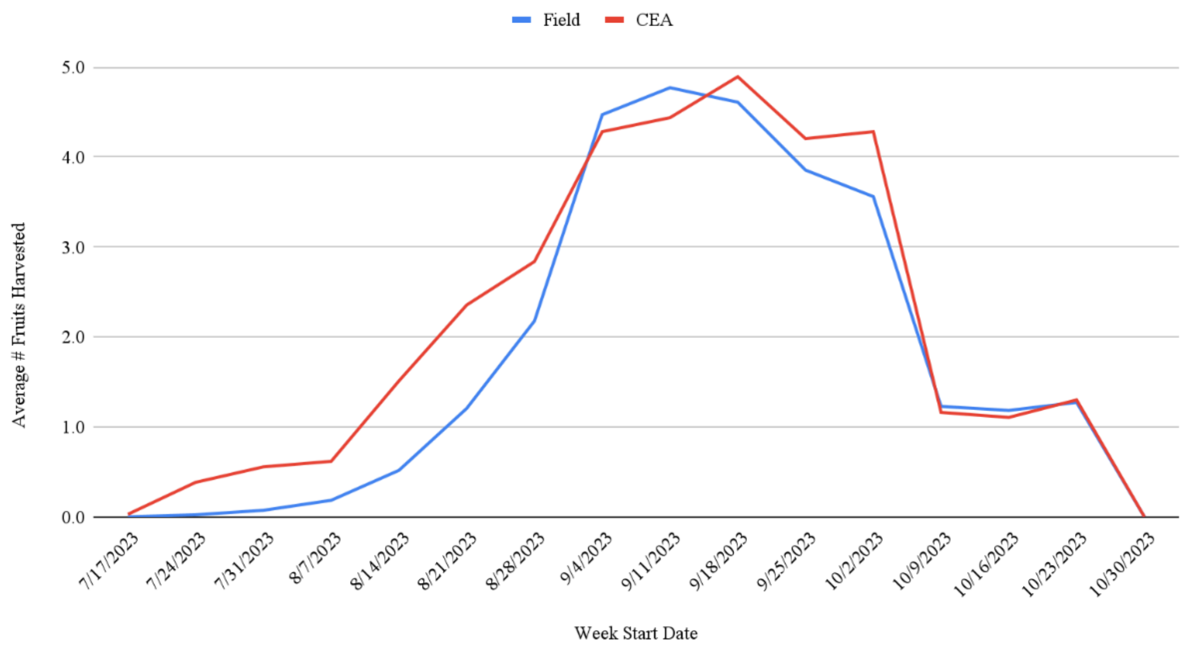


Figure 1.6. Effect of propagation method within specific cultivars on the average quantity of strawberries harvested (#) per plant throughout the 2023 production period by week.

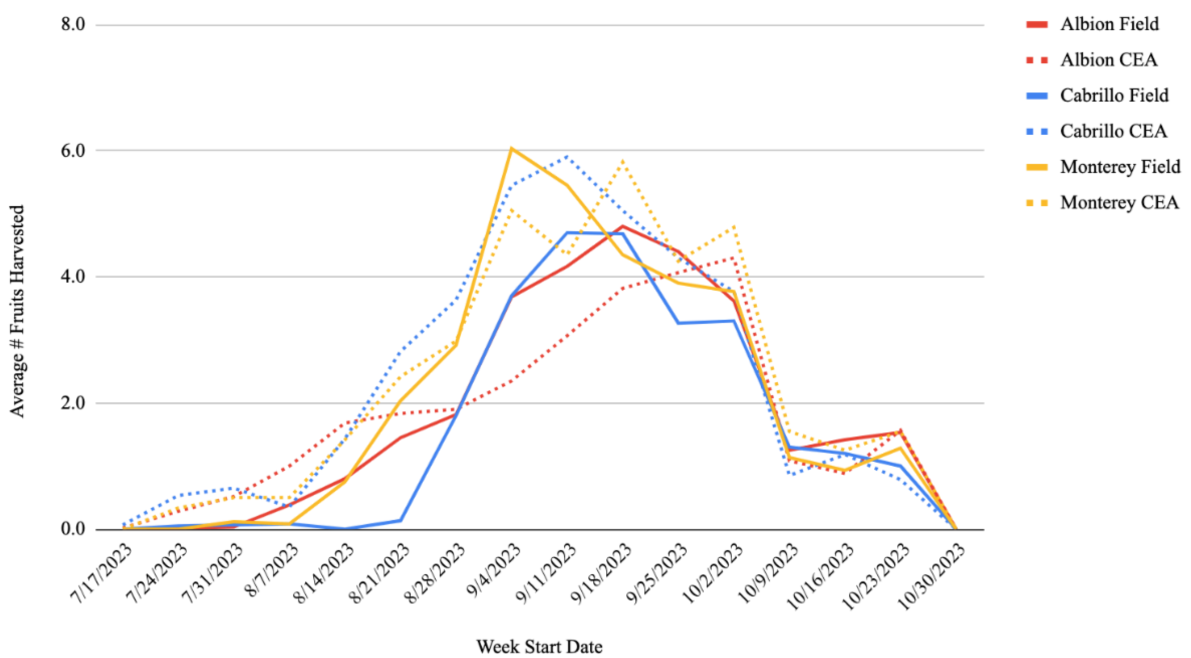
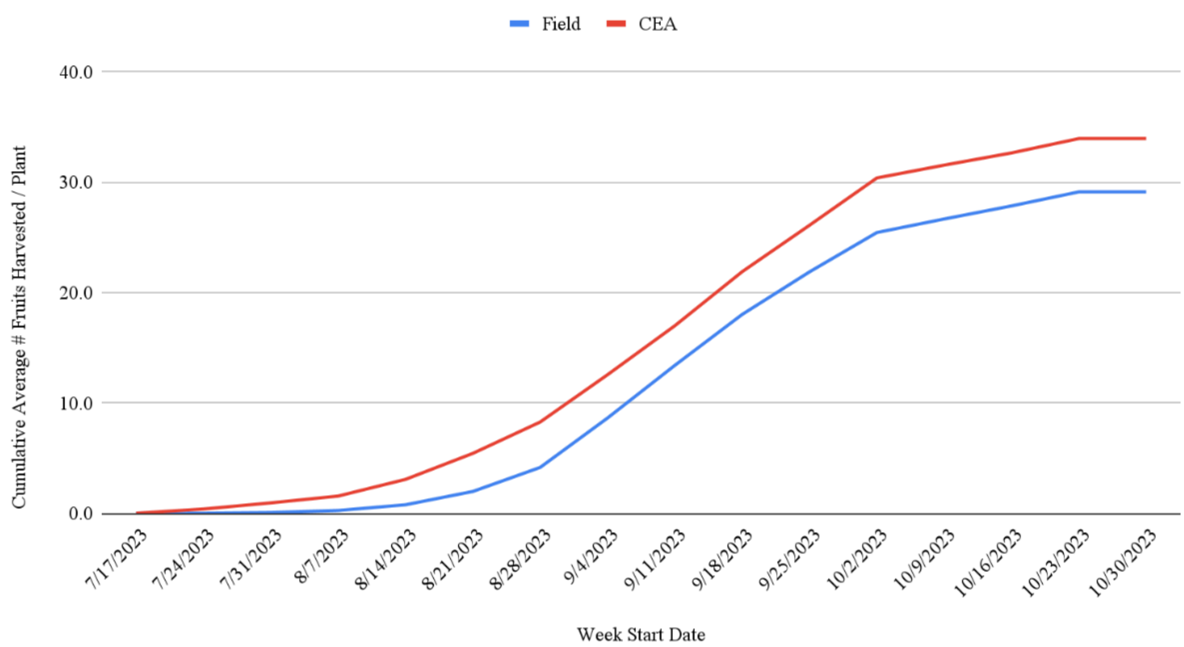


Figure 1.7. Effect of propagation method comparing CEApp and Fbrp on the cumulative average quantity of strawberries harvested (#) per plant throughout the 2023 production period by week.



1.5.3 Overall Mass of Fruits Harvested - Yield

Propagation method (p = 0.0029) significantly affected yield (Table 1.10). On average, CEApp yielded an estimated 129.3 g, or 21.6%, more per plant than Fbrp throughout the production period (Table 1.10). Cultivar and the interaction between cultivar and propagation method did not significantly affect yield (Table 1.10). There was no significant difference in the overall mass of fruits harvested between cultivars (Table 1.10).

When looking at the differences in yield by propagation method within cultivars, on average 'Cabrillo' CEApp had significantly higher yields per plant than 'Cabrillo' Fbrp (p = 0.0019) (Table 1.10). Over the production period, 'Cabrillo' CEApp yielded an estimated 236.8 g, or 34.7%, more per plant than 'Cabrillo' Fbrp (Table 1.10). There was no significant difference in the yield between 'Albion' or 'Monterey' CEApp and 'Albion' Fbrp (Table 1.10).

Over the production period, CEApp and Fbrp followed a similar trend (Figure 1.8). The yield steadily increased from the start of the period until it reached a peak on 4 September 2023 for both propagation methods (Figure 1.8). Following this, yields from both CEApp and Fbrp decreased (Figure 1.8). After 9 October 2023, yields for both CEApp and Fbrp stayed at ~10 g/plant per week until the end of the production period (Figure 1.8). CEApp had a noticeably higher average mass harvested compared to Fbrp, most notably in the early season between 17 July 2023 and 21 August 2023 (Figure 1.8). 'Cabrillo' CEApp consistently had a higher average mass harvested compared to 'Cabrillo' Fbrp from the start of the production period until 2 October 2023 (Figure 1.9). 'Albion' CEApp had a lower but more consistent mass harvested throughout the production period than 'Albion' Fbrp (Figure 1.9).

The cumulative average overall mass harvested for both CEApp and Fbrp followed similar trends (Figure 1.10). Throughout the entire production period, CEApp consistently had a higher cumulative average quantity harvested than Fbrp (Figure 1.10). The difference between CEApp and Fbrp was noticeable starting from the beginning of the production period (Figure 1.10). Following 11 September 2023, the difference between CEApp and Fbrp gradually increased (Figure 1.10).

Table 1.10. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average overall mass of strawberry fruit harvested (g) per plant throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Fruit Mass Harvested/Plant (g)	SE	p-value
Propagation Method			p = 0.0029†*
CEA	399.5	45.4	
Field	313.3		
Cultivar			p = 0.15†
Albion	320.5 a[1]	48.9	
Cabrillo	376.3 a		
Monterey	372.4 a		
Cultivar by Propagation Method			p = 0.09†
Albion CEA	329.3	58.2	p = 0.68‡
Albion Field	311.6		
Cabrillo CEA	455.2	58.2	p = 0.0019‡*
Cabrillo Field	297.3		
Monterey CEA	414.0	58.2	p = 0.06‡
Monterey Field	330.9		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 1.8. Effect of propagation method on the overall average mass of strawberries harvested (#) per plant throughout the 2023 production period by week.

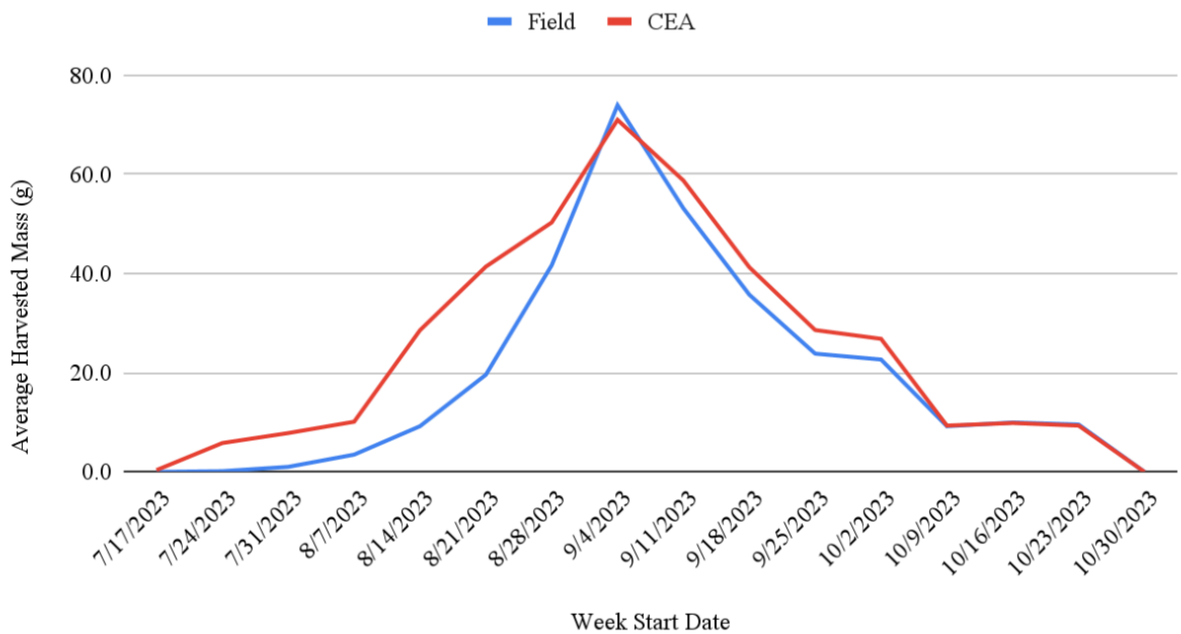


Figure 1.9. Effect of propagation method within specific cultivars on the average overall mass of strawberries harvested (g) per plant throughout the 2023 production period by week.

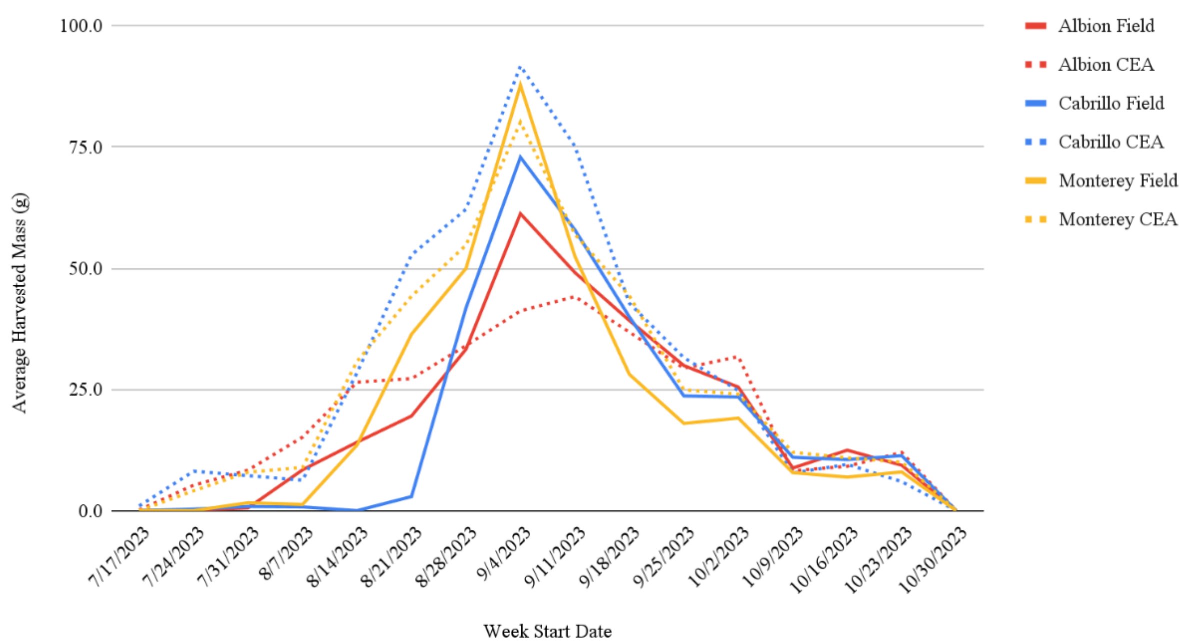
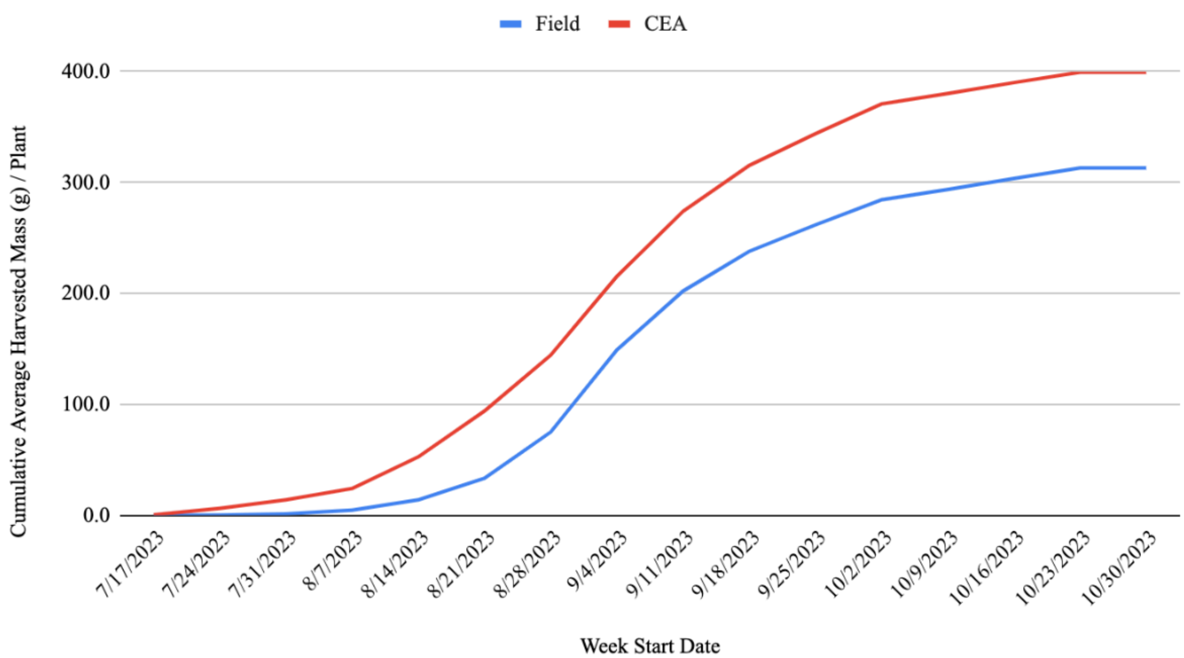


Figure 1.10. Effect of propagation method on the average cumulative mass of strawberries harvested (g) per plant throughout the 2023 production period by week.



1.5.4 Largest Fruit Harvested

There was no significant effect from propagation method, cultivar, or the interaction between propagation method and cultivar on the largest fruit harvested (Table 1.11). There was also no significant difference in the average largest fruit harvested between cultivars (Table 1.11). There was no significant effect observed from propagation method within cultivar (Table 1.11).

Average largest fruit mass steadily increased from the start of the production period until 4 September 2023 for both CEApp and Fbrp (Figure 1.11). Following 4 September 2023, average largest fruit mass steadily decreased for both treatments until stalling between 25 September 2023 and 9 October 2023 (Figure 1.11). There was a slight uptick for both treatments on 16 October 2023 before continuing to decline until the end of the production period (Figure

1.11). For all cultivars, CEApp had a more consistent average largest fruit size than Fbrp (Figure 1.12). ‘Albion’ CEApp and ‘Albion’ Fbrp had a larger spike than other cultivars in average largest fruit during the week of 16 October 2023 (Figure 1.8).

Table 1.11. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average largest strawberry produced (g) per plot/harvest throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of Largest Fruit (g)	SE	p-value
Propagation Method			p = 0.32†
CEA	21.5	0.901	
Field	20.6		
Cultivar			p = 0.11†
Albion	21.9 a[1]	1.0	
Cabrillo	21.5 a		
Monterey	19.6 a		
Cultivar by Propagation Method			p = 0.34†
Albion CEA	22.4	1.25	p = 0.52‡
Albion Field	21.4		
Cabrillo CEA	21.1	1.25	p = 0.62‡
Cabrillo Field	21.9		
Monterey CEA	20.9	1.25	p = 0.12‡
Monterey Field	18.4		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 1.11. Effect of propagation method on the average largest strawberry (g) per harvest/plot throughout the 2023 production period by week.

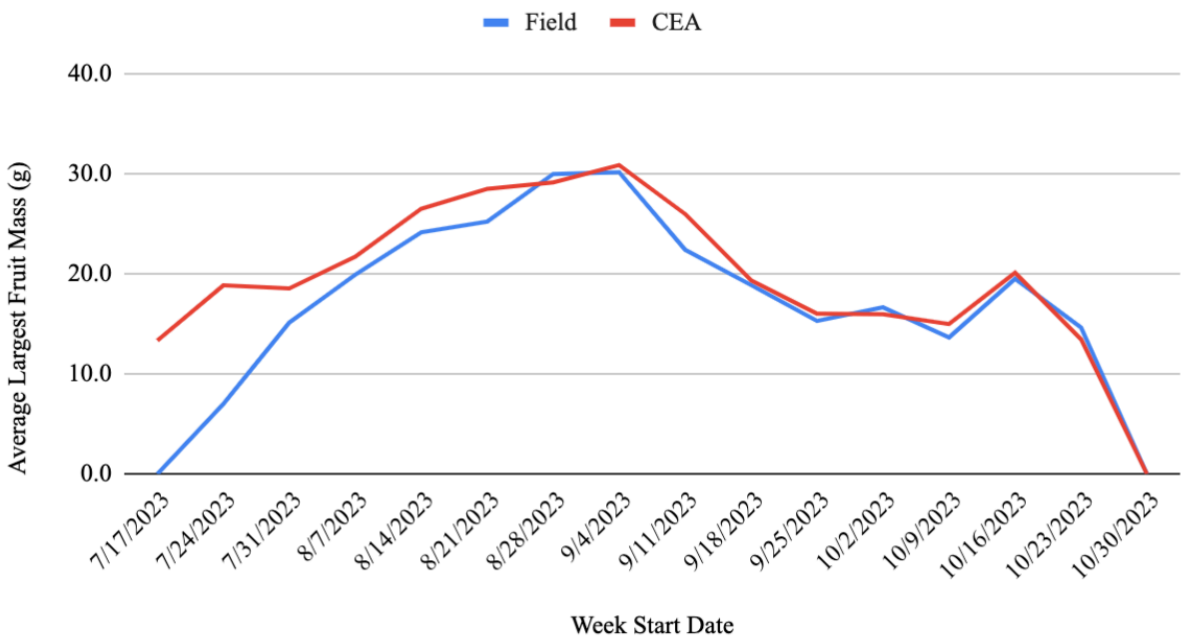


Figure 1.12. Effect of propagation method within specific cultivars on the average largest strawberry (g) per harvest/plot throughout the 2023 production period by week.

1.5.5 Average Individual Fruit Mass

Propagation method significantly affected the average individual fruit mass (p = 0.0052) (Table 1.12). On average, CEApp produced fruits that were 1.2 g, or 9.4%, heavier than fruits from Fbrp (Table 1.12). There was no significant difference in average individual fruit mass between cultivars (Table 1.12). There was also no significant effect observed from cultivar or the interaction between cultivar and propagation method on average individual fruit mass (Table 1.12).

When looking at differences from propagation effect within cultivars, ‘Monterey’ CEApp produced significantly more massive fruits than Fbrp ‘Monterey’ plants (p = 0.0060) (Table 1.12). On average, ‘Monterey’ CEApp produced 1.9 g, or 15.1%, larger fruits than ‘Monterey’ Fbrp (Table 1.12). There were no significant differences in individual fruit mass between propagation methods in ‘Albion’ or ‘Cabrillo’ (Table 1.12).

CEApp and Fbrp followed similar trends for average individual fruit mass throughout the production period (Figure 1.13). From the start of the production period, average individual fruit mass increased for both CEApp and Fbrp. Following the week of 28 August 2023 average individual fruit mass began to decrease for both CEApp and Fbrp (Figure 1.13). The average individual fruit mass slightly increased again between 2 October 2023 and 16 October 2023 before declining to zero (Figure 1.13). CEApp of all tested cultivars had a more consistent average individual fruit mass than Fbrp throughout the production period (Figure 1.14). ‘Cabrillo’ Fbrp had a noticeably inconsistent average individual fruit mass compared to the other cultivars and propagation methods between 24 July 2023 and 4 September 2023 (Figure 1.14). Average individual fruit mass peaked in the first half of the production period for all cultivars and propagation methods, but the date of the peak varied (Figure 1.14).

Table 1.12. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average individual strawberry fruit mass (g) per harvest/plot throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean Individual Fruit Mass/Plot (g)	SE	p-value
Propagation Method			p = 0.0052†*
CEA	12.7	0.332	
Field	11.5		
Cultivar			p = 0.19†
Albion	12.4 a[1]	0.373	
Cabrillo	11.4 a		
Monterey	10.8 a		
Cultivar by Propagation Method			p = 0.30†
Albion CEA	12.7	0.476	p = 0.25‡
Albion Field	12.0		
Cabrillo CEA	12.7	0.476	p = 0.23‡
Cabrillo Field	11.9		
Monterey CEA	12.6	0.476	p = 0.0060‡*
Monterey Field	10.7		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 1.13. Effect of propagation method on the average individual strawberry mass (g) per harvest/plot throughout the 2023 production period by week.

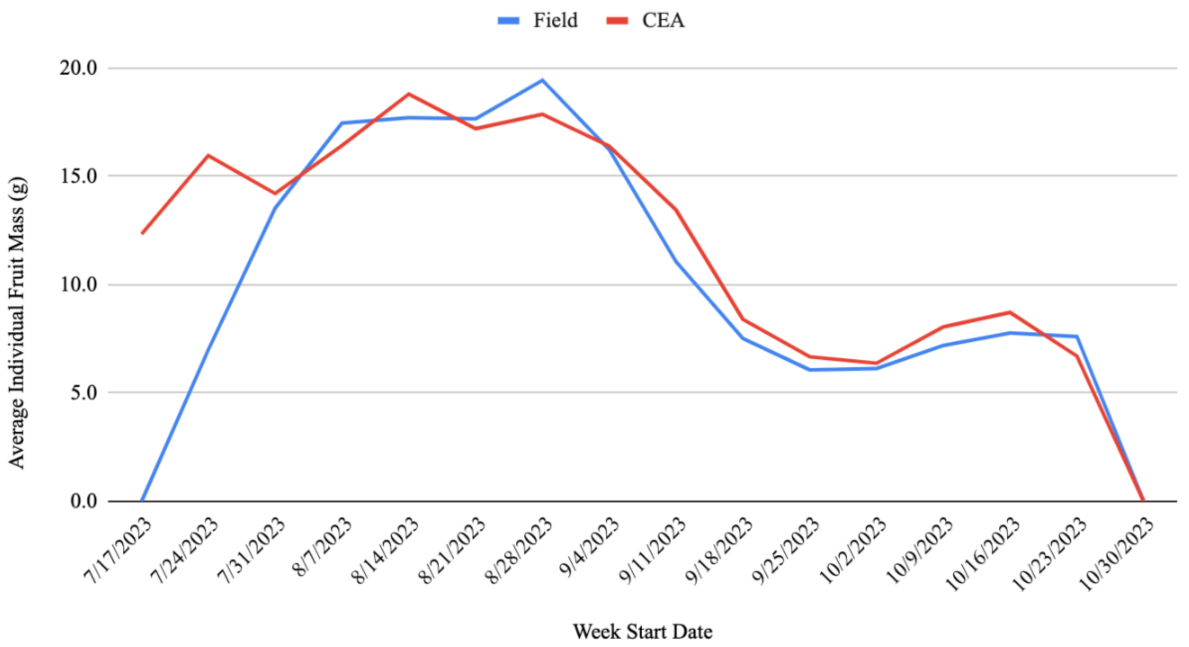
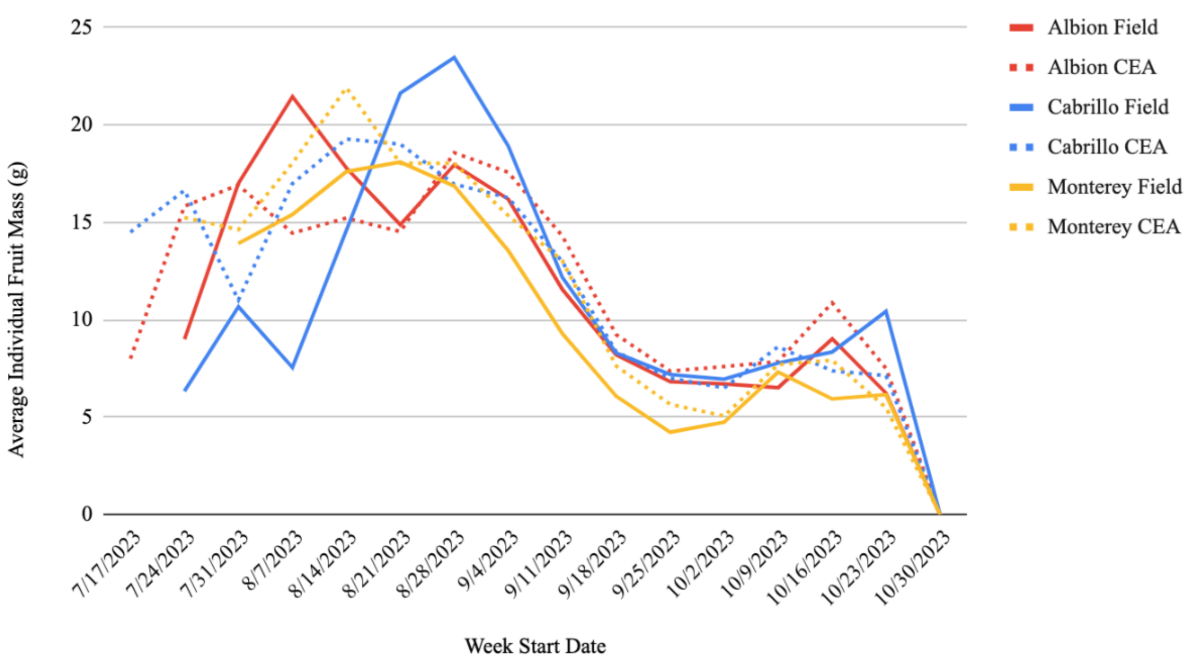


Figure 1.14. Effect of propagation method within specific cultivars on the average individual strawberry mass (g) per harvest/plot throughout the 2023 production period by week.



1.5.6 Field Effects on Fruit Harvest Qualities

The effect of row varied between the different measured fruit qualities (Table 1.13; Table 1.14, Table 1.15, Table 1.16). For the quantity of fruits harvested, it was found that variation between rows accounted for 46.4% of the variation within the data (Table 1.13). For overall mass harvested it was calculated that variation between rows was responsible for 41.4% of the variation within the data (Table 1.14). For the largest fruit harvested, variation between rows was responsible for 27.8% of the variation (Table 1.15). For average individual fruit mass, row was responsible for 21.2% of the variation in the data (Table 1.16).

Table 1.13. Intraclass Correlation Coefficient from the random effect of row on quantity of strawberries harvested throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	46.4%
Adjusted ICC	46.4%
Unadjusted ICC	30.9%

Table 1.14. Intraclass Correlation Coefficient from the random effects of row on overall mass of strawberries harvested throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	41.4%
Adjusted ICC	41.4%
Unadjusted ICC	26.3%

Table 1.15. Intraclass Correlation Coefficient from the random effect of row on largest strawberry fruit throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	27.8%
Adjusted ICC	27.8%
Unadjusted ICC	21.9%

Table 1.16. Intraclass Correlation Coefficient from the random effect row on average individual fruit mass throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	21.2%
Adjusted ICC	21.2%
Unadjusted ICC	11.9%

1.5.7 Weather and Harvest Measures

The daily high air temperatures for the 2023 production period followed a similar trend as the historical average daily high temperatures (Figure 1.15). During the production period, air temperature was highest on 4 September 2023, 31.1 ºC, and was lowest on 22 October 2023, 5.0 ºC (Figure 1.15). These measures were both more extreme than the average temperatures for their dates (Figure 1.15). Between 2 September 2023 and 7 September 2023, there was a spike in the highest temperature compared to the historical average (Figure 1.15). From the beginning of the production period to 2 September 2023, daily high air temperatures were fairly consistent and following 7 September 2023, the daily high air temperatures steadily declined, following a similar trend to the average data (Figure 1.15). Between 29 September 2023 and 7 October 2023, there was a spike in temperature up to 28.5ºC, which was higher than average for that time of year (Figure 1.15). Nighttime low temperatures during the production period were not as consistent as the season average, but followed a similar trend (Figure 1.15).

The total precipitation was higher than expected from the start of the production period until 18 August 2023 (Figure 1.16). On 24 September 2023, total precipitation was 0.6 in higher than the average precipitation for the date (Figure 1.16). During the period between 7 September 2023 and 13 September 2023 experienced high levels of precipitation compared to the historical averages (Figure 1.16). Following this, precipitation was lower than expected for the remainder of the production period (Figure 1.16).

Figure 1.15. Actual and average daily air temperatures recorded from the Geneva Agritech North weather station. Averages are from years 2019-2023

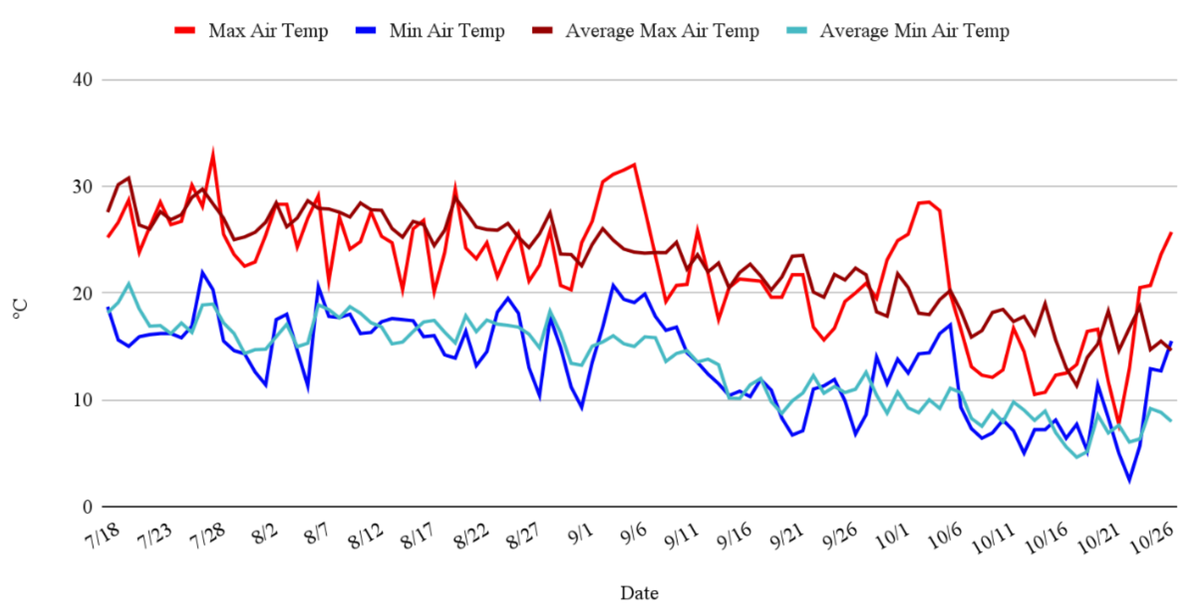
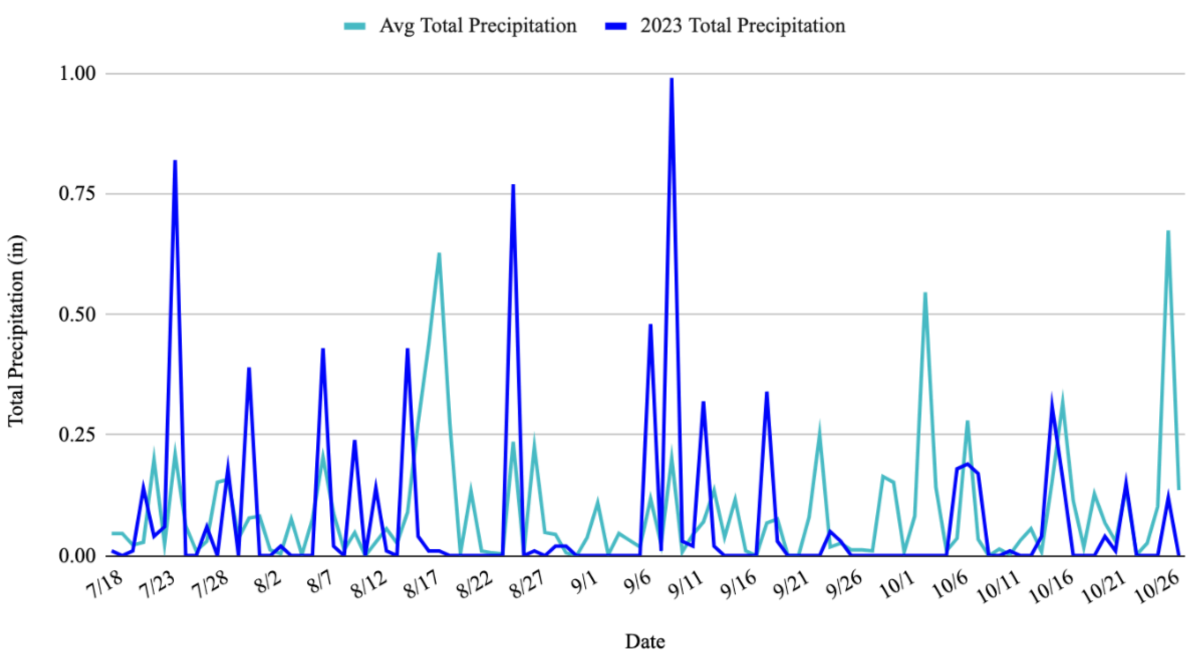


Figure 1.16. Actual and average daily total precipitation (in) recorded from the Geneva Agritech North weather station. Averages are from years 2019-2023



1.6 Discussion

1.6.1 Vegetative Analysis Discussion

CEApp resulted in significant increases in branch crown quantities and dry mass in strawberry plants regardless of cultivar (Table 1.3; Table 1.4). Runner counts were not significantly affected by CEApp (Table 1.2). At the start of the growing season, the CEApp were further developed than the Fbrp, which were just emerging from dormancy. This may have allowed for the CEApp to grow into larger plants than Fbrp during the growing season. Other studies looking at vegetative differences between bare-root and plug plants found similar results (Torres-Quezada et al., 2020; Cocco et al., 2011) A replicate trial where Fbrp is started earlier in cold frames or in a greenhouse could help determine if the effects on branch crown incidence and dry mass were because of the propagation method per se or the plant developmental stage at planting. Despite the potential effect of the developmental stage, these results are representative of growth characteristics of CEApp versus Fbrp in commercial production as growers would receive propagules in the same condition as in this study.

A grower would need to consider that CEApp planted fields would have larger plants with more branch crowns and consider the potential effects on their production system. In spring planting systems the plants may be manageable throughout the production period, but if CEApp are used in fall plantings the plants may become even larger than observed in this study. Future research on the differences of effect of CEApp in spring plantings vs fall plantings could provide valuable insight into how CEApp physically responds to overwintering.

The tested cultivars responded differently to CEApp. 'Albion' was not significantly affected by CEApp in runner count, branch crown incidence, or dry mass (Table 1.2; Table 1.3; Table 1.4). 'Cabrillo' was significantly affected by CEApp in branch crown incidence but not in runner counts or dry mass (Table 1.2; Table 1.3; Table 1.4). 'Monterey' was significantly affected by CEApp in dry mass and branch crown incidence but not in runner counts (Table 1.2; Table 1.3; Table 1.4). As these effects were not uniform across cultivars, growers would need cultivar specific information when deciding whether CEApp or Fbrp would be most appropriate for their production system.

Observationally, in the last month of the production period, CEApp 'Monterey' plants had denser canopies with more leaves than 'Monterey' Fbrp, which is likely why 'Monterey' CEApp had much higher dry mass. Smaller fruit sizes were also observed as the canopy thickened, likely as a result of the increasing number of branch crowns and leaves drawing energy away from fruit production (Figure 1.14). The smaller fruit sizes were not observed with 'Cabrillo' despite 'Cabrillo' CEApp having significantly more branch crowns than 'Cabrillo'

Fbrp (Figure 1.14). The difference between 'Monterey' and 'Cabrillo' in the number of leaves produced per crown is possibly genetic, which led to a much denser canopy for 'Monterey' and could explain why 'Cabrillo' CEApp did not have significantly higher dry mass than 'Cabrillo' Fbrp. Also, observationally, 'Monterey' plants from both propagation methods had noticeably thicker canopies than 'Albion' or 'Cabrillo' plants, supporting this theory. A larger study with more cultivars could help predict which cultivars are more likely to develop higher numbers of branch crowns and denser canopies. Future studies could also measure leaf canopy of a wider variety of strawberry cultivars propagated either as CEApp or Fbrp to test if the phenomena observed in this trial were because of cultivar or propagation method.

Since higher numbers of branch crowns are associated with dense leaf canopies, CEApp plants may have higher levels of foliar disease incidence than Fbrp. Growers should consider this when planting CEApp to appropriately plan for pest management and irrigation. Reduced fruit size and quality can also be a concern in plants with higher numbers of branch crowns, but harvest analyses showed that CEApp had better fruit harvest qualities than Fbrp. This could indicate that the positive effects of CEApp are so impactful that they can negate the potential negative effects of an increased leaf canopy. Further studies could evaluate foliar disease incidence of CEApp compared to Fbrp to determine if foliar disease incidence rates of CEApp are different than expected.

While not the main objective of this study, the differences in vegetative traits between cultivars are important. While branch crown incidence and dry mass were significantly affected by cultivar, the quantity of runners produced per plant was not (Table 1.2; Table 1.3; Table 1.4). Previous studies have also observed differences in branch crown counts between cultivars (McWhirt, 2021). This suggests that plant size is cultivar specific, but the number of runners a plant will produce is not affected by propagation method, and is more likely influenced by environmental conditions such as temperature and/or daylength. Future studies could compare the number of runners produced per plant from a wider variety of cultivars to determine if this is the case or if genetics plays a larger role that was not observed here due to a limited cultivar sample.

The ICC values for vegetative measures were either unable to be calculated because the model estimated between row variance was ≈0 or were lower than ICC values for harvest measures (Table 1.5; Table 1.6; Table 1.7; Table 1.13; Table 1.14; Table 1.15; Table 1.16). One explanation of why vegetative measures were less affected by variation between rows is because the data was either collected once at the end of the season, or only a few times during the season depending on the measure. This contrasts with the harvest data which was collected multiple times per week throughout the season. After planting, both Fbrp and CEApp had to acclimate to the field, but there was a lag period of Fbrp plant growth compared to CEApp because of Fbrp dormancy. Different microclimates between rows caused by localized soil differences, irrigation differences, or slopes in the field may have affected how quickly CEApp and Fbrp acclimated and may have affected the ability of Fbrp to grow after breaking dormancy. Observationally, as the season progressed, Fbrp quickly caught up to CEApp. Measures in the early season may have captured these differences in establishment, adding to the variation between rows, but since vegetative measures were only evaluated a few times or once at the end of the season, this data reflects plant growth after having the opportunity to catch up, which could result in a lower variance in data between rows.

1.6.2 Harvest Analysis Discussion

CEApp had a significant increase in the quantity of fruits harvested, overall mass of fruits harvested, and average individual fruit mass in strawberry plants compared to Fbrp, regardless of cultivar (Table 1.9; Table 1.10; Table 1.12). There was no significant difference between CEApp and Fbrp in the average largest fruit produced per harvest (Table 1.11). Previous studies investigating the differences between strawberry transplant types had similar results with plug plants producing significantly higher yields (Gaisser et al., 2024; Weber 2021; Cocco et al., 2020; Torres-Quezada et al., 2020).

In a 2022 survey of New York State wholesale strawberry pricing, Park (2023) found that the average wholesale strawberry price per pound was $4.00. If the same planting density used in this study was scaled up to an acre, the density would be 17500 plants per acre. If a grower decided to grow CEApp over Fbrp, they could pay $0.76 more per plant and still expect to make the same profit (Table 1.17). Even though the CEApp may be more expensive initially than Fbrp, their increase in yield would allow growers to still profit.

Table 1.17. Economic comparison of the difference in mass harvested (yield) value between controlled environment propagated plug plants (CEApp) and field propagated bare-root plants (Fbrp). The cost of strawberries was fixed at $4.00/lb, or $0.0088/g, based on wholesale value from a 2022 survey of New York State berry growers (Park, 2023). Planting density per acre based on the planting density of this study was determined to be 17500 plants per acre.

Estimated Marginal Mean Overall Mass Harvested (g)		**Difference**
CEApp	399.5	+ 86.2 g
Fbrp	313.3	
Overall Mass Harvested per Acre (17500 plants/acre) (g)		
CEApp	6,991,250	+ 1,508,500 g
Fbrp	5,482,750	
Value per Acre ($0.0088/g) ($)		
CEApp	61,523.00	+ $13,275
Fbrp	48,248.20	
Value per plant ($)		
CEApp	3.52	+ $0.76
Fbrp	2.76	

At the start of the growing season, the CEApp were further developed than the Fbrp, which were from cold storage and relatively dormant. This likely allowed the CEApp to establish a more robust root system that could better support the production of fruit than Fbrp. This is supported by the early season harvest data in which the largest differences between CEApp and Fbrp harvest parameters were observed. Future studies could start Fbrp earlier in a greenhouse or cold frame to see if the effects on harvest measurements were because of the propagation method per se, or the plant developmental stage of CEApp at planting. Differences in root mass between CEApp and Fbrp could also be examined to determine if CEApp has a more extensive root system, which would support a larger crop load and potentially improve harvest parameters. Despite the potential effects of plant establishment and dormancy, the test field was planted with the plants as a grower would receive them. Therefore, this study is a good approximation of real-world strawberry production systems.

The cumulative quantity of fruits harvested and overall mass harvested data both followed similar trends. For both measures, CEApp consistently had a higher cumulative average quantity harvested than Fbrp and the difference between CEApp and Fbrp was noticeable starting from the beginning of the production period (Figure 1.7; Figure 1.10). The overall harvest mass is likely correlated with the quantity of fruits harvested so the similarity of the two analyses is logical. This data may be valuable to growers as it provides insight into when in the season they can expect to have a certain amount of fruit. In the cumulative plots it is also easier to visualize the overall differences between CEApp and Fbrp than in the overall season trend plots as the day to day variation is less prominent.

Comparing the harvest measure trends throughout the production period to air temperatures and precipitation during trials, there were trends that appeared correlated. For average overall mass harvested, the first and second peaks of mass harvested line up with both spikes in daily high temperature (Figure 1.8; Figure 1.15). Also, when looking at average largest fruit, the second peak in largest fruit lined up with the second spike in daily high temperature (Figure 1.11; Figure 1.15). These correlations may indicate that daily high temperatures have an effect on fruit mass. Previous studies have also observed increased yields in relatively high temperatures (Gaisser 2024; Palencia et al., 2013) It was also observed that average individual fruit mass increased around periods of heavy rainfall (Figure 1.13; Figure 1.16). This may be because of the plants uptaking more water, which allowed for the fruits to become larger and/or heavier.

Specific cultivars were not affected by CEA propagation equally (Table 1.9; Table 1.10; Table 1.11; Table 1.12). ‘Albion’ CEApp was not significantly different from ‘Albion’ Fbrp in any of the harvest measures (Table 1.9; Table 1.10; Table 1.11; Table 1.12). ‘Cabrillo’ was significantly affected by CEApp in quantity harvested and overall mass harvested, but there were no significant differences between ‘Cabrillo’ CEApp and Fbrp for average largest fruit per harvest or average individual fruit mass over the season (Table 1.9; Table 1.10; Table 1.11; Table 1.12). However, the trend in almost all cases was for CEApp to produce more and larger fruit on average than Fbrp except for the number of fruit in ‘Albion’ (Table 1.9; Table 1.10; Table 1.12). The relatively small size of this study may have prevented observed differences between CEApp and Fbrp from being significant in some cases. A repeat trial with a larger sample size may show more significant results.

The ‘Cabrillo’ Fbrp were visibly stressed upon arrival and took longer to get established than Fbrp plants of different cultivars. This could explain why ‘Cabrillo’ CEApp had a significantly higher quantity of fruits and overall mass harvested as they were established and actively growing for longer in the field trial than ‘Cabrillo’ Fbrp. This hypothesis is also supported by the large difference in performance between CEApp and Fbrp during the early season for quantity harvested and overall fruit mass. A repeat trial with healthier ‘Cabrillo’ Fbrp would help determine if the results seen are from a greater effect of CEApp on ‘Cabrillo’ or if they are from healthier plants being compared against stressed plants. Harvest qualities such as largest fruit and average individual fruit mass may be more affected by genotype than environment, which may be why there was no significant difference between propagation methods observed from these qualities despite the initial condition of the ‘Cabrillo’ Fbrp.

‘Monterey’ CEApp had significantly higher average individual fruit masses than ‘Monterey’ Fbrp (Table 1.12). There was no significant difference between ‘Monterey’ CEApp and Fbrp in quantity harvested, overall mass harvested, or largest fruit (Table 1.9; Table 1.10; Table 1.11). The difference between ‘Monterey’ CEApp and Fbrp was most prevalent during the first two thirds of the production period, so the difference could potentially be due to the plug plants being able to establish themselves faster than the bare-root plants (Figure 1.13). A repeat trial with more similarly established 'Monterey' CEApp and Fbrp could show if the effects of the propagation are the cause of the difference or if it was because of the difference in plant establishment.

Therefore, when a grower is choosing which cultivars to grow, they may or may not observe a difference in harvest qualities between CEApp and Fbrp. Cultivar specific information is required for growers to make informed decisions on sourcing plants due to differences in cost and planting procedures.

There was no significant effect from across the cultivars in quantity of fruits harvested, overall mass harvested, largest fruit, or average individual fruit mass. But when cultivars were compared individually, there were some differences (Table 1.9; Table 1.10; Table 1.11, Table 1.12). For the quantity of fruits harvested measure, 'Monterey' produced significantly more fruits than 'Albion' (Table 1.9). Since all of the cultivars in the study were developed for the same strawberry growing systems, it is likely that similar harvest traits were selected for during the breeding process. One potential conclusion is that with their current stage of cultivar development, strawberries have reached their maximum harvest potential. Future research could look at harvest traits from a wider selection of cultivars to see if cultivar significantly affects such measures.

The adjusted ICC for the variation in the data from row for quantity harvested was 46.4%, overall mass harvested was 41.4%, largest fruit mass 27.8%, and average individual fruit mass was 21.2% (Table 1.13; Table 1.14; Table 1.15; Table 1.16). While these numbers may seem high, they provide insight on how much variation can occur even within a singular planting site. The level of variance in the data may be due to differences in irrigation, soil content, air flow, or any seemingly minute irregularities between the rows. Observationally, the plants in row 3 appeared to be less vigorous than plants in the other three rows and plants in rows 4 and 5 appeared to be more vigorous than plants in rows 3 or 6 which may be the reason for the variation in the different measures between rows. The values for quantity harvested and overall mass harvested are similar as well as the values for largest fruit and average individual fruit mass. This suggests that the overall quantity of fruit produced is more affected by the variation between rows than measures of individual fruit size. One theory as to why this may be the case is that individual fruit size is more impacted by genotype than environment and the quantity of fruit produced is more impacted by environment.

1.6.2 General Discussion and Conclusion

Overall, relative to Fbrp, CEApp were more vigorous plants. This was seen with the higher quantities of branch crowns and dry mass as well as the increased quantity harvested, overall mass harvested, and average individual mass of strawberries (Table 1.3; Table 1.4, Table 1.9; Table 1.10; Table 1.12). The benefits of CEApp in regards to harvest were most noticeable in the early season which could give growers an advantage against others growing Fbrp (Table 1.9; Table 1.10; Table 1.12).

The results of this study support the application of CEApp as a replacement for Fbrp in commercial strawberry production systems. If growers choose to grow CEApp instead of Fbrp they can expect increased yields and larger fruits, especially in the early season. By utilizing CEApp, the risk of introducing soil-borne pathogens is negated, further reducing crop loss and the need for intensive control measures. The production of CEApp is also not reliant on soil fumigation, ensuring that plants will be available even as restrictions on fumigants continue to tighten. Also, because CEApp are produced in a controlled environment plug plants can be produced year-round, allowing for plants to be accessible for spring or fall plantings in colder climates so that growers in the region can compete with larger operations on the West Coast.

In summary, the utilization and applications of CEApp solve many of the current struggles with the current strawberry production system. While there is still much research to be done on CEApp development and understanding, it is a promising alternative to the current standard propagation method.

1.7 References

Berrycrop Agro LLP. (n.d.). https://berrycrop.in/product-category/plants/

Chellemi, D. O. (2014). Plant health management: Soil fumigation. *Encyclopedia of Agriculture and Food Systems*, 456–459. https://doi.org/10.1016/b978-0-444-52512-3.00250-3

Cocco, C., Andriolo, J. L., Cardoso, F. L., Erpen, L., & Schmitt, O. J. (2011). Crown size and transplant type on the strawberry yield. *Scientia Agricola*, 68(4), 489–493. https://doi.org/10.1590/s0103-90162011000400015

Cocco, C., Faedi, W., Magnani, S., Maltoni, M. L., Stagno, F., Turci, P., Quacquarelli, I., Ferré, A.-S., & Baruzzi, G. (2020). Effects of plug plants and bare-root plants on strawberry field performance, fruit quality traits and health-promoting compounds. *Journal of Berry Research*, 10(2), 145–156. https://doi.org/10.3233/jbr-190389

ICC: Intraclass Correlation Coefficient (ICC) in performance: Assessment of regression models performance. (2024). *R Package Documentation*. https://rdrr.io/cran/performance/man/icc.html

ERS annual report, FY 2018. (2018). *USDA ERS*. https://www.ers.usda.gov/about-ers/plans-and-accomplishments/ers-annual-report-fy-2018/

Forcelini, B. B., & Peres, N. A. (2018). Widespread resistance to QoI fungicides of *Colletotrichum acutatum* from strawberry nurseries and production fields. *Plant Health Progress*, 19(4), 338–341. https://doi.org/10.1094/php-08-18-0050-rs

Gaisser, R., Kuehn, K., & Pritts, M. (2024). Novel low tunnel coverings and plant type affect productivity of day-neutral strawberries. *HortTechnology*, 34(3), 381–387. https://doi.org/10.21273/horttech05409-24

Guthman, J. (2016). Paradoxes of the border: Labor shortages and farmworker minor agency in reworking California's strawberry fields. *Economic Geography*, 93(1), 24–43. https://doi.org/10.1080/00130095.2016.1180241

Guthman, J., & Jiménez-Soto, E. (2021). Socioeconomic challenges of California strawberry production and disease resistant cultivars. *Frontiers*. https://www.frontiersin.org/articles/10.3389/fsufs.2021.764743/full

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. https://doi.org/10.1094/phyto-11-19-0406-ia

James, K. M., Sargent, D. J., Whitehouse, A., & Cielniak, G. (2022). High‐throughput phenotyping for breeding targets—current status and future directions of strawberry trait automation. *Plants, People, Planet*, 4(5), 432–443. https://doi.org/10.1002/ppp3.10275

Koike, S. T., Gordon, T. R., Daugovish, O., Ajwa, H., Bolda, M., & Subbarao, K. (2013). Recent developments on strawberry plant collapse problems in California caused by *Fusarium* and *Macrophomina*. *International Journal of Fruit Science*, 13(1–2), 76–83. https://doi.org/10.1080/15538362.2012.697000

Marin, M. V., Seijo, T., Mertely, J., & Peres, N. A. (2019). First report of crown rot caused by *Phytopythium helicoides* on strawberry in the Americas. *Plant Disease*, 103(10), 2696. https://doi.org/10.1094/pdis-03-19-0658-pdn

McWhirt, A. (2021). Spring 2021 Strawberry crop monitoring: Impacts of high GDD accumulation in November of 2020. *University of Arkansas Research & Extension*. https://www.uaex.uada.edu/farm-ranch/crops-commercial-horticulture/horticulture/ar-fruit-veg-nut-update-blog/posts/HighGDDImpact.aspx

Nakagawa, S., Johnson, P. C., & Schielzeth, H. (2017). The coefficient of determination r^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*, 14(134), 20170213. https://doi.org/10.1098/rsif.2017.0213

Noncitrus fruits and nuts - 2021 summary. (2022). *USDA NASS*. https://www.nass.usda.gov/Publications/Todays_Reports/reports/ncit0522.pdf

Oliveira, M. S., Amiri, A., Zuniga, A. I., & Peres, N. A. (2017). Sources of primary inoculum of *Botrytis cinerea* and their impact on fungicide resistance development in commercial strawberry fields. *Plant Disease*, 101(10), 1761–1768. https://doi.org/10.1094/pdis-02-17-0203-re

Orde, K. and Sideman, B., (Eds.). (2023) Strawberry production guide for the Northeast, Midwest, and Eastern Canada (2nd ed.). *University of New Hampshire Cooperative Extension*. https://scholars.unh.edu/extension/1670/

Palencia, P., Martínez, F., Medina, J. J., & López-Medina, J. (2013). Strawberry yield efficiency and its correlation with temperature and solar radiation. *Horticultura Brasileira*, 31(1), 93–99. https://doi.org/10.1590/s0102-05362013000100015

Park, K. S. (2023). 2022 New York Berry Price Information. *NYS Berry Growers Association*. https://dyson.cornell.edu/wp-content/uploads/sites/5/2023/04/2022_NYS_Berry_Prices_EB_rev-VD.pdf.

Pettit, T. R., & Pegg, G. F. (1994). Sources of crown rot (*Phytophthora cactorum*) infection in strawberry and the effect of cold storage on susceptibility to the disease. *Annals of Applied Biology*, 125(2), 279–292. https://doi.org/10.1111/j.1744-7348.1994.tb04969.x

Poling, E. B. (2012). Strawberry Plant Structure and Growth Habit. *Cornell Cooperative Extension*. http://www.hort.cornell.edu/expo/proceedings/2012/Berries/Berry%20Plant%20Structure%20Poling.pdf

Santos, B., Stanley, C., Whidden, A., Salame-Donoso, T., Whitaker, V., Hernandez-Ochoa, I., Huang, P.-W., & Torres-Quezada, E. (2012). Improved sustainability through novel water management strategies for strawberry transplant establishment in Florida, United States. *Agronomy*, 2(4), 312–320. https://doi.org/10.3390/agronomy2040312

Shaw, D. V., & Larson, K. D. (2006). Strawberry plant named ‘Albion’. USPP16228P3

Shaw, D. V., & Larson, K. D. (2017). Strawberry plant named ‘Cabrillo’.

US20160227687P1

Shaw, D. V., & Larson, K. D. (2009). Strawberry plant named ‘Monterey’. USPP19767P2

Strawberryplants.org. (2022). Are bare root strawberries or strawberry plant plugs better? https://strawberryplants.org/are-bare-root-strawberries-or-strawberry-plant-plugs-better/

Thomas, D. (2019). California Strawberry Nursery Industry. *Cal Poly AEPS 400.* https://www.youtube.com/watch?v=JfV54nzIOL4

Torres-Quezada, E. A., Zotarelli, L., Whitaker, V. M., Darnell, R. L., Morgan, K., & Santos, B. M. (2020). Production techniques for strawberry plugs in West-central Florida. *HortTechnology*, 30(2), 238–247. https://doi.org/10.21273/horttech04529-19

USDA Plant Hardiness Zone Map (2023). *USDA plant hardiness zone map.* https://planthardiness.ars.usda.gov/

USDA NASS. (2017). *USDA National Agricultural Statistics Service*. https://www.nass.usda.gov/AgCensus/

USDA Web Soil Survey. (2023). *USDA National Resources Conservation Service*. https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx

Weber, C. A. (2021). Strawberry crown plugs provide flexibility and improved performance in cold climate plasticulture production. *Agronomy*, 11(8), 1635. https://doi.org/10.3390/agronomy11081635

Yue, C., Gallardo, R. K., Luby, J., Rihn, A., McFerson, J. R., McCracken, V., Whitaker, V. M., Finn, C. E., Hancock, J. F., Weebadde, C., Sebolt, A., & Iezzoni, A. (2014). An evaluation of U.S. strawberry producers trait prioritization: Evidence from audience surveys. *HortScience*, 49(2), 188–193. https://doi.org/10.21273/hortsci.49.2.188

CHAPTER 2

An Evaluation of Fruit Properties Based on Chemical Analysis in Novel Controlled Environment Propagated Plug Plants in Strawberry

2.1 Abstract

The strawberry industry's future success faces challenges related to farm profitability and viability, including issues such as fumigation bans, plant availability, and the spread of soil-borne pathogens. **F**ield propagated **b**are-**r**oot **p**lants (**Fbrp**) are widely used in commercial strawberry production systems, but criticism surrounds their seasonal availability, sustainability and potential for introducing soil-borne diseases into previously disease-free fields. **C**ontrolled **E**nvironment **A**griculture propagated **p**lug **p**lants (**CEApp**) are an emergent and promising alternative to Fbrp for strawberry field production. However, there is limited research on the differences in fruit properties from strawberries propagated via the novel method grown in a field setting.

To assess whether CEApp can serve as a viable alternative to Fbrp, a field trial was conducted in the summer of 2023, evaluating three strawberry cultivars, Albion, Cabrillo, and Monterey, propagated as CEApp or Fbrp. The study focused on comparing fruit properties based on chemical analyses, specifically brix, titratable acidity (TA), the brix to TA ratio, and pH.

Overall, the study found that CEApp performed equally to Fbrp across all metrics. However, within specific cultivars, 'Albion' propagated via CEApp had significantly lower ºBx compared to Fbrp. For all other measures within 'Albion', as well as across 'Cabrillo' and 'Monterey', CEApp and Fbrp performed equally. In conclusion, the study supports CEApp as a

viable alternative to Fbrp for strawberry production and suggests that CEApp offers additional fruit quality benefits beyond those inherent to the propagation method.

2.2 Introduction

Strawberry (*Fragaria* x *ananassa*) is a widely consumed specialty fruit crop known for its sweet and aromatic flavor. It is consumed fresh and is also often processed into products such as jam, yogurt, and ice cream. Qualities such as sugar content, acidity, and the ratio between sugars and acidity have been found to be important biochemical aspects of strawberry fruit that influence fruit flavor and customer opinions of the overall fruit quality (Jouquand et al., 2008). Strawberries with higher levels of sugars and relatively lower levels of acid are perceived to taste sweeter and are most preferable to consumers (Batista-Silva et al., 2018; Jouquand et al., 2008). Previous studies have found that factors besides genotype, such as environment, can affect such qualities (Osatuke, 2020) which has prompted research into understanding if propagation method may also have an effect on fruit flavor and quality.

Measures such as sugar content, titratable acidity (TA), the ratio of sugar to acid, and pH are able to provide insight on how a fruit will taste and therefore how likely a consumer is to accept and continue to purchase a fruit. In fruit such as strawberries, sugar content is typically measured as the concentration of total soluble solids (TSS) and is reported in units of degrees Brix (ºBx). One ºBx is equivalent to 1g sucrose per 100ml of water in a solution. The recommended acceptable ºBx for strawberries is considered to be 7-9 (Kubota and Kroggel, 2019). Strawberry fruit acidity is another important aspect of flavor. TA, expressed as citric acid equivalent for strawberry, indicates how sour or tart a fruit will taste. The UC-Davis Postharvest Technology Center recommends that the desirable TA for strawberries is a maximum of 8 g/L

(Mitcham, 2023). The ratio of ºBx to TA represents if a consumer will interpret the primary flavor of a strawberry fruit to be sweet or sour, and how much sweetness is perceived. When the ratio of ºBx:TA exceeds 1.0, the strawberry fruit is perceived as sweet (Kubota and Kroggel, 2019).

Modifications to strawberry cropping systems are often driven by factors other than fruit flavor quality. Agronomic traits such as improved yields, enhanced disease resistance, and fruit appearance are often the targets of cropping optimization as they are more economically driven. Since people only visually assess fruit prior to purchase, the production of many large attractive fruits will in theory lead to a higher number of purchases than the production of fewer or less attractive fruits. While objectively beautiful fruit may sell, if the flavor is lacking it will not encourage repeat purchases. When working to further optimize strawberry production systems it is important to continue to factor in consumer preference and eating quality as well as agronomic traits in order to ensure the future production of fruit is of a high quality.

Commercial strawberry fruit production begins with the production of plantlets which are produced by nurseries primarily in California, North Carolina, and eastern Canada and are then shipped to growers around the United States (Holmes, 2024). The current standard propagation system of strawberry plantlets is costly, time consuming, unsustainable, and does not reliably produce pathogen free plantlets. The process begins by producing certified clean (disease free) mother plants in vitro in tissue culture facilities. Once the mother plants have been removed from tissue culture and have been acclimated, the most common propagation method requires the mother plants to be planted in methyl-bromide (MB) treated fields for 2-3 years from where daughter plants are harvested to be sold as bare-root plantlets (Thomas, 2019).

While this production system has been effective in the past, it has many drawbacks. There are many known adverse health effects on humans associated with soil fumigants which has raised public concern surrounding the safety of fruit resulting from facilities that utilize such practices (Conroy et al., 2022). Fumigation has become increasingly regulated and, in some regions, has been completely banned (Chellemi, 2014). Also, despite fumigation treatments, daughter plants still come into contact with soil-borne pathogens and carry latent infections, spreading pathogens globally (Pettitt and Pegg, 1994; Marin et al., 2019; Forcelini and Peres, 2018 Oliveira et al., 2017).

Another problem with the current production system is the limited window of strawberry plant availability. In cold climate regions, such as New York State, there is a limited planting window of quality strawberry planting material due to the production timing so that plants are often unavailable when they are needed (Weber, 2021). This puts local growers at a disadvantage by making it more difficult to compete with large scale commercial growers on the West Coast.

To address the known problems with the current standard production method, a controlled environment based strawberry propagation system has been proposed as an alternative. By utilizing a controlled environment-based system, strawberry plantlets can be produced year-round and will never come into contact with soil during the propagation process, allowing for a higher level of certainty that plants are, in fact, free of disease. Also, because of the inherent soillessness of the propagation system there is no need for MB applications, making the novel system more environmentally sustainable, a safer environment for farm workers, and protected from the looming risk of tightening fumigation restrictions.

To fully understand if controlled environment propagation is a viable alternative to field production, it is important to gain an understanding of potential differences in fruit quality from strawberry plants of the same genotype propagated via the two different methods. To test this, a field trial consisting of Albion, Cabrillo, and Monterey strawberry cultivars propagated either as CEApp or as Fbrp was performed at Cornell Agritech in Geneva NY. Fruit quality traits including ºBx, TA, the ratio between ºBx and TA, and pH were measured.

2.3 Materials and Methods

2.3.1 Plant Material and Trial Establishment

Three strawberry cultivars, Albion, Cabrillo, and Monterey, were used in this study (Table 2.1). CEApp for all cultivars were sent from North Carolina State University for the trial and arrived at the research station on 17 May 2023. 'Albion' and 'Monterey' Fbrp were obtained on 17 May 2023 from Indiana Berry in Plymouth, IN and 'Cabrillo' Fbrp were obtained from EZ Grow in Langton, ON on 23 May 2023. Prior to planting, the roots of the Fbrp were soaked in tap water for 24 hours to ensure proper hydration, and the plug plants were thoroughly watered with deionized water. Because of problems with shipping, the 'Cabrillo' Fbrp were visibly stressed upon arrival. In order to ensure enough plants survived transplanting, the plants were sorted to plant the healthiest plants. Additionally, extra Fbrp were potted in plug trays in growing media and were placed in a cold frame on the same planting date to grow as potential replacement plants of the same developmental stage in the case of failure in the field. On 6 July 2023 plants that did not survive transplanting in the field were replaced with cold frame grown plants if available.

Table 2.1: Description of Strawberry Cultivars Used in the Trial

Cultivar	Breeding Name	Patent Number	Origin	Pedigree	Flowering Type	Year Released
Albion	CN220	USPP16228P3	Univ. of California	Diamante x Cal 94.16-1	Day-neutral	2004
Cabrillo	CN236	US20160227687P1	Univ. of California	Cal 3.149-8 x Cal 5.206-5	Day-neutral	2015
Monterey	CN222	USPP19767P2	Univ. of California	Albion x Cal 97.85-6	Day-neutral	2008

2.3.2 Site Description

The trial was performed in the summer of 2023 in adjacent plots within the same field at Cornell AgriTech at the New York State Agricultural Experiment station in Geneva, NY, USA (42°52'14.3" N, 77°02'38.6" W) in the USDA hardiness zone 6a (USDA Plant Hardiness Zone Map, 2023). The field consists of Honeoye loam soil (mesic Glossic Hapludalfs) and has a grade of 3%-8% (USDA Web Soil Survey, 2023). Prior to the trial establishment, the field was planted with grain rye (*Secale cereale*) as a cover crop. The cover crop was then mowed and plowed under, and then rotovated prior to bed formation. The site was unfumigated.

2.3.3 Experimental Design

The plots were arranged in rows in a randomized split-plot block design with four replications within a larger strawberry planting. Each row consisted of six plots (three CEApp and three Fbrp plots per row) representing each cultivar (whole plots) and propagation method. Their positions were randomly assigned within rows (Figure 2.1). Each split plot consisted of 15-25 plants depending on plant availability.

Figure 2.1: Layout of the 2023 field trial by plots and rows arranged as a randomized split-plot block design with four replications.

Row 3	Albion Test	Albion Control	Cabrillo Test	Cabrillo Control	Monterey Test	Monterey Control
Row 4	Monterey Test	Monterey Control	Albion Test	Albion Control	Cabrillo Test	Cabrillo Control
Row 5	Cabrillo Test	Cabrillo Control	Monterey Test	Monterey Control	Albion Test	Albion Control
Row 6	Albion Control	Albion Test	Cabrillo Control	Cabrillo Test	Monterey Control	Monterey Test

2.3.4 Crop Management

Rows consisted of raised beds which were 15 cm high and 61 cm wide that were spaced 1.7 m center to center and were covered with 5 ft wide white plastic (Filmtech Corp., Allentown, PA, USA). Irrigation was supplied via a single center 10 mm T-tape drip line with 30 cm emitter spacing (Rivulis Irrigation, San Diego, CA). Within each row, plants were planted in double-offset rows with 30 cm spacing between plants and rows.

Strawberry plants were grown under low tunnels in a plasticulture system with drip irrigation. Plants were added to the field on 22 May 2023 and 23 May 2023. After planting, flowers were removed from the plants as they appeared for 4 weeks, until June 19th, to allow for adequate vegetative growth. Low tunnels were put up on 27 July 2023. Fruit harvesting began on 18 July 2023 and occurred three times per week to prevent overripening, mitigate loss due to opportunistic pathogens, and reduce pest incidence.

Rows were fertigated via the drip line starting after planting with 20-20-20 fertilizer, (Jack's Fertilizer, JR Peters, Allentown, PA, USA, 20N-20P-20K) on a weekly basis, supplying approximately 5 lb/acre N per week equivalent throughout the growing season. Irrigation was supplied three times per week for an application of 2.5 cm of water per week prior to the fruiting phase. During the fruiting phase irrigation was increased to 5 cm per week. Weeds were removed by hand as needed throughout the season. No other significant pest management strategies were applied to the trial.

2.3.5 Fruit Harvesting Practices

Within each plot, fifteen contiguous strawberry plants were flagged for fruit collection to maintain consistency as the number of strawberry plants within each plot varied due to dieback and/or availability of plants. Throughout the harvest period ripe fruits were harvested by hand every Monday, Wednesday, and Friday as weather permitted. Fruits were harvested once they reached commercial maturity (75%-100% red), which was assessed visually. Fruits that would typically be classified as 'unmarketable', due to pest damage or otherwise, were still harvested as the goal of the study was to analyze the differences between the two propagation methods rather than to assess the overall quality of the plants. In cases of unavoidable conflict, fruits were harvested the next day. Once harvested the fruits were counted, massed, and then frozen for later chemical analysis. The last harvest date was 27 October 2023.

2.3.6 Fruit Processing

After collecting data on the fruits, representative samples of strawberry fruits harvested from an entire plot were stored in plastic deli containers and frozen in a -20 ºC frost free freezer until processed. Due to freezer malfunctions there were three instances where fruits were inadvertently thawed. Instances of postharvest disease due to thawed fruits sitting at room temperature were recorded.

To perform chemical analysis that was representative of an entire harvested plot, the frozen strawberry fruits were homogenized. Fruits were thawed at room temperature and then pureed using a laboratory blender. Once pureed, the samples were refrozen until further processed.

2.3.7 Brix

ºBx data were collected for each sampling day for each of the cultivars. Frozen strawberry samples, consisting of the harvested fruit from an individual plot on a specific harvest date, were thawed and then pureed using a laboratory blender at high speed until visibly homogenized. The puree was then allowed to rest until supernatant separated from the solids. Supernatant was placed directly onto a digital refractometer (SPER Scientific LTD, Scottsdale, AZ) according to manufacturer instructions. The remaining sample was then frozen at -20ºC to be used for further analysis.

2.3.8 Titratable Acidity and pH

Titratable acidity and pH data were analyzed for each harvest sample for each of the cultivars. Frozen pureed samples in 50 ml Falcon Tubes were thawed at room temperature. Once thawed, the samples were centrifuged at 3000 rpm for three minutes to separate the supernatant from the solids. 2 mL of supernatant was then extracted using a pipette and was added to an empty 50 ml Falcon tube. Each sample of supernatant was then put in a 55 ºC water bath for 30 minutes. Following this, 18 ml of DI water was added to each tube. Titratable acidity and pH were measured using a Mantech MT-30. Titratable acidity was determined through titration with 0.1 M NaOH to an endpoint pH of 8.21. Results were calculated as the mass of citric acid (g) / mL of strawberry juice (Sadler and Murphy, 2010). Approximating the density of the juice to the density of water (1g/mL) then gives a mass percentage. The results were reported as % citric acid as citric acid is the primary acid found in strawberry fruits.

2.3.9 Weather Data

Weather data were obtained from the Network for Environmental and Weather Applications (NEWA). Specifically, the Geneva (Agritech North), NY weather station data were used. For historical daily weather averages, weather data from the Geneva (Agritech North), NY weather station data were averaged by day for years 2019-2023.

2.3.10 Statistical Analysis

Using R version 4.4.0 (R Core Team 2021) linear mixed model analyses from R packages ‘lme4’ and ‘lmertest’ were used to evaluate the effects of propagation method, cultivar, and the interaction between propagation method and cultivar on ºBx, TA, the ratio between ºBx and TA, and pH over the 2023 production period. The overall production period average values per plot for each measure were used for statistical analysis. The differences between CEApp and Fbrp within specific cultivars were also analyzed. Row was specified as a random effect. The ‘DHARMa’ R package was used to map the residuals of the data output from the mixed models to determine if the distribution of the residuals matched the expected distribution. Estimated marginal means were calculated using the ‘emmeans’ R package. They were calculated by fitting the linear mixed model to the data, extracting the fixed effects of the model, computing predicted values for each factor level or combination of factors, and then averaging over the model predicted random effects using the ‘emmeans’ package software. Estimated marginal means were used instead of recorded means in order to correct for effects from variation between rows on specific measures. Because estimated marginal means provide a way to interpret the fixed effects of the model while still accounting for the variability between rows, they allow for a better comparison of the relationship between the tested variables. The ‘performance’ R package was used to calculate Intraclass correlation coefficients (ICC) which were used to quantify the impact of the random effect (variation between rows) on the various measures. ICC quantifies the random effect variance in comparison to the total variance. Adjusted ICC values only account for the random effect variance while unadjusted ICC values account for the random effect variance as well as the fixed effect variances (Nakagawa et al., 2017).

Figure 2.2. Formulas for the adjusted and unadjusted Intraclass Correlation Coefficient (ICC) values

$$ICC_{adj} = \frac{\sigma^2_{RE}}{\sigma^2_{RE} + \sigma^2_{\varepsilon}}$$

$$ICC = \frac{\sigma^2_{RE}}{\sigma^2_{RE} + \sigma^2_{FE} + \sigma^2_{\varepsilon}}$$

σ^2_{RE} is the variance of the random effect (an output of the model fitting)

σ^2_{ε} is the variance of the model residual (an output of the model fitting)

σ^2_{FE} is the sum of the variances explained by each of the fixed effects (an output of the model fitting)

2.4 Results

2.4.1 Brix

Cultivar ($p < 0.0001$) significantly affected ºBx (Table 2.2). 'Albion', 'Cabrillo', and 'Monterey' all had significantly different estimated marginal average ºBx from each other (Table 2.2). The estimated marginal average ºBx throughout the production period from all harvested fruit was 8.34 for 'Albion', 7.77 for 'Cabrillo', and 8.85 for 'Monterey' (Table 2.2). There was no significant effect on ºBx from propagation method or the interaction between cultivar and propagation method (Table 2.2).

Within specific cultivars, 'Albion' CEApp had significantly lower ºBx than Fbrp 'Albion' plants ($p = 0.037$) (Table 2.2). On average, 'Albion' Fbrp had 0.4º, or 4.7%, higher ºBx than

‘Albion’ CEApp (Table 2.2). There was no significant difference in ºBx between propagation methods in ‘Cabrillo’ and ‘Monterey’.

On average, ºBx declined from the beginning of the production period until 18 September 2023 (Figure 2.3). Following 18 September 2023 ºBx increased except for a dip during the week of 9 October 2023 (Figure 2.3). CEApp of all tested cultivars had more consistent ºBx than Fbrp throughout the production period (Figure 2.4).

Table 2.2. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average strawberry fruit ºBx per harvest/plot throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean ºBx/Plot	SE	p-value
Propagation Method			p = 0.34†
CEA	8.27	0.124	
Field	8.37		
Cultivar			p < 0.0001†*
Albion	8.34 b[1]	0.134	
Cabrillo	7.77 a		
Monterey	8.85 c		
Cultivar by Propagation Method			p = 0.10†
Albion CEA	8.14	0.159	p = 0.04‡*
Albion Field	8.54		
Cabrillo CEA	7.85	0.159	p = 0.33‡
Cabrillo Field	7.68		
Monterey CEA	8.81	0.159	p = 0.69‡
Monterey Field	8.88		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 2.3. Effect of propagation method on the average strawberry ºBx per harvest/plot throughout the 2023 production period by week.

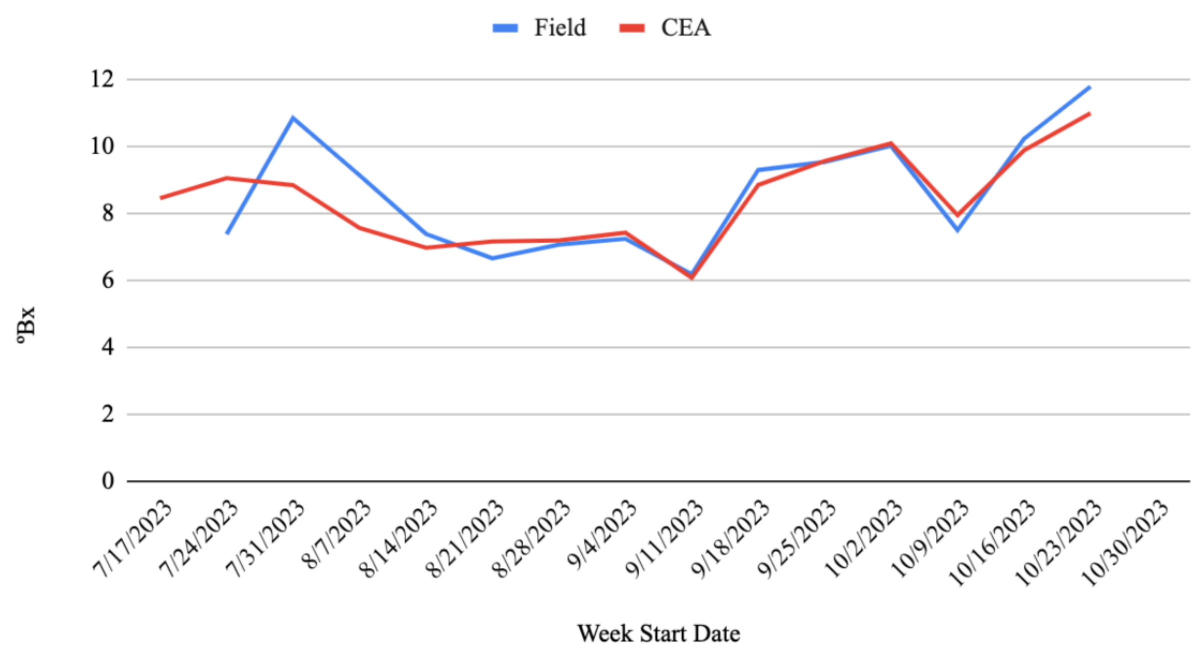


Figure 2.4. Effect of propagation method within specific cultivars on the average strawberry ºBx per harvest/plot throughout the 2023 production period by week.

2.4.2 Titratable Acidity

Cultivar significantly impacted titratable acidity ($p = 0.0010$) (Table 2.3). 'Albion' and 'Cabrillo' had significantly different estimated marginal average titratable acidity from each other (Table 2.3). Fruit from 'Albion' plants had an estimated marginal average titratable acidity of 8.88% citric acid and fruit from 'Cabrillo' had an estimated marginal average titratable acidity of 7.98% citric acid (Table 2.3). The estimated marginal average titratable acidity of 'Monterey' was not significantly different from the estimated marginal average titratable acidity of 'Albion' or 'Cabrillo' (Table 2.3). Propagation method and the interaction between cultivar and propagation method had no significant effect on titratable acidity (Table 2.3). There were no significant differences between CEApp and Fbrp when looking at specific cultivars (Table 2.3).

For both CEApp and Fbrp, titratable acidity started off high at the beginning of the production period and decreased until 4 September 2023 (Figure 2.5). Following this, titratable acidity steadily increased for plants of both propagation methods until starting to decrease again on 25 September 2023 (Figure 2.5). There was a sharp upward trend in titratable acidity for both CEApp and Fbrp from 9 October 2023 until the end of the production period (Figure 2.5). Both 'Albion' CEApp and 'Albion' Fbrp had a sharp spike in titratable acidity between 11 September 2023 and 2 October 2023 (Figure 2.6). 'Cabrillo' CEApp had noticeably lower titratable acidity than the other cultivar and propagation method combinations, most notably from the start of the season until 21 August 2023 (Figure 2.6).

Table 2.3. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average strawberry fruit titratable acidity per harvest/plot throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean of % Citric Acid	SE	p-value
Propagation Method			p = 0.76†
CEA	8.40	0.203	
Field	8.44		
Cultivar			p = 0.0010†*
Albion	8.88 b[1]	0.217	
Cabrillo	7.98 a		
Monterey	8.41 ab		
Cultivar by Propagation Method			p = 0.44†
Albion CEA	8.94	0.255	p = 0.63‡
Albion Field	8.81		
Cabrillo CEA	7.81	0.255	p = 0.23‡
Cabrillo Field	8.15		
Monterey CEA	8.44	0.255	p = 0.83‡
Monterey Field	8.38		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 2.5. Effect of propagation method on the average strawberry titratable acidity as % citric acid per harvest/plot throughout the 2023 production period by week.

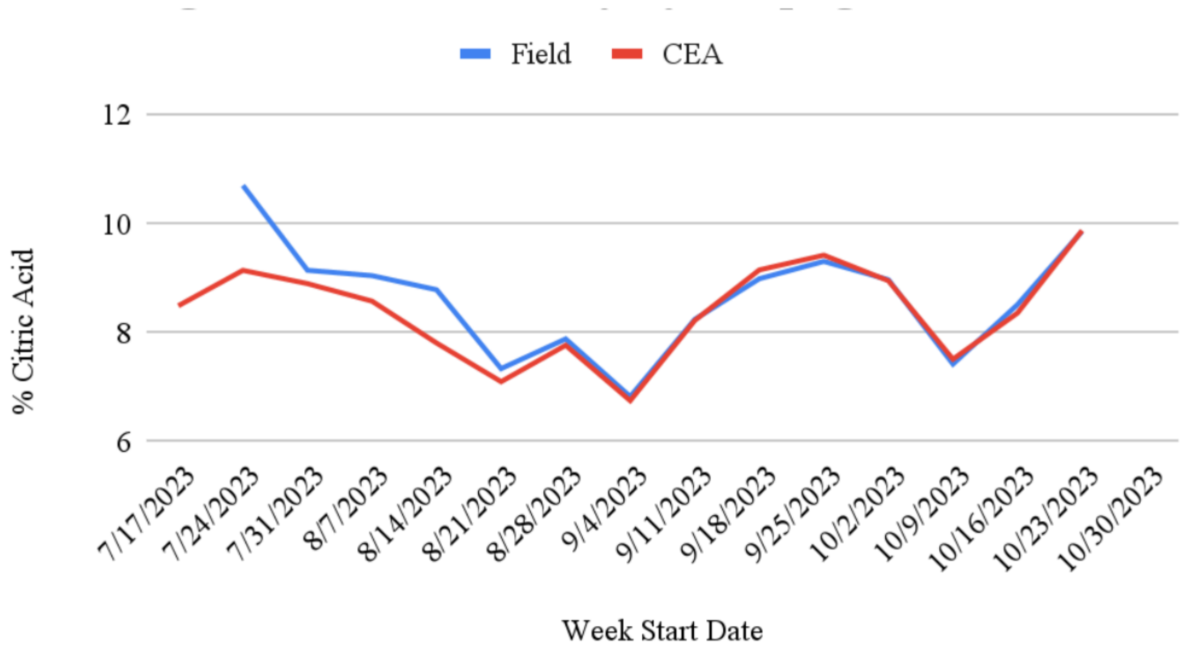
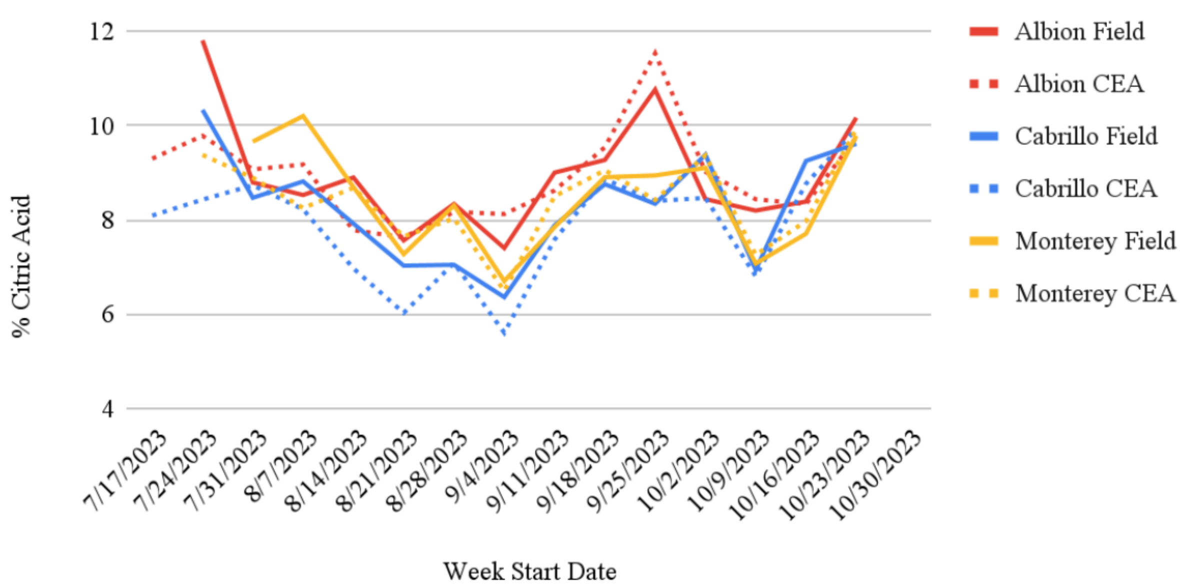


Figure 2.6. Effect of propagation method within specific cultivars on the average strawberry titratable acidity as % citric acid per harvest/plot throughout the 2023 production period by week.



2.4.3 Brix:TA

Cultivar had a significant impact on the ratio between Brix and TA ($p < 0.0012$) (Table 2.4). 'Monterey' plants had a significantly different estimated marginal mean ºBx:TA than 'Albion' and 'Cabrillo' plants (Table 2.4). 'Albion' plants had an estimated marginal mean ºBx:TA ratio of 0.99, 'Cabrillo' plants had a predicted average ratio of 1.01, and 'Monterey' plants had an estimated marginal mean ratio of 1.07 (Table 2.4). There was no significant difference in estimated average ºBx:TA between 'Albion' and 'Cabrillo' plants (Table 2.4). There was no significant effect on titratable acidity observed from propagation method or the interaction between cultivar and propagation method (Table 2.4). There were no significant differences observed between CEApp and Fbrp within specific cultivars (Table 2.4).

CEApp and Fbrp followed a similar trend throughout the production period (Figure 2.7). ºBx:TA was overall increasing from the start of the production period until 4 September 2023 (Figure 2.7). ºBx:TA was its highest for both CEApp and Fbrp near the end of the production period on 16 October 2023 (Figure 2.7). Within cultivars, CEApp and Fbrp followed similar trends throughout the production period (Figure 2.8).

Table 2.4. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average strawberry fruit brix:TA throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean °Bx:TA	SE	p-value
Propagation Method			p = 0.52†
CEA	1.02	0.0171	
Field	1.03		
Cultivar			p = 0.0012†*
Albion	0.99 a[1]	0.0186	
Cabrillo	1.01 a		
Monterey	1.07 b		
Cultivar by Propagation Method			p = 0.29†
Albion CEA	0.97	0.0226	p = 0.14‡
Albion Field	1.01		
Cabrillo CEA	1.02	0.0226	p = 0.45‡
Cabrillo Field	1.00		
Monterey CEA	1.07	0.0226	p = 0.72‡
Monterey Field	1.08		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 2.7. Effect of propagation method on the average strawberry ºBx:TA per harvest/plot throughout the 2023 production period by week.

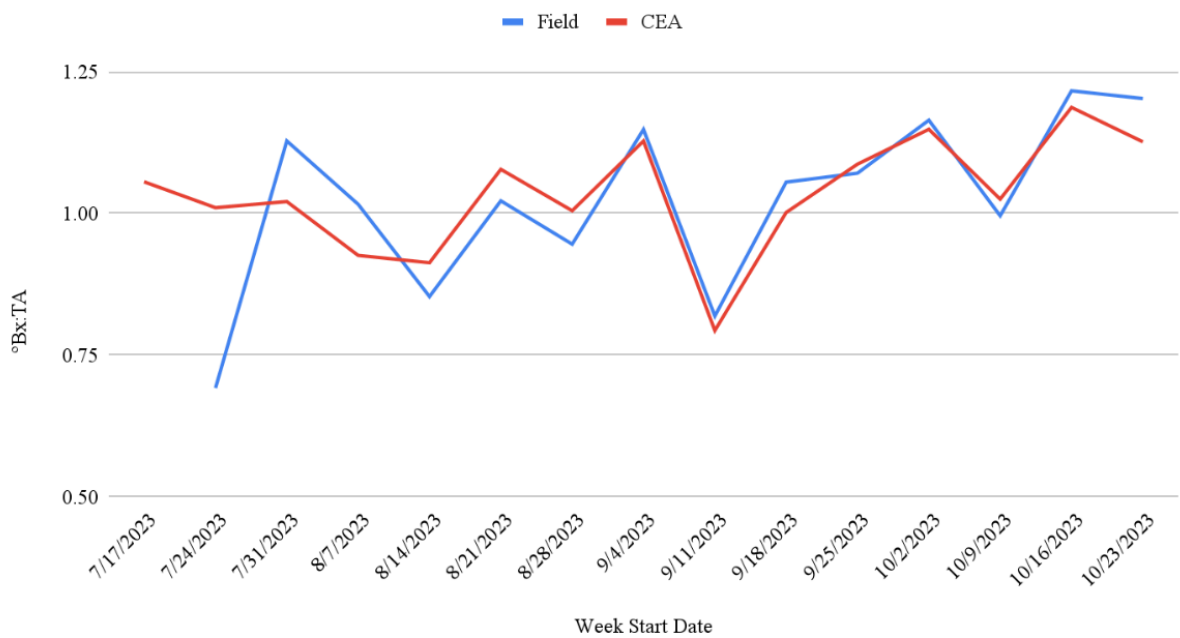
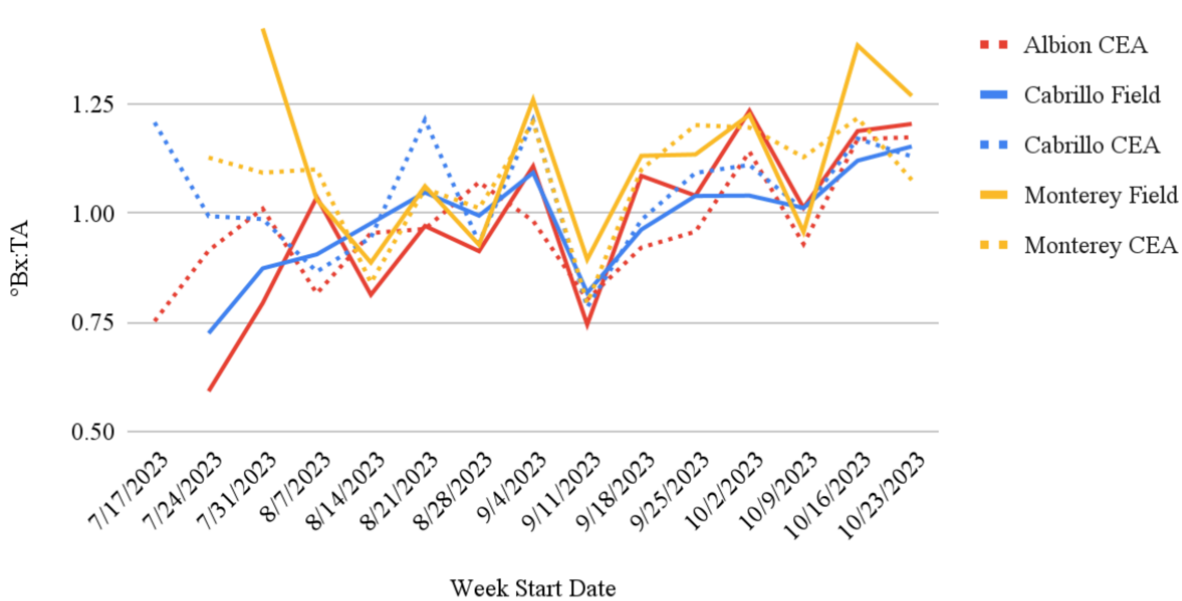


Figure 2.8. Effect of propagation method within specific cultivars on the average strawberry ºBx:TA per harvest/plot throughout the 2023 production period by week.



2.4.4 pH

Cultivar had a significant impact on pH ($p < 0.0001$) (Table 2.5). Propagation method and the interaction between cultivar and propagation method had no significant effect on pH (Table 2.5). There were no significant differences between CEApp and Fbrp when comparing within specific cultivars (Table 2.5). ‘Albion’, ‘Cabrillo’, and ‘Monterey’ plants all had significantly different predicted average pH values throughout the production period (Table 2.5). ‘Albion’ had an estimated marginal average pH of 3.534, ‘Cabrillo’ 3.443, and ‘Monterey’ 3.556 (Table 2.5).

CEApp and Fbrp had very consistent estimated average pH throughout the production period (Figure 2.9). During the week of 31 July 2023 Fbrp had a spike in pH value (Figure 2.9). When comparing estimated average pH of the two propagation methods within the tested cultivars, CEApp had more consistent pH throughout the production period than Fbrp (Figure 2.10).

Table 2.5. Effects from propagation method, cultivar, and the interaction between cultivar and propagation method on the average strawberry fruit pH per harvest/plot throughout the 2023 production period based on linear mixed model analysis.

Treatment	Estimated Marginal Mean pH/Plot	SE	p-value
Propagation Method			p = 0.47†
CEA	3.509	0.0079	
Field	3.513		
Cultivar			p < 0.0001†*
Albion	3.534 b[1]	0.0083	
Cabrillo	3.443 a		
Monterey	3.556 c		
Cultivar by Propagation Method			p = 0.32†
Albion CEA	3.53	0.0095	p = 0.43‡
Albion Field	3.54		
Cabrillo CEA	3.44	0.0095	p = 0.22‡
Cabrillo Field	3.45		
Monterey CEA	3.56	0.0095	p = 0.43‡
Monterey Field	3.55		

† p-value is evaluating the significance of the effect of the overall treatment.

‡ p-value is evaluating the significance of propagation method within a specific cultivar.

* Indicates a significant difference based on a Likelihood Ratio Test for a mixed model. An α value of < 0.05 was used as the threshold to determine significance.

[1] Values followed by the same letter are not significantly different based on comparisons by the Tukey method for comparing a family of 3 estimates. An α value of < 0.05 was used as the threshold to determine significance.

Figure 2.9. Effect of propagation method on the average strawberry pH per harvest/plot throughout the 2023 production period by week.

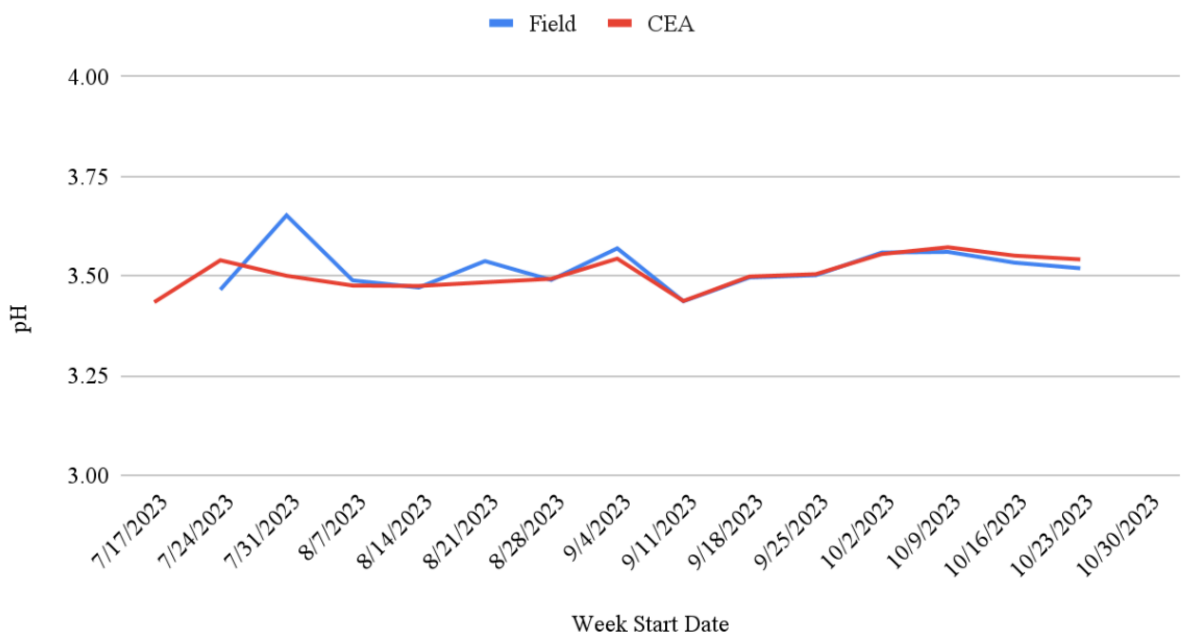


Figure 2.10. Effect of propagation method within specific cultivars on the average strawberry pH per harvest/plot throughout the 2023 production period by week.

2.4.5 Effect of Field on Chemical Measures

Row was found to have varying levels of effect on the variation found within the data for the different chemical measures (Table 2.6; Table 2.7; Table 2.8; Table 2.9). For ºBx, row was responsible for 41.2% of the variation within the data (Table 2.6). For titratable acidity, row was responsible for 44.8% of the variation within the data (Table 2.7). For ºBx:TA, row was responsible for 35.8% of the variation found within the data (Table 2.8). For pH, row was responsible for 53.8% of the variation found within the data (Table 2.9).

Table 2.6. Intraclass Correlation Coefficient from the random effect of variation between rows on average strawberry fruit ºBx throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	41.2%
Adjusted ICC	41.2%
Unadjusted ICC	13.0%

Table 2.7. Intraclass Correlation Coefficient from the random effect of variation between rows on average titratable acidity (% citric acid) throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	44.8%
Adjusted ICC	44.8%
Unadjusted ICC	28.3%

Table 2.8. Intraclass Correlation Coefficient from the random effect of variation between rows on brix:TA throughout the 2023 production period

Random Effect Variables	Intraclass Correlation Coefficient
Row	35.8%
Adjusted Total Effect	35.8%
Unadjusted Total Effect	21.0%

Table 2.9. Intraclass Correlation Coefficient from the random effect of variation between rows on pH throughout the 2023 production period.

Random Effect Variables	Intraclass Correlation Coefficient
Row	53.8%
Adjusted ICC	53.8%
Unadjusted ICC	6.7%

2.4.6 Weather and Chemical Measures

The daily high air temperatures for the 2023 production period followed a similar trend as the historical average daily high temperatures (Figure 2.11). During the production period, air temperature was highest on 4 September 2023, 31.1 ºC, and was lowest on 22 October 2023, 5.0 ºC. These measures were both more extreme than the average temperatures for their dates (Figure 2.11). Between 2 September 2023 and 7 September 2023, there was a spike in the highest temperature compared to the historical average (Figure 2.11). From the beginning of the production period to 2 September 2023, daily high air temperatures were fairly consistent and following 7 September 2023, the daily high air temperatures steadily declined, following a similar trend to the average data (Figure 2.11). Between 29 September 2023 and 7 October 2023, there was a spike in temperature up to 28.5ºC, which was higher than average for that time of year (Figure 2.11). Nighttime low temperatures during the production period were not as consistent as the season average, but followed a similar trend (Figure 2.11).

The total precipitation was higher than expected from the start of the production period until 18 August 2023 (Figure 2.12). On 24 September 2023, total precipitation was .6 in higher than the average precipitation for the date (Figure 2.12). During the period between 7 September 2023 and 13 September 2023 experienced high levels of precipitation compared to the historical averages (Figure 2.12). Following this, precipitation was lower than expected for the remainder of the production period (Figure 2.12)

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Figure 2.11. Actual and average daily air temperatures recorded from the Geneva Agritech North weather station. Averages are from years 2019-2023

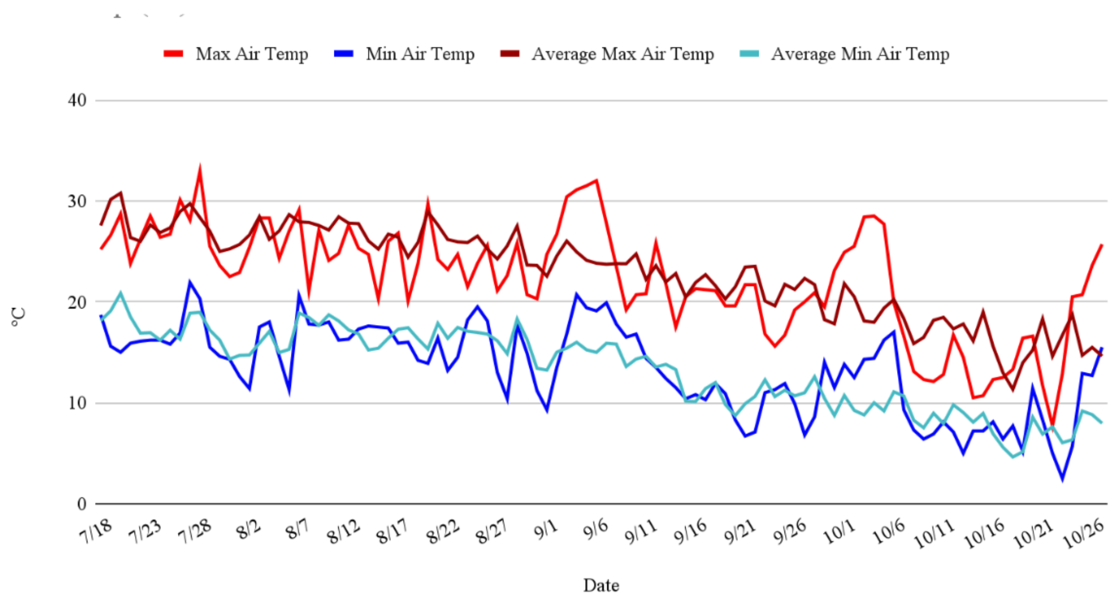
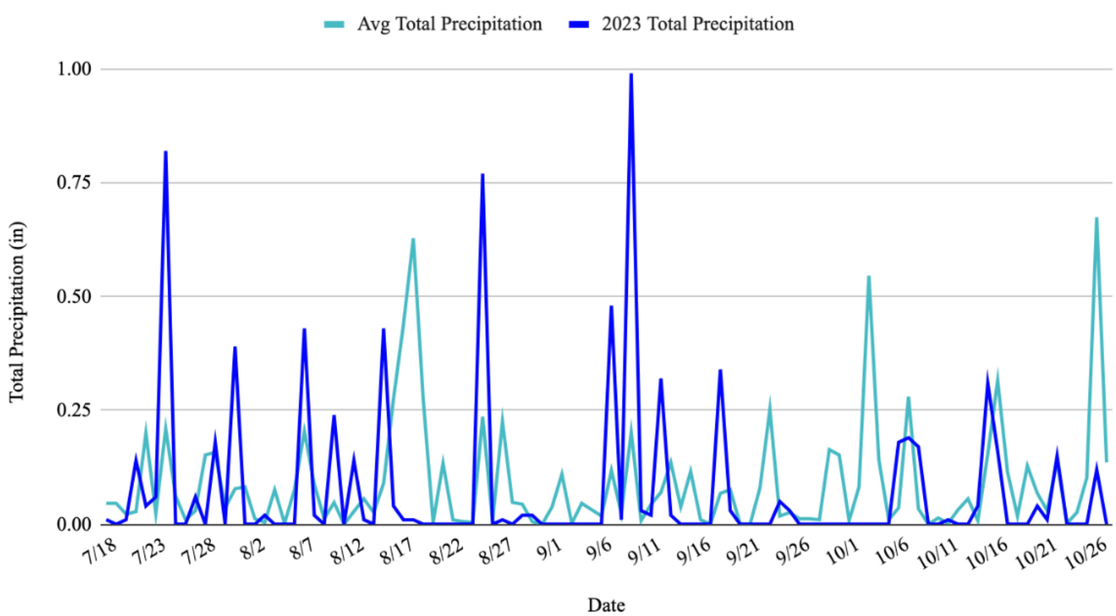


Figure 2.12. Actual and average daily total precipitation (in) recorded from the Geneva Agritech North weather station. Averages are from years 2019-2023



2.5 Discussion

CEApp did not result in significant differences in ºBx, titratable acidity, pH or ºBx:TA in strawberry fruit regardless of cultivar (Table 2.2; Table 2.3; Table 2.4; Table 2.5). Cocco et al. (2020) found similar results when comparing strawberry bare-root and plug plants. Another study looking at the effect of propagation method on fruit quality also saw no difference in ºBx or TA from fruits of strawberry plants propagated via different methods (Capocasa et al., 2018). One potential conclusion is that fruit chemical properties were not affected by the method of which the plants were propagated and because the CEApp and Fbrp of each cultivar shared the same genotype, they produced fruits of the same chemical profile. CEApp and Fbrp also produced fruits under the same environmental conditions which may indicate that fruit chemical properties are more affected by the production environment than the initial propagation method. Throughout the production period, ºBx, TA, ºBx:TA, and pH were much more consistent than the other harvest measures (Table 1.9; Table 1.10; Table 1.11; Table 1.12; Table 2.2; Table 2.3; Table 2.4; Table 2.5), supporting the theory that chemical properties of strawberries are not sensitive to different methods of initial plant propagation.

The tested cultivars responded very similarly to the effects of CEApp, with one exception (Table 2.2; Table 2.3; Table 2.4; Table 2.5). There were no significant differences between CEApp and Fbrp for ºBx in 'Cabrillo' or 'Monterey', but 'Albion' CEApp was associated with a significantly lower average ºBx (Table 2.2). One study found a similar result of lower average ºBx in plug plants (Cocco et al., 2020). While 'Albion' CEApp had a significantly lower average ºBx than 'Albion' Fbrp, the average ºBx was still above the acceptable level (8.0). Therefore, growers can still expect to produce a high quality fruit that will still be accepted by the consumer despite a lower sugar content. Future research could include sensory trials to determine if people can taste differences in ºBx of the magnitudes observed in this study.

ºBx and TA increased as total precipitation decreased (Figure 2.3; Figure 2.5; Figure 2.12). This may be due to the sugars and acids becoming more concentrated since there would be less water in the fruit with less rainfall. Other studies have also found that weather affects fruit chemical measures such as ºBx and TA (Osatuke, 2020; Mihálka et al., 2020; Gündüz and Özbay 2018) To harvest more flavorful fruits, growers could harvest plants before predicted storms to avoid the flavor becoming diluted. Future research could look at ºBx and TA from strawberry plants under different irrigation schedules to investigate this theory.

For all quality variables measured, cultivar was significant (Table 2.2; Table 2.3; Table 2.4; Table 2.5), suggesting that fruit flavor is genotype specific. This is logical as it is one of the inherent reasons to develop new cultivars, to explore and develop different phenotypic qualities such as flavor.

The adjusted ICC values from the various chemical measures showed that there was a large amount of variation between rows (Table 2.6; Table 2.7; Table 2.8; Table 2.9). This may suggest that there were small environmental differences between rows such as differences in irrigation, soil content, air flow, or any other irregularities. The adjusted ICC values for all chemical measures were similar, ranging from 35.8% - 53.8% (Table 2.6; Table 2.7; Table 2.8; Table 2.9), which suggest that environmental differences affect chemical measures similarly. Many of the measures were inherently related to each other, such as TA and pH, which also may help explain why the adjusted ICC values were similar.

2.6 References

Batista-Silva, W., Nascimento, V. L., Medeiros, D. B., Nunes-Nesi, A., Ribeiro, D. M., Zsögön, A., & Araújo, W. L. (2018). Modifications in organic acid profiles during fruit development and ripening: Correlation or causation? *Frontiers in Plant Science*, 9. https://doi.org/10.3389/fpls.2018.01689

Capocasa, F., Balducci, F., Marcellini, M., Bernardini, D., Navacchi, O., & Mezzetti, B. (2018). Comparing nursery behavior, field plant yield and fruit quality of in vitro and in vivo propagated strawberry mother plants. *Plant Cell, Tissue and Organ Culture*, 136(1), 65–74. https://doi.org/10.1007/s11240-018-1492-8

Chellemi, D. O. (2014). Plant health management: Soil fumigation. *Encyclopedia of Agriculture and Food Systems*, 456–459. https://doi.org/10.1016/b978-0-444-52512-3.00250-3

Cocco, C., Faedi, W., Magnani, S., Maltoni, M. L., Stagno, F., Turci, P., Quacquarelli, I., Ferré, A.-S., & Baruzzi, G. (2020). Effects of plug plants and bare-root plants on strawberry field performance, fruit quality traits and health-promoting compounds. *Journal of Berry Research*, 10(2), 145–156. https://doi.org/10.3233/jbr-190389

Conroy, D., Frethey-Bentham, C., Gan, C., Errmann, A., & Young, J. (2022). The lesser evil: Consumer acceptance of X-ray irradiation compared with alternative phytosanitary treatments. *Appetite*, 175, 106085. https://doi.org/10.1016/j.appet.2022.106085

Forcelini, B. B., & Peres, N. A. (2018). Widespread resistance to QoI fungicides of *Colletotrichum acutatum* from strawberry nurseries and production fields. *Plant Health Progress*, 19(4), 338–341. https://doi.org/10.1094/php-08-18-0050-rs

Gündüz, K., & Özbay, H. (2018). The effects of genotype and altitude of the growing location on physical, chemical, and phytochemical properties of strawberry. *Turkish Journal of Agriculture and Forestry*, 42(3), 145–153. https://doi.org/10.3906/tar-1706-65

Holmes, G. J. (2024). The California strawberry industry: Current trends and future prospects. *International Journal of Fruit Science*, 24(1), 115–129. https://doi.org/10.1080/15538362.2024.2342900

ICC: Intraclass Correlation Coefficient (ICC) in performance: Assessment of regression models p erformance. (2024). *R Package Documentation*. https://rdrr.io/cran/performance/man/icc.html

Jouquand, C., Chandler, C., Plotto, A., & Goodner, K. (2008). A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality. *Journal of the American Society for Horticultural Science*, 133(6), 859–867. https://doi.org/10.21273/jashs.133.6.859

Kubota, C., & Kroggel, M. (2019). Fruit quality. *Controlled Environment Berry Production Information*. https://u.osu.edu/indoorberry/fruit-quality/

Mihálka, V., Székelyhidi, R., Kapcsándi, V., & Király, I. (2020). Sugar and acid content of organically and conventionally grown strawberry (*Fragaria × ananassa*). *Gradus*, 7(2), 225–229. https://doi.org/10.47833/2020.2.agr.042

Mitcham, E. J. (2023). Strawberry - recommendations for maintaining postharvest quality. *Postharvest research and extension center.* https://postharvest.ucdavis.edu/produce-facts-sheets/strawberry

Nakagawa, S., Johnson, P. C., & Schielzeth, H. (2017). The coefficient of determination r2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*, 14(134), 20170213. https://doi.org/10.1098/rsif.2017.0213

Oliveira, M. S., Amiri, A., Zuniga, A. I., & Peres, N. A. (2017). Sources of primary inoculum of *Botrytis cinerea* and their impact on fungicide resistance development in commercial strawberry fields. *Plant Disease*, 101(10), 1761–1768. https://doi.org/10.1094/pdis-02-17-0203-re

Osatuke, A. C. (2020). Soil management and site and their effects on strawberry fruit quality and yield. https://doi.org/https://doi.org/10.7298/ktk1-7r58

Pettitt, T. R., & Pegg, G. F. (1994). Sources of crown rot (*Phytophthora cactorum*) infection in strawberry and the effect of cold storage on susceptibility to the disease. *Annals of Applied Biology*, 125(2), 279–292. https://doi.org/10.1111/j.1744-7348.1994.tb04969.x

Sadler, G. D., & Murphy, P. A. (2010). pH and titratable acidity. *Food Science Texts Series,* 219–238. https://doi.org/10.1007/978-1-4419-1478-1_13

Shaw, D. V., & Larson, K. D. (2006). Strawberry plant named ‘Albion’. USPP16228P3

Shaw, D. V., & Larson, K. D. (2017). Strawberry plant named ‘Cabrillo’. US20160227687P1

Shaw, D. V., & Larson, K. D. (2009). Strawberry plant named ‘Monterey’. USPP19767P2

Thomas, D. (2019). California Strawberry Nursery Industry. *Cal Poly AEPS 400*. https://www.youtube.com/watch?v=JfV54nzIOL4

USDA Plant Hardiness Zone Map (2023). *USDA Plant Hardiness Zone Map.* https://planthardiness.ars.usda.gov/

USDA Web Soil Survey. (2023). *USDA National Resources Conservation Service*. https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx

Weber, C. A. (2021). Strawberry crown plugs provide flexibility and improved performance in cold climate plasticulture production. *Agronomy*, 11(8), 1635. https://doi.org/10.3390/agronomy11081635