# SURVEY OF SOILBORNE PATHOGENS INFECTING STRAWBERRY IN OXNARD, CALIFORNIA & EFFECT OF PLANTING DATE AND CHILL TREATMENT ON YIELD OF STRAWBERRY.

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# ABSTRACT

Survey of Soilborne Pathogens Infecting Strawberry in Oxnard, California & Effect of Planting Date and Chill Treatment on the Yield of Strawberry.

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Survey to quantify the frequency of the five main pathogens (*Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae* races 1 and 2, *Verticillium dahliae*, and *Phytophthora* spp.) infecting strawberry in the Oxnard growing district of California. *M. phaseolina* was the most prevalent pathogen detected in diseased plants in the Oxnard growing district, present in 67.6% of samples positive for at least one pathogen. *F. oxysporum* f. sp. *fragariae* race 1 (35.3%), *V. dahliae*(8.8%), *F. oxysporum* f. sp. *fragariae* race 2 (5.9%) and *Phytophthora* spp. (2.9%) followed at lower prevalence. Associations were found between fewer drip irrigation lines and the presence of *M. phaseolina and F. oxysporum* f. sp. *fragariae* race 2. No associations were found between pathogen presence and mulch color, organic/conventional, or soil type. To quantify the effect of planting date and chill treatment on strawberry yield, plug plants sourced from North Carolina State University were planted at Cal Poly, San Luis Obispo. Earliest planting optimized fruit yield and the number of branch crowns for both 'Monterey' and 'Fronteras' plug plants. Chilling treatment increased overall yield and number of branch crowns, and decreased plant mortality. This trial also proved the feasibility of producing high quality strawberry plug plants in North Carolina, shipping to California, and producing fruit during typical production season.


Keywords*: Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae*, *Verticillium dahliae*, *Phytophthora* spp., recombinase polymerase amplification, bareroot transplant,

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# CHAPTER 1. LITERATURE REVIEW

## 1.1 Introduction

This two-part literature review provides insight into the background and purpose of the trials conducted in Chapters 2 and 3 of this thesis. In part one of this literature review (1.1), the California strawberry fruit industry is described, along with the impact of soilborne pathogens, current management, and diagnostic tools. This information serves as a foundation for the soilborne pathogen disease survey in Chapter 2. In part two of this literature review (1.2), the California strawberry nursery industry is described, as well as strawberry plant physiology and variability among cultivars. This portion of review will serve as background for the trials outlined in Chapter 3.

### 1.1.2 Strawberry cultivation

The garden strawberry (*Fragaria* × *ananassa*) has its origins in Europe and North America, where wild species were initially found. Cultivation of strawberries dates back centuries, with the modern garden strawberry resulting from crossbreeding efforts in the 18th century between *Fragaria virginiana* from North America and *Fragaria chiloensis* from Chile (Whitaker et al. 2020). These early breeding efforts aimed to improve fruit size, flavor, and disease resistance. Historically, strawberries have been grown in various regions worldwide, including Europe, North America, and parts of South America, adapting to diverse climates and soil conditions (Hancock 2020). The evolution of strawberry cultivation has led to the development of numerous cultivars tailored to different growing environments and desirable traits.

The strawberry industry has a large economic impact on California's Gross Domestic Product having become an increasingly popular specialty crop (Hernández-Martínez et al. 2023). California is the leading producer of strawberries in the United States, with the industry

concentrated primarily in three districts: Oxnard, Santa Maria, and Watsonville-Salinas (USDA 2023). Oxnard and Santa Maria are notable for their dual planting seasons, with crops planted in both fall and summer to maximize yield and extend the supply of fruit over a broader timeframe (California Strawberry Commission 2023). Watsonville-Salinas, however, focuses mainly on fall-planted strawberries due to its cooler climate and later frost dates (Holmes 2024). The economic impact of California strawberries is significant, contributing over $3.5 billion annually to the state's agricultural revenue and supporting thousands of jobs throughout the supply chain (Galinato et al. 2019). California strawberries not only dominate the domestic market but also play a crucial role in the global strawberry trade, supplying fresh berries year-round to consumers worldwide (California Strawberry Commission 2023).

California's strawberry growers implement advanced agricultural techniques to improve yield and quality. Plastic mulch is extensively used for weed control, soil moisture conservation, and accelerating fruit ripening (Holmes 2024). Bareroot transplants, sourced primarily from nurseries located in Northern California's Sierra Nevada foothills, benefit from natural chill hours, crucial for maximizing productivity throughout the growing season (Hochmuth 2017). Drip irrigation systems play a pivotal role in delivering precise amounts of water and nutrients directly to the root zone, enhancing water efficiency and minimizing the risk of water stress during critical growth stages (Shaw et al. 2012). These sustainable practices not only optimize resource utilization but also contribute to the long-term economic sustainability of strawberry production in California.

The phase-out of methyl bromide, an ozone-depleting substance previously used extensively in agriculture, has had profound implications for the strawberry industry. Historically, methyl bromide was favored for its efficacy in controlling soilborne pests, weeds, and pathogens, crucial in the production of high-value crops like strawberries (Noling 2011). However, its ozone-depleting properties led to international agreements, such as the Montreal Protocol in 1987,

mandating its gradual phase-out. The United States, including California where most of the nation's strawberries are grown, committed to reducing and ultimately eliminating its use (US EPA 2023).

The phase-out of methyl bromide posed significant challenges for strawberry growers, who relied heavily on this chemical for soil sanitation and pest control. Alternative fumigants like chloropicrin (Tri-Clor) and 1,3-dichloropropene (Telone) emerged as substitutes, but these have their own environmental and regulatory challenges (Fennimore et al. 2014). Transitioning away from methyl bromide required innovative approaches in pest management and soil health. Integrated pest management (IPM) strategies such as biological control agents, crop rotation, and breeding resistance cultivars have become essential tools for growers to maintain yields and quality while reducing reliance on chemical fumigants (Galinato et al. 2019; Shaw et al. 2012).

### 1.1.3 Diseases caused by soilborne pathogens

The history of soilborne pathogens affecting strawberry production in California dates to the early 20th century, coinciding with the onset of commercial cultivation. As cultivation practices intensified, the industry faced escalating challenges from soilborne pathogens such as *Verticillium dahliae, Phytophthora* spp., *Macrophomina phaseolina*, and *Fusarium oxysporum* f. sp. *fragariae*. Verticillium wilt, caused by *V. dahliae*, emerged as a significant threat, leading to symptoms of wilting and decline in strawberry plants (Smith 1965). *Phytophthora* species, notably *P. cactorum* and *P. fragariae*, exacerbated issues by causing root and crown rot, thriving in moist conditions and persisting in soil and plant debris (Brasier 2022). *M. phaseolina* rapidly colonizes plant crown tissue in the hot, dry conditions of typical summer fruit production and summer planting. *F. oxysporum* f. sp. *fragariae*, responsible for Fusarium wilt, further complicates production by infecting roots, colonizing the vascular tissue, hindering plant growth and eventually killing the plant (Henry 2017). These pathogens not only reduced yields but also

burden costly management strategies, prompting the adoption of various chemical and cultural practices to mitigate their impact.

#### 1.1.3.1 Verticillium wilt

Verticillium wilt, caused by *V. dahliae*, was first identified as a significant plant disease in the early 1900s (Wilhelm 1955). *V. dahliae* affects strawberries by invading their vascular system to disrupt water and nutrient transport, leading to leaf wilting, stunting, reduced plant vigor and eventually plant death (Basu 1961).

*V. dahliae* is taxonomically classified within the kingdom Fungi, phylum Ascomycota, class Sordariomycetes, order Glomerellales, and family Plectosphaerellaceae (Pegg and Brady 2002). This fungal species is characterized by its filamentous hyphae and conidiophores, which produce conidia in verticils or whorls, hence its genus name "*Verticillium*" (Fradin and Thomma 2006). Its taxonomic placement is based on morphological features, genetic analyses, and its role as a plant pathogen affecting a wide range of hosts (Pegg and Brady 2002).

*V. dahliae* exhibits a broad host range, infecting numerous economically important crops and plants worldwide. It is notorious for causing vascular wilt diseases in crops such as strawberries, tomatoes, potatoes, and cotton, among others (Fradin and Thomma 2006). The fungus enters its host plants through roots and colonizes the vascular system, leading to wilting and necrosis. Its ability to infect a wide range of hosts is facilitated by the production of specialized structures called microsclerotia, which can persist in soil and plant debris for decades, contributing to its survival and spread (Wheeler et al. 2019).

*V. dahliae* has been documented across a broad geographic range, including regions such as North America, Europe, Asia, and Australia (Fradin and Thomma 2006; López-Escudero and Mercado-Blanco 2011; Pegg and Brady 2002). In North America, it is prevalent in strawberry-growing regions of California and the Pacific Northwest (Basu 1961). In Europe, the pathogen is

commonly found in strawberry fields in countries such as Spain and the Netherlands (López-Escudero and Mercado-Blanco 2011). In Asia, occurrences have been noted in strawberry-producing areas of Japan and China (Fradin and Thomma 2006). The widespread distribution of *V. dahliae* underscores its adaptability to various climates and agricultural practices, necessitating region-specific management strategies to minimize its impact on crop productivity.

The disease cycle of *V. dahliae* begins with the survival of the fungus as resilient microsclerotia in soil and plant debris (Klosterman et al. 2009). When conditions are favorable, these microsclerotia germinate and produce hyphae that penetrate plant roots (Hiemstra and Harris 1998). Inside the root, the fungus colonizes the vascular tissue, spreading upwards through the xylem, where it obstructs water and nutrient transport (Bhat and Subbarao 1999). This colonization leads to characteristic wilting symptoms in infected plants as water uptake becomes impaired (Basu 1961). As infected plants senesce, they release new microsclerotia into the soil, completing the disease cycle and contributing to the persistence of *V. dahliae* in the soil (Hiemstra and Harris 1998).

Disease management for Verticillium wilt in strawberry production encompasses several strategies. Crop rotation with non-host plants such as broccoli and Brussels sprouts is a foundational approach used to break the disease cycle and reduce pathogen populations in soil between strawberry growing seasons (Njoroge et al. 2009). However, crop rotation in production systems is often done with host plants such as lettuce, subsequently increasing the level of inoculum (Njoroge et al. 2009). Soil fumigation is the most common and effective method of managing Verticillium wilt. It is a non-selective treatment that reduces the pathogen population in the soil prior to planting (Smith 1965). Another significant tactic involves planting resistant cultivars that exhibit varying degrees of tolerance to Verticillium wilt, thereby reducing plant loss and inoculum levels (Shaw et al. 2010).

#### 1.1.3.2 Phytophthora crown and root rot

*Phytophthora* species are plant pathogens belonging to the oomycetes class, often causing devastating disease in a wide range of plants including crops and ornamentals. They thrive in moist environments, infecting roots and causing rot that can lead to plant mortality.

*Phytophthora* spp. belong to the kingdom Stramenopila, phylum Oomycota, and order Peronosporales. They are characterized by coenocytic, filamentous hyphae and ovoid sporangia, infecting a wide range of plants including potatoes, tomatoes, peppers, strawberries, oaks, and citrus, which results in significant global economic losses (Brasier 2022; Erwin and Ribeiro 1996). *Phytophthora* was first identified in the Netherlands in the mid-19th century and is one of the key factors responsible for the Great (Irish Potato) Famine in the mid-1800s. Today, *Phytophthora* species can be found in countries across all continents, affecting crops and natural ecosystems alike due to their ability to thrive in various climates and environments.

The disease cycle of *Phytophthora* spp. begins with the production of thick-walled oospores or chlamydospores in soil or plant debris, acting as survival structures (Brasier 2022). Under favorable conditions (moisture, moderate temperatures, and susceptible host plants) these spores germinate, producing hyphae that can directly infect roots or produce sporangia (Erwin and Ribeiro 1996). Sporangia release motile zoospores that swim in water films to new infection sites such as root tips or wounds. Upon contact with host tissues, zoospores encyst, germinate, and penetrate plant cells directly or via appressoria, establishing infection in the root cortex (Erwin and Ribeiro 1996). The pathogen then spreads through the vascular system, disrupting water and nutrient transport and causing symptoms like wilting, stunting, and plant death (Brasier 2022). As infected plants decay, *Phytophthora* spp. produce new oospores or sporangia that are released into the soil or surrounding environment, perpetuating the disease cycle (Brasier 2022).

Managing *Phytophthora* diseases requires integrated strategies due to the pathogen's resilience and broad host range. Cultural practices include crop rotation with non-host plants and proper sanitation to remove infected plant debris (Brasier 2022). Chemical control involves the use of fungicides applied preventively or curatively to target *Phytophthora* spp. in soil or on plants (Erwin and Ribeiro 1996). Resistant cultivars are bred to withstand *Phytophthora* infections, though resistance may vary among species and strains (Erwin and Ribeiro 1996). Improving soil drainage and avoiding waterlogged conditions can mitigate *Phytophthora* impacts on plants (Brasier 2022). Biological control agents show potential in managing *Phytophthora* diseases, although practical applications vary (Brasier 2022).

1.1.3.3 Macrophomina root rot

*M. phaseolina*, first identified in 1886 (Dhingra and Sinclair 1978), is a highly versatile fungal pathogen known for infecting over 90 plant species worldwide (Pennerman et al. 2024). Commonly referred to as charcoal rot or Macrophomina root rot, it poses a significant threat to strawberries (Koike et al. 2016). This pathogen's ability to survive in arid environments and persist in crop residues exacerbates its impact, causing root rot and wilt symptoms that can severely reduce crop yields due to plant necrosis (Dhingra and Sinclair 1978)

*M. phaseolina* is a fungal pathogen classified within the Ascomycota phylum, specifically belonging to the class Sordariomycetes and the order Botryosphaeriales. It is further categorized under the family Botryosphaeriaceae. This phytopathogenic fungus is characterized by its ability to form dark, microsclerotia, which are instrumental in its reproductive cycle (Dhingra and Sinclair 1978). Taxonomically, *M. phaseolina* has been studied extensively due to its significant impact on a wide range of agricultural crops worldwide.

*M. phaseolina* exhibits a broad host range, infecting at least 97, including many economically important crops such as soybeans, watermelon, sorghum, sunflower, strawberry,

and various legumes (Pennerman et al. 2024). This wide host range contributes to its status as a prominent soilborne pathogen capable of causing severe losses in agriculture. The fungus infects both monocot and dicot plants, with varying degrees of susceptibility among different cultivars and plant species (Ghosh et al. 2018).

*M. phaseolina* is globally distributed and has been reported in numerous regions with suitable environmental conditions for its growth and survival (Sarr et al. 2014). It thrives in warm and dry climates, as well as temperate climates during periods of warmer weather (Zveibil et al. 2012).

The disease cycle of *M. phaseolina* typically begins with the survival of the fungus in soil and plant debris as thick-walled, melanized survival structures called microsclerotia. These microsclerotia serve as the primary inoculum source, found to persist in soil without a host for up to 15 years and remain viable under adverse environmental conditions such as drought and high temperatures (Gupta et al. 2012).

When environmental conditions become favorable, such as during periods of high soil temperatures (above 30°C) and water stress, hyphae germinate and infect crown and root tissue, causing characteristic symptoms such as wilting, crown discoloration, and ultimately plant death (Smith and Carvil 1997).

As the infected host plant dies, *M. phaseolina* undergoes saprophytic growth, producing new microsclerotia on infected tissues. These microsclerotia are subsequently released into the soil upon plant decomposition, completing the disease cycle and ensuring the persistence of the fungus in soil (Aldrich-Wolfe et al. 2015). The presence of alternative hosts and crop residues further facilitates the survival and spread of *M. phaseolina*, complicating disease management strategies.

Managing *M. phaseolina* in strawberries involves integrated strategies to minimize its impact on crop yield and quality. Cultural practices such as crop rotation with non-host plants and sanitation of tools and equipment can reduce inoculum levels in the soil (Basu 1961). Additionally, selecting disease-resistant strawberry cultivars can significantly decrease the likelihood of infection (Maas 1998). Implementing proper irrigation management to avoid water stress and maintaining optimal soil fertility levels also play crucial roles in mitigating disease incidence (Smith and Wyllie 1999).

1.1.3.4 Fusarium wilt

*F. oxysporum* f. sp. *fragariae* race 1, the causal agent of Fusarium wilt in strawberries, was first described in the early 1960s (Maas 1998) and first identified in California in 2006 (Koike et al. 2009). *F. oxysporum* f. sp. *fragariae* race 2 was first discovered in the fall of 2022 in Oxnard, California (Henry et al. 2023). This fungal pathogen infects through the roots and colonizes the vascular system, leading to symptoms such as chlorosis, wilting, and eventual death of the plant.

*F. oxysporum* species complex is a diverse group of fungi that include both pathogenic and nonpathogenic lineages (Burkhardt et al. 2019). *F. oxysporum* f. sp. *fragariae* is highly specialized to infect strawberries (*Fragaria* spp.). It affects cultivated strawberries (*Fragaria* × *ananassa)* as well as wild strawberry species (*Fragaria vesca*), causing significant economic losses in strawberry production worldwide (Maas 1998).

*F. oxysporum* f. sp. *fragariae* race 1 has a global distribution wherever strawberries are cultivated. It is particularly prevalent in regions with intensive strawberry production systems, including California, Europe, and parts of Asia (Maas 1998; Viljoen et al. 2019). *F. oxysporum* f. sp. *fragariae* race 2 has only been identified in the Oxnard, California growing district. The pathogen's distribution is influenced by factors such as climate, soil conditions, and agricultural

practices, with outbreaks often exacerbated by the presence of susceptible cultivars and conducive environmental conditions.

The disease cycle of *F. oxysporum* f. sp. *fragariae* begins with the survival of the fungus in the soil and infected plant debris as chlamydospores, which are thick-walled resting structures capable of withstanding adverse environmental conditions (Maas 1998). Upon encountering a suitable host, the chlamydospores germinate and produce hyphae that penetrate the roots. In addition, sporodochia can produce airborne conidia that infect petiole tissue (Henry et al. 2023). The fungus then colonizes the vascular tissues, producing microconidia and macroconidia that cause blockages and disrupting water and nutrient transport (Pastrana et al. 2019). As the infection progresses, characteristic symptoms of Fusarium wilt appear, including yellowing, wilting, and eventual death of the plant (Farr et al. 2001). The pathogen spreads through the plant's vascular system, aided by water movement, and produces new chlamydospores on infected plant tissues. These chlamydospores accumulate in the soil, where they serve as an inoculum source for future infections, completing the disease cycle (Henry et al. 2017).

Management practices for Fusarium wilt include several integrated strategies. Limiting the occurrence of back-to-back strawberry planting and instead rotating with non-host plant species helps reduce soil inoculum levels (Koike et al. 2009). Disease-free planting material and *F. oxysporum* f. sp. *fragariae* race 1 resistant strawberry cultivars can prevent initial infections (Gordon 2017). There are no known cultivars with resistance to *F. oxysporum* f. sp. *fragariae* race 2 (Henry 2024, *personal communication*). Biological control agents, such as beneficial fungi and bacteria, can also be introduced to compete with or antagonize the pathogen (Fravel 2005), but there is currently no widespread adoption of this practice in the industry due to poor or inconsistent performance. Additionally, maintaining proper irrigation and drainage practices minimizes plant stress and reduces the likelihood of infection (Ploetz 2015).

### 1.1.4 Diagnostic assays

To diagnose the described pathogens, soil or plant tissue samples are collected and analyzed via traditional plating and molecular assays. Traditional plating involves inserting plant material on the surface of selective and semi-selective media. Protocols for soil analysis include both incorporating soil in media and plating, as well as on the surface of media. These selective and semi-selective media are designed to inhibit the growth of non-target organisms, allowing for the isolation and identification of specific pathogens based on their morphological and biochemical characteristics. Molecular assays for pathogen detection include Recombinase Polymerase Amplification, High Resolution Melting, and quantitative real-time Polymerase Chain Reaction (Wang et al. 2021).

Recombinase Polymerase Amplification was developed in 2006 as a method to detect pathogens in samples with minimal sample preparation and reduced time compared to molecular assay in use at the time such as Polymerase Chain Reaction (Rohrman and Richards-Kortum 2015). Unlike traditional PCR, RPA does not require thermal cycling, making it faster and more suitable for field diagnostics. It is also considered a more affordable option for diagnostics (Rohrman and Richards-Kortum 2015) in comparison to its counterparts. Due to these attributes, RPA was utilized as the diagnostic method for the survey outlined in Chapter 2. This method uses recombinase enzymes to pair primers with the target DNA, followed by strand displacement and DNA polymerase activity to amplify the DNA (Fig. 1). RPA can be used to detect the presence of viruses, bacteria, and protozoa infecting both animals and plants (Rohrman and Richards-Kortum 2015).

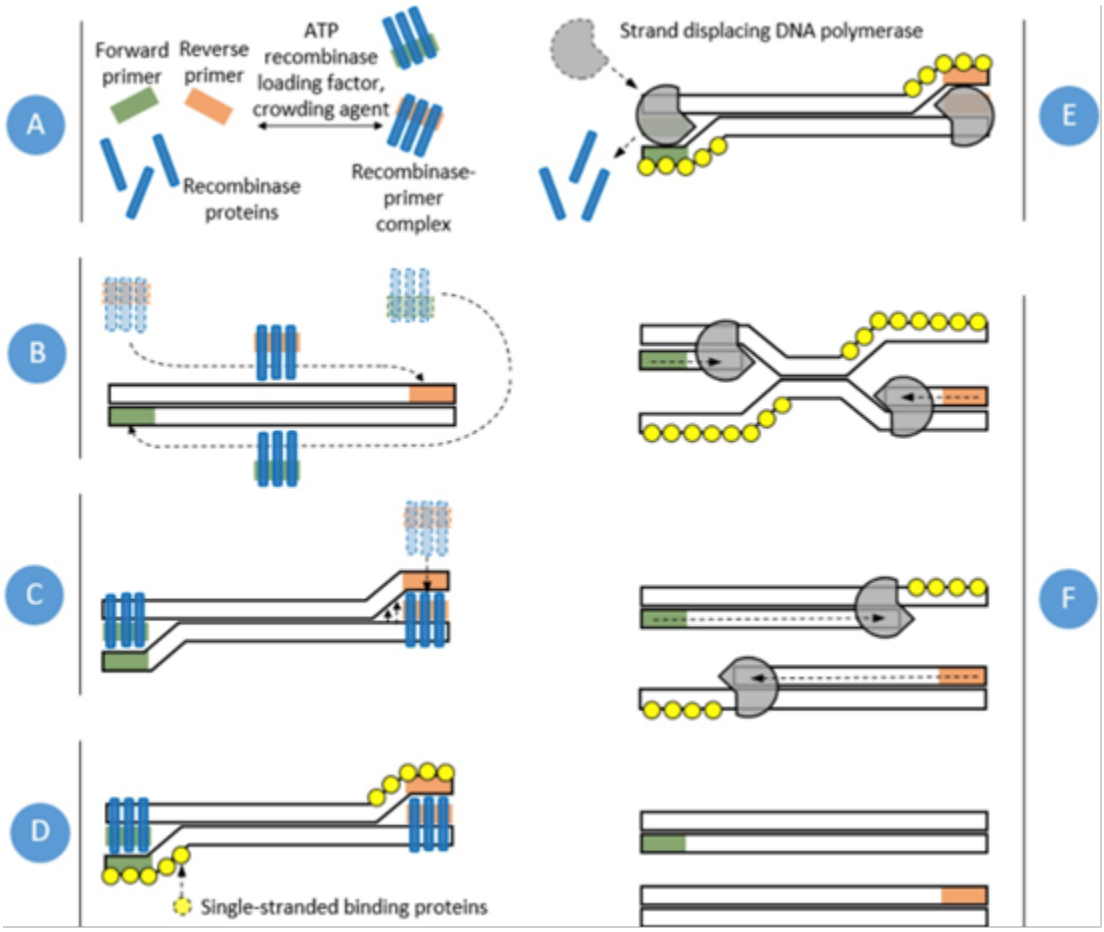


Figure 1. RPA amplification scheme. Recombinase proteins form complexes with each primer (A), which scans DNA for homologous sequences (B). The primers are then inserted at the cognate site by the strand-displacement activity of the recombinase (C) and single stranded binding proteins stabilize the displaced DNA chain (D). The recombinase then disassembles leaving the 3′-end of the primers accessible to a strand displacing DNA polymerase (E), which elongates the primer (F). Exponential amplification is achieved by cyclic repetition of this process (Lobato and O'Sullivan 2018).

While RPA is rapid and frequently utilized for accurate results in diagnostic labs for, with the ability to detect 200 femtogram of purified pathogen DNA, clinical sensitivity is low in comparison to its clinical specificity (Munawar 2020). This means that RPA is more likely to detect a false negative than a false positive. In addition, RPA can be inhibited by high concentrations of genomic DNA and requires extensive optimization of primer concentrations to

prevent target suppression (Lobato and O'Sullivan 2018). Despite the potential disadvantages, RPA is still recognized as an accurate and effective method to pathogen diagnosis of strawberry samples.

### 1.2.1 California strawberry nurseries

California strawberry nurseries play a critical role in the countries' strawberry fruit production industry (California Strawberry Commission 2023). California strawberry nurseries are predominantly located in the high-elevation areas of Northern California's Sierra Foothills, where cooler temperatures and longer daylight hours are ideal for the production of daughter plants (California Strawberry Commission 2023). Natural chill hours, which refer to the cumulative number of hours below 7.2°C, are crucial for strawberry plants as they promote dormancy and enhance the plant's fruiting potential during fruit production (Strand 2008). California's diverse microclimates ensure that nurseries can select sites with adequate chill hours to meet the chilling requirements of different strawberry cultivars, ensuring robust and productive plants for commercial growers (U.S. Department of Agriculture 2023).

In the California strawberry industry, bareroot strawberry transplants are widely utilized for their cost-effectiveness and ease of handling (Pritts and Handley 1998). Strawberry plants are transplanted into bare soil in rows, typically 36 cm between plant lines and 30-46 cm between plants within a line (Holmes 2024). Optional stolon density in this field setting is approximately 951,000 plants per hectare (Holmes 2024). Transplants are harvested during their dormant period, typically from early September to early November, and stored at -1°C to -2°C to maintain dormancy until planting (Galletta and Maas 1990). Bareroot transplants can be viable for up to 9 months in long-term storage (-1°C to -2°C), however plant health and vigor can be reduced, especially if stored beyond five months (Lieten et al. 2005). The exposed roots of bareroot transplants require meticulous handling during planting to ensure proper soil coverage and moisture retention, which are crucial for rapid establishment and growth (Poling 1993). Bareroot

transplants offer a robust start to the growing season and are easily acclimated to fruiting field conditions (Strand 2008).

Plug strawberry transplants are commonly utilized for small-scale commercial growing in the eastern region of the United States (Hancock 1999). Grown in small, individual containers filled with a soil or soilless medium, these transplants are typically started from daughter plants in controlled greenhouse environments (Durner et al. 2002).

### 1.2.2 Strawberry physiology

Strawberry plants are herbaceous perennials characterized by their low-growing habit and compound leaves, which consist of three leaflets with serrated edges (Darnell et al., 2003). The plants have a fibrous root system that spreads shallowly in the soil, providing stability and nutrient absorption (Hancock 1999). Strawberry plants produce a central crown from which leaves, flowers, and stolons emerge. The flowers are typically white with five petals and a receptacle that develops into the strawberry. Strawberry fruit are an aggregate accessory fruit composed of multiple tiny individual fruit, or achenes, embedded on the surface (Hancock and Bringhurst 1981). Efficient photosynthesis and nutrient uptake are crucial for the plant's overall health and fruit production, requiring adequate sunlight, water, and soil fertility (Mass 1998).

### 1.2.3 Day Neutrality

Day neutrality in strawberries is a genetic trait that allows certain cultivars to initiate flowering and fruit production regardless of photoperiod, or daylength. This trait is controlled by a set of genes that respond differently to light cues compared to short-day or day-neutral varieties (Durner et al. 1984). In day-neutral cultivars, the flowering process is regulated by a continuous low-level expression of the flowering locus T (FT) gene, which enables the plants to flower under varying day lengths (Iwata et al. 2012). This characteristic allows day-neutral strawberries to produce fruit throughout the growing season (Guttridge 1985). By bypassing the photoperiodic

flowering control, these cultivars can be used in a wider range of geography and climates (Hancock 1999).

Extensive research has been conducted to understand the underlying mechanisms of day neutrality and improve the performance of these strawberry varieties. Studies have shown that day-neutral strawberries exhibit a more consistent and extended flowering period, leading to a prolonged fruiting season compared to their short-day counterparts (Durner et al. 1984). Additionally, advancements in molecular breeding have allowed scientists to identify and select for genes associated with day neutrality, enhancing the breeding process for better yield, disease resistance, and fruit quality (Gaston et al. 2013). The adaptability of day-neutral strawberries to various environmental conditions makes them a crucial asset in the face of climate change, as they can maintain productivity despite shifts in seasonal patterns (Hancock 1999). Consequently, the cultivation of day-neutral strawberries supports more sustainable agricultural practices by providing a stable and reliable fruit supply (Guttridge 1985; Durner 1984).

### 1.2.4 Stolon production

Daughter plant production is a critical aspect of strawberry plant propagation and growth. Stolons, or runners, are horizontal stems that grow from the main plant and produce new daughter plants at their nodes (Strik and Proctor 1988). The genetic control of runner production involves a complex interplay between genes that regulate growth and development. For example, the interaction between genes such as GA20ox, which is involved in gibberellin biosynthesis, and SP, a floral repressor, plays a significant role in balancing vegetative growth and fruit production (Mouhu et al. 2013). This vegetative reproduction method allows for the rapid spread and establishment of new daughter plants in a nursery setting (Galletta and Maas 1990). Commercial growers utilize this natural cloning process to maintain genetic consistency and ensure uniform crop characteristics (Pritts and Handley 1998). In commercial fruit production, proper

management of runner production involves timely pinching or cutting to control plant density and optimize fruit yield, as runner growth can divert energy away from fruit production and negatively impact overall productivity (Poling 1993).

### 1.2.5 Chill hours

Strawberry plants require a certain number of chill hours, or exposure to temperatures between 0 to 7°C, to break dormancy and promote robust fruit production and flowering (Durner 1999). The number of chill hours required varies among cultivars, but most cultivars need approximately 200 to 300 hours to ensure optimal performance (Strik et al. 2007). Insufficient chill hours can lead to irregular flowering, reduced fruit size, and lower yields (Hancock 1999). The effect of chilling hours on fruit yield has yet to be quantified for cultivars grown in high volume in recent years such as 'Monterey'. Growers must carefully monitor and manage chilling requirements, especially in regions with mild winters, to achieve consistent and high-quality fruit production (Lieten 2002). Techniques such as supplemental chilling through refrigeration or selecting low-chill cultivars can help mitigate challenges related to inadequate natural chilling (Voth and Bringhurst 1990).

Vernalization is the process by which strawberry plants undergo a period of cold exposure to induce flowering and enhance fruit production (Diel et al. 2017). This cold treatment triggers physiological changes that prepare the plant for the reproductive phase, ensuring synchronized and prolific flowering when temperatures rise (Darnell et al. 2003). Vernalization requirements vary among strawberry cultivars, with some needing extended periods of up to 400 hours of cold exposure to initiate flowering (Strik and Proctor 1988). Commercial growers often manipulate vernalization through controlled environments or field management practices to optimize bloom timing and maximize yields (Hancock 1999). Understanding and implementing

effective vernalization strategies is essential for achieving consistent and profitable strawberry production (Pritts and Handley 1998).

### 1.2.6 Strawberry cultivars

The development of different strawberry cultivars has been driven by the need to improve yield, fruit quality, disease resistance, and adaptability to various climates and growing conditions. Breeding programs worldwide have focused on creating cultivars that can meet the specific demands of both commercial growers and consumers (Hancock 1999). Traditional breeding methods, including cross-pollination and selection, have been used alongside modern techniques such as genetic marker-assisted selection and biotechnology to expedite the development of superior cultivars (Finn et al. 2013). Notable breeding programs, such as those at the University of California Davis and the University of Florida, have produced cultivars such as 'Monterey,' 'Portola', and 'Florida Radiance,' which are widely cultivated for their high yields, large fruit size, and disease resistance (Shaw & Larson 2008; Whitaker et al. 2011). In California, there are also proprietary cultivars produced at companies such as Driscoll's, Incorporated (Watsonville, CA), Plant Sciences, Incorporated (Watsonville, CA), GoodFarms, Incorporated (San Diego, CA) and California Berry Cultivars, L.L.C. (French Camp, CA)

The selection of strawberry cultivars will significantly determine yield and overall plant physiology. Different cultivars exhibit varied responses to environmental conditions, management practices, and pest and disease pressures (Lieten 2002). For instance, day-neutral cultivars such as ‘Monterey' and 'San Andreas' produce fruit continuously throughout the growing season, leading to higher overall yields compared to short-day cultivars such as ‘Fronteras’ and ‘Petaluma’, which have a more limited, early-season fruiting period (Durner et al. 2002). Additionally, the genetic makeup of a cultivar determines its vigor, root system development, and nutrient uptake efficiency, all of which are critical factors in achieving high yields and robust plant health (Maas 1998). Selecting the appropriate cultivar for specific growing conditions and

production goals is thus a crucial decision for commercial strawberry growers aiming to maximize productivity and profitability (Hokanson and Finn 2000).

Physiological characteristics can greatly vary among strawberry cultivars. Different cultivars have distinct growth habits, including variations in leaf size, canopy architecture, and root system development, which influence their adaptability to different growing systems and environmental stresses (Fletcher 1917). For example, cultivars with a more compact growth habit and efficient root systems are better suited to high-density planting and containerized growing systems (Finn et al. 2013). Furthermore, the inherent disease resistance and pest tolerance of a cultivar can make it more desirable to growers (Pritts & Handley 1998). The physiological traits of each cultivar, therefore, play a pivotal role in determining its suitability for specific production systems and its overall performance in the field (Shaw and Larson 2008).

### 1.2.7 Planting timing

Research on planting dates for strawberries has consistently shown that the timing of planting significantly affects yield, fruit quality, and overall plant development. Earlier planting dates enhance fruit yield and size due to a longer growing season, allowing plants more time to establish and produce flowers (Mauere & Umeda 1999). Strawberries planted earlier in the optimal planting window exhibit increased vigor and higher productivity compared to those planted later (Rice 1990). It is important to note that optimal planting times also vary depending on the specific cultivar, with some performing best when planted early and others thriving with later planting dates (Anna et al. 2003). While there are trials conducted on optimal planting timing of strawberries, there have yet to be studies conducted in California utilizing cultivars that are currently farmed in high volume.

## 1.3 Conclusion

This literature review offers an understanding of the context and objectives of the material discussed in Chapters 2 and 3 of this thesis. The prevalence of the described soilborne pathogens affecting strawberry in the Oxnard growing district was previously unknown. The results of the soilborne pathogen survey in Chapter 2 will conclude the statewide prevalence and serve as a decision-making tool for strawberry growers, breeders, and industry members. With the understanding that many physiological responses are cultivar-dependent, the results of the field trials in Chapter 3 will give California strawberry growers insight into to the responses of 'Monterey', the most commonly grown to planting time cultivar statewide (California Strawberry Commission 2024).

# CHAPTER 2- SURVEY OF SOILBORNE PATHOGENS INFECTING STRAWBERRY IN OXNARD, CA

## 2.1 Abstract

There are five major soilborne pathogens of strawberries in California, but their distribution and prevalence in the Oxnard production district are unknown. To quantify the frequency of these pathogens, 93 symptomatic strawberry plant samples were collected from 82 fields in the Oxnard growing district between November 2022 and December 2023. Each sample consisted of eight plants exhibiting moderate to severe plant collapse. DNA was extracted from crown tissue from each plant for recombinase polymerase amplification (RPA) to detect *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae* races 1 and 2, *Verticillium dahliae*, and *Phytophthora* spp. Root, petiole and crown tissue from plant samples in which no pathogens were detected by RPA was plated on semi-selective media to verify the absence of the five pathogens and screen for other pathogenic fungi. At least one of the five pathogens was detected in 68 of the 93 samples (73.1%). *M. phaseolina* was the most prevalent pathogen detected in diseased plants in the Oxnard growing district, present in 67.6% of samples positive for at least one pathogen. *F. oxysporum* f. sp. *fragariae* race 1 (35.3%), *V. dahliae*(8.8%), *F. oxysporum* f. sp. *fragariae* race 2 (5.9%) and *Phytophthora* spp. (2.9%) followed at lower prevalence. Associations were found between fewer drip irrigation lines and the presence of *M. phaseolina and F. oxysporum* f. sp. *fragariae* race 2. No associations were found between pathogen presence and mulch color, organic/conventional, or soil type.

## 2.2 Introduction

The strawberry is an economically important crop in California that supports a $3.4 billion industry (California Strawberry Commission 2023). Soilborne pathogens represent a substantial source of economic loss for the strawberry industry, causing widespread plant

mortality while leading to increased costs for disease management (Katan 2017). Historically, there are five main soilborne pathogens known to infect strawberry: *Verticillium dahliae*, *Phytophthora* spp., *Macrophomina phaseolina*, and *Fusarium oxysporum* f. sp. *fragariae.* races 1 and 2. The current prevalence of these five pathogens in the California strawberry industry was unknown, prompting a survey in which strawberry fields in the Watsonville-Salinas district of California were sampled during the 2021 growing season and tested for these pathogens (Steele et al. 2022). In a continued effort to survey all three growing districts, an additional survey was conducted on 2021 fall-planted and 2022 summer-planted strawberry in the Santa Maria district (Steele et al. 2023).

In addition to the five major soilborne pathogens, samples were also screened for root knot nematodes. These microscopic roundworms invade the plant roots, forming galls or "knots," which disrupt the plant's ability to absorb water and nutrients. As a result, infected plants often exhibit stunted growth and chlorosis that can be easily interpreted as symptoms of a soilborne pathogen. In other cropping systems such as tomato and melon, the damage caused by root-knot nematodes can be exacerbated by secondary infections, such as fungal or bacterial pathogens, which further diminish plant health (Holterman 2017; Galvez et al. 2019). It is yet to be confirmed if this occurs in strawberries or if there is a relation between nematode and fungi presence.

The Oxnard district is a critical region for California strawberry production, making up 20.7% of the state's fall-planted acreage and 40.2% of summer-planted acreage (California Strawberry Commission 2024). The Oxnard growing district has the highest percentage of proprietary cultivars (63.9% of fall-planted acreage and 79.1% of summer-planted acreage) of the three growing districts (Watsonville: 49.0% of fall-planted acreage and Santa Maria: 22.1% of fall-planted and 22.0% of summer-planted acreage) (California Strawberry Commission 2024). A survey of diseased strawberry plants in the Oxnard growing district was critical in summarizing

the relative prevalence of these five pathogens statewide and providing a roadmap for future research on soilborne pathogens affecting strawberry.

## 2.3 Materials and methods

To evaluate the distribution and prevalence of the five major soilborne pathogens of strawberry (*Fragaria* × *ananassa* Duch.) in the Oxnard production district, strawberry fields were scouted for plant mortality via ground and satellite imagery. To maximize the sample size and represent as much of the growing district's acreage as possible, both fall- and summer-planted fields were included. Fields were sampled during peak to late fruit production when conditions favor disease development and disease symptoms can be observed visually. In addition to pathogen detection, the following data on growing practices were collected for each field: cultivar, proportion of symptomatic plants in the sample areas, symptoms observed, Global Positioning System (GPS) coordinates, if the field was organic or conventional, number of drip irrigation lines, soil type, and plastic mulch color. Soil type of each field was determined using GPS coordinates to locate the field on SoilWeb (California Soil Resource Lab, UC Davis/UC-ANR/USDA NRCS, https://casoilresource.lawr.ucdavis.edu/gmap/). Correlations between these field variables and pathogen presence were assessed using nominal logistic regression (JMP Version 17 statistical software; SAS Institute Inc., Cary, NC).

Field samples consisted of eight plants exhibiting moderate to severe plant collapse, stunting, or foliar necrosis. Plants were collected from the most diseased area of the field (Fig. 1). In total, 93 symptomatic strawberry plant samples were collected from 82 fields in Ventura County between Nov 2022 and December 2023. Two samples were taken from the same ranch if there were two different cultivars planted. This represents 17.7% of 2022 summer-planted acreage (n=22), 23.0% of 2022 fall-planted acreage (n=23), and 67.6% of 2023 summer planted acreage (n=48) (Fig. 2).

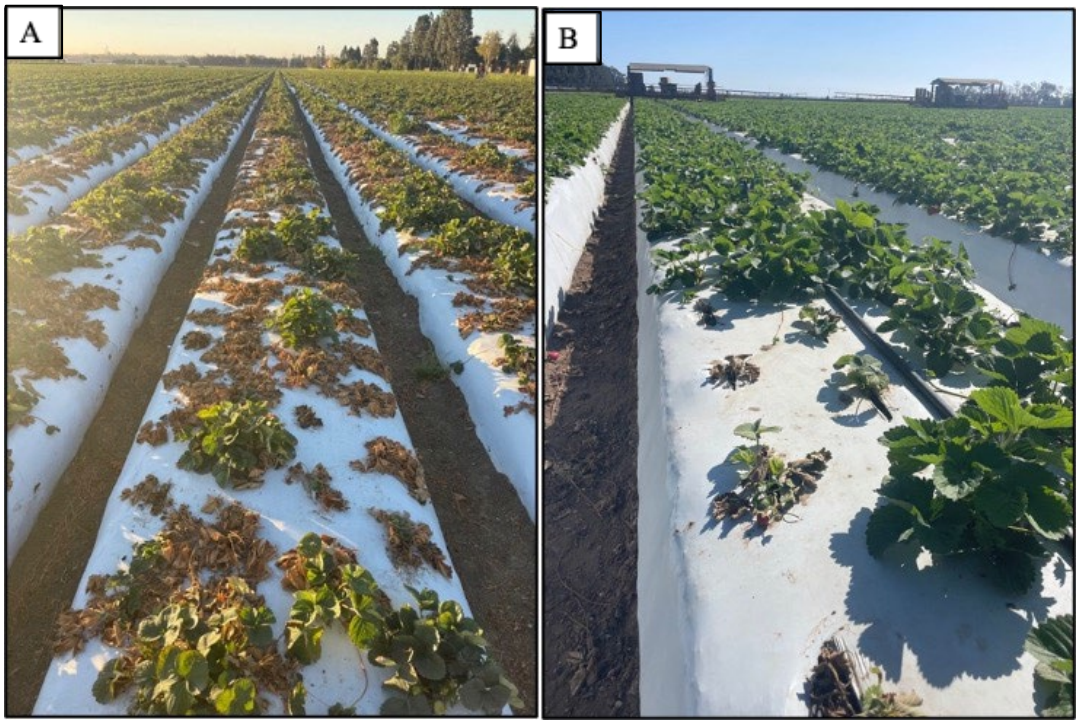


Figure 2. Plant mortality in a sampled field with high disease incidence (A), and typical disease incidence (B) encountered during the survey.

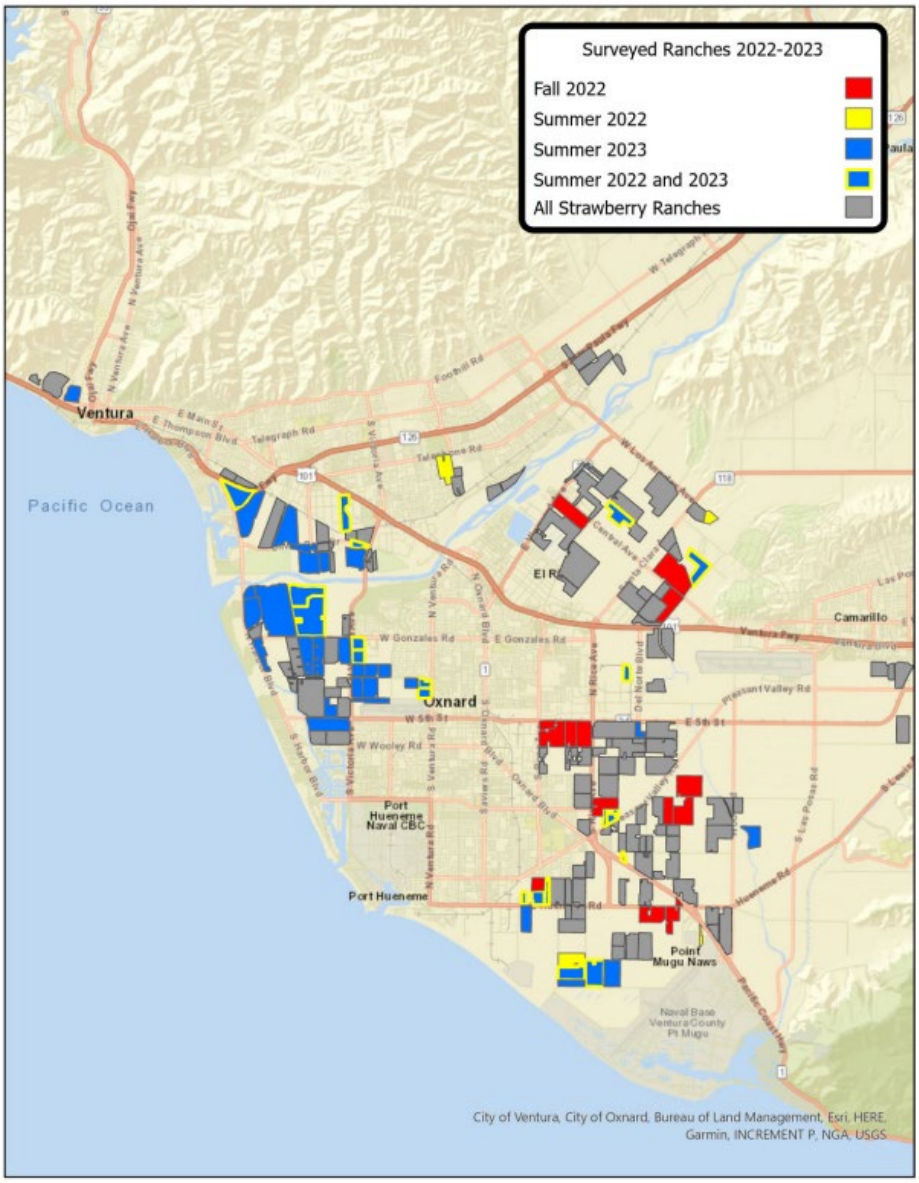


Figure 3. Map of sampled acreage.

Plants were processed at the Cal Poly Strawberry Center Pathology Lab in San Luis Obispo, CA. Recombinase polymerase amplification (RPA), a molecular diagnostic technique, was used to detect *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae* race 1, *Fusarium oxysporum* f. sp. *fragariae* race 2, *Verticillium dahliae*, and *Phytophthora* spp. in each sample. For RPA diagnostics, DNA was extracted from 1 $cm^3$ pieces of crown tissue from each plant in the sample by manually macerating the tissue in general extract buffer (Agdia, Elkhart,

IN, U.S). This ensured the DNA extraction was representative of all the plants in the sample. DNA extracts were stored in sterile, 1.5 mL Eppendorf tubes at -20°C. RPA was conducted on each sample in duplicate using TwistAmp exo kit (TwistDX Limited, Maidenhead, U.K.) and Axxin T-16 ISO RPA machine (Axxin, Fairfield, Australia). Previously developed protocols were employed for *V. dahliae* (Martin, F., *unpublished*, based on previously published primers from Bilodeau et al., 2012), *M. phaseolina* (Burkhardt et al. 2018), *F. oxysporum* f. sp. *fragariae* race 1 (Burkhardt et al. 2019), *F. oxysporum* f. sp. *fragariae* race 2 (Henry, *unpublished*) and *Phytophthora* spp. (Miles et al. 2015). Cox primers and probe (Miles et al. 2015) were added to each reaction to detect plant DNA. A positive and negative control reaction was included in each run of samples.

In cases where both technical replicates of RPA were negative for all pathogens, root, petiole, and crown tissue from plant samples were plated on half-strength acidified potato dextrose agar (APDA) as a general purpose medium, Sorensen's NP-10 (Sorenson et al., 1991) for *V. dahliae* and *M. phaseolina*, and PARP (pimaricin + ampicillin + rifampicin + pentachloronitrobenzene [PCNB] agar; Kannwischer and Mithcell 1978) for *Phytophthora* and *Pythium* spp. to verify the absence of the five pathogens and screen for other pathogenic fungi and Oomycetes. Plant tissue was surface disinfected with 2% sodium hypochlorite for 2 min, washed three times in sterile deionized water for 30 s, dried on sterile paper towel, then plated as follows: four approximately 1-$cm^3$ crown pieces each on two plates of half-strength APDA, four crown pieces on NP-10, four petiole pieces approximately1.5-cm long on NP-10, four crown pieces on PARP, and four approximately 2-cm symptomatic secondary or tertiary root pieces on PARP. The plates were incubated at room temperature and monitored for any fungal growth. Of the 25 samples that tested negative after conducting RPA, all 25 were negative for *M. phaseolina*, *V. dahliae*, and *Phytophthora* spp. by traditional plating techniques.

2.4 Results

*M. phaseolina* was the most prevalent pathogen detected in diseased plants in the Oxnard growing district, present in 67.6% of samples positive for at least one pathogen. *F. oxysporum* f. sp. *fragariae* race 1 (35.3%), *V. dahliae* (8.8%), *F. oxysporum* f. sp. *fragariae* race 2 (5.9%) and *Phytophthora* spp. (2.9%) followed at lower prevalence (Fig. 3). During plant processing, root-knot nematodes (*Meloidogyne* spp.) were observed in two of the 2023 summer-planted samples. While root-knot nematodes were not a focus of this survey, they have also been observed in samples submitted to the Cal Poly Strawberry Center diagnostic service in recent years (Hewavitharana, *unpublished*) and in the soilborne pathogen survey of Santa Maria (Steele et al. 2023).

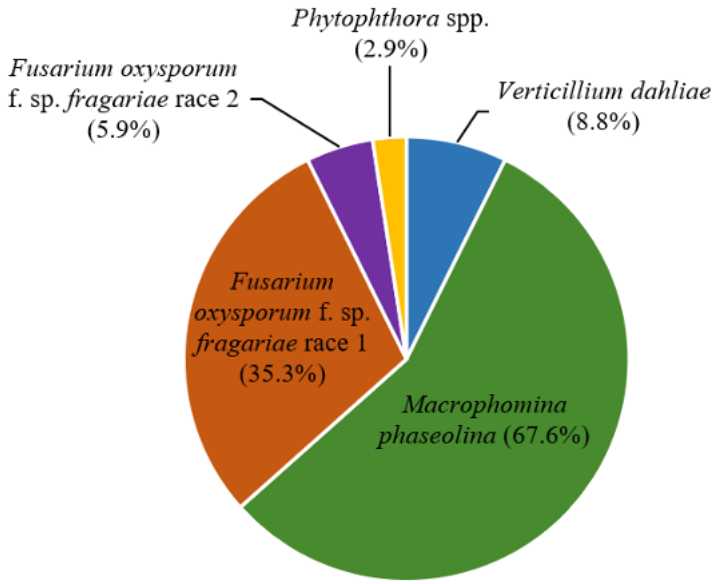


Figure 4**.** Prevalence of the five tested pathogens in all positive samples from Oxnard, CA.

In both 2022 and 2023 summer-planted plant samples, *M. phaseolina* was the most frequently found pathogen. In 2022 fall-planted samples, *F. oxysporum* f. sp. *fragariae* race 1 was the most frequently found pathogen (Fig. 4).

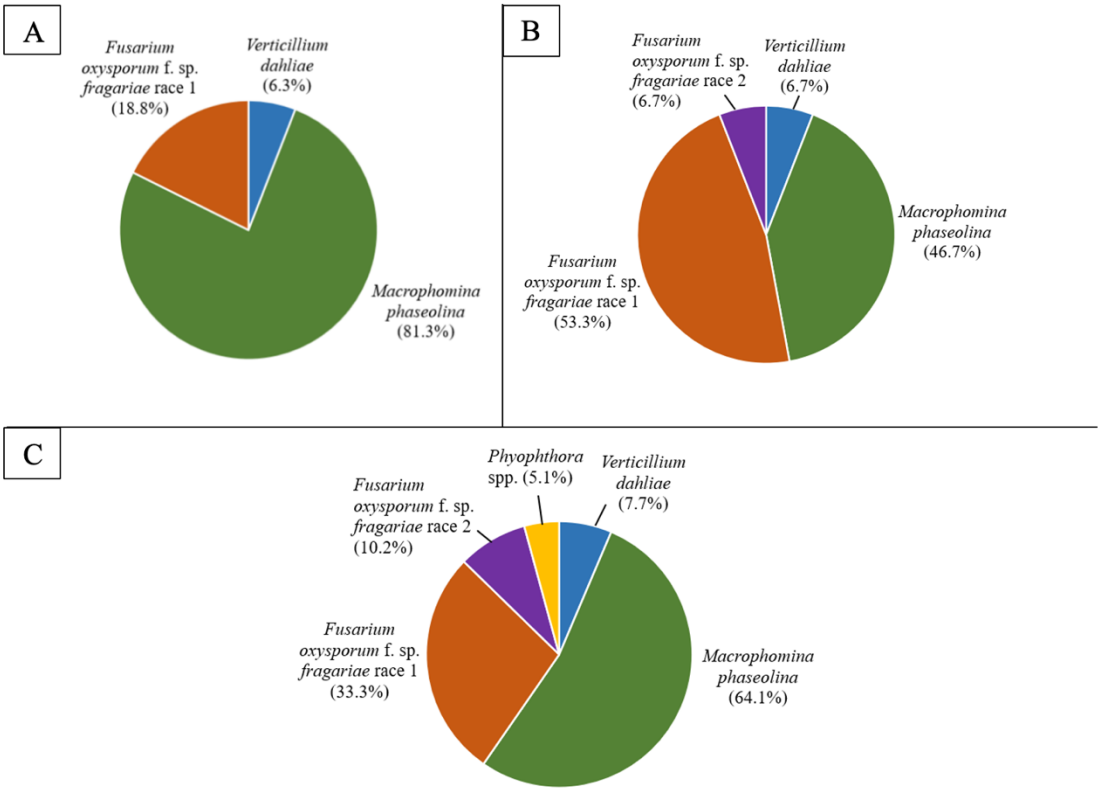


Figure 5. Prevalence of the five major pathogens across summer 2022 (A), fall 2023 (B), and summer 2023 (C) plantings.

Of 93 plant samples, 68 (73.1%) were positive for at least one of the five pathogens tested utilizing RPA. Of the 25 (26.8%) negative plant samples, 4 were positive for *Pythium* spp. when plated on semi-selective media. The 21 other samples were confirmed to be negative for all pathogens when plated on semi-selective media. Eleven samples (11.8%) were positive for two pathogens and zero samples were positive for three or more pathogens (Table 1).

Table 1. Number of samples for which each pairwise combination of the five major pathogens was detected. This includes result of all 93 samples.

| | *M. phaseolina* | *V. dahliae* | *F. oxysporum* f. sp.*fragariae* race 1 | *F. oxysporum* f. sp. *fragariae* race 2 | *Phytophthora*spp. |
|---|---|---|---|---|---|
| *M. phaseolina* | | 1 | 6 | 0 | 0 |
| *V. dahliae* | | | 2 | 1 | |
| *F. oxysporum* f. sp.*fragariae* race 1 | | | | 0 | 1 |
| *F. oxysporum* f. sp. *fragariae* race 2 | | | | | 0 |
| *Phytophthora* spp. | | | | | |

Cultivars from fall 2022 and summer 2023 planted samples are listed in Table 2. ‘Rosalind’ was the most predominantly sampled cultivar from the Oxnard district, making up 16.1% of samples (n=15).

Table 2. Cultivars sampled from 2022 fall-planted and 2023 summer-planted samples.

| **Cultivar** | **Breeding program** | **Number of samples** | **Day-neutrality** |
|---|---|---|---|
| Rosalind | Driscoll’s | 15 | Short-Day |
| Portola | UC Davis | 12 | Day-Neutral |
| Fronteras | UC Davis | 11 | Short-Day |
| Unnamed Selection | Plant Sciences | 6 | NA |
| Pomona | Driscoll’s | 6 | Short-Day |
| Unnamed Selection | NA | 4 | NA |
| Lucille | Driscoll’s | 4 | Short-Day |
| Amado | Driscoll’s | 3 | Short-Day |
| Petaluma | UC Davis | 2 | Short-Day |
| Petra | Driscoll’s | 2 | Day-Neutral |
| Marquis | Driscoll’s | 2 | Day-Neutral |
| BG 10.3169 | Plant Sciences | 1 | Short-Day |
| BG 4.367 | Plant Sciences | 1 | Short-Day |
| Bonanza | Driscoll’s | 1 | Short-Day |

In a multivariate model assessing field variables and pathogen detection, there were no significant associations found between pathogen, color of plastic mulch, organic/conventional, or soil type

(Table 3). Colors of plastic mulch included white (66.7% of samples), black (16.5%), skunk (8.4%), green (5.6% of samples), and clear (2.8%). All summer-planted fields were white mulch. Soil types included loam (81.5% of samples), sand (15.4%), and clay (3.1%). A much higher proportion of samples were farmed conventionally (83.6%) than organically (16.4%). There was a statistically significant association between fewer drip lines and presence of both *M. phaseolina* and *F. oxysporum* f. sp. *fragariae*race 2 (Table 3). The categories for number of drip lines included four (14.1% of samples), three (57.7%), and two (28.2%).

Table 3. Summary of multivariate model assessing field variable correlations to pathogen detection of 2022 fall-planted and 2023 summer-planted samples. Asterisk indicates statistical significance.

| **Factor** | **Response** | ***P*-value** |
|---|---|---|
| Plastic color | *M. phaseolina* | 0.3394 |
| | *V. dahliae* | 0.5539 |
| | *F. oxysporum* f. sp. *fragariae* race 1 | 0.1979 |
| | *F. oxysporum* f. sp. *fragariae* race 2 | 0.5763 |
| | *Phytophthora* spp. | 0.8915 |
| Number of drip lines | *M. phaseolina* | 0.0310* |
| | *V. dahliae* | 0.9624 |
| | *F. oxysporum* f. sp. *fragariae* race 1 | 0.9685 |
| | *F. oxysporum* f. sp. *fragariae* race 2 | 0.0233* |
| | *Phytophthora* spp. | 0.4668 |
| Organic/conventional | *M. phaseolina* | 0.7675 |
| | *V. dahliae* | 0.5592 |
| | *F. oxysporum* f. sp. *fragariae* race 1 | 0.1504 |
| | *F. oxysporum* f. sp. *fragariae* race 2 | 0.3305 |
| | *Phytophthora* spp. | 0.4287 |
| Soil type | *M. phaseolina* | 0.1509 |
| | *V. dahliae* | 0.6801 |
| | *F. oxysporum* f. sp. *fragariae* race 1 | 0.3928 |
| | *F. oxysporum* f. sp. *fragariae* race 2 | 0.3824 |
| | *Phytophthora* spp. | 0.7765 |

## 2.5 Discussion

*Macrophomina phaseolina* was the most frequently detected pathogen in strawberry plant samples from the Oxnard growing district. In the Santa Maria district survey conducted in 2022, *M. phaseolina* was also the most prevalent pathogen detected in plant samples. The first report of *M. phaseolina* in California was in 2005 in the Oxnard district and has now become a widespread and problematic pathogen to strawberry (Koike 2008). In both 2022 and 2023 summer-planted samples, *M. phaseolina* was the most prevalent while *F. oxysporum* f. sp. *fragariae* race 1 was most prevalent in 2022 fall-planted samples. This observed trend confirms that pathogen prevalence is closely associated with occurrence of disease conducive conditions. The hot, dry summer conditions in Oxnard, CA, combined with the region's naturally warm climate, create a more favorable environment for *M. phaseolina*. In addition, *M. phaseolina* was more commonly found in fields with fewer drip lines. This may be attributed to lower soil moisture distribution often seen in a two-drip line to four-plant line system (Daugovish et al. 2016).

The proportion of samples collected that tested negative for all major soilborne pathogens (26.8%) is similar to that of the soilborne pathogen surveys conducted in Santa Maria and Watsonville-Salinas; 21% (Steele et al. 2023) and 26% (Steele et al. 2022) respectively. In addition, the Cal Poly Strawberry Center diagnostic service receives symptomatic plant samples that are negative for all major soilborne pathogens (21% in 2022, 20% in 2023, and 21% in 2024) (Hewavitharana, *unpublished*).

*F. oxysporum* f. sp. *fragariae* race 2 was first identified in Oxnard in the fall of 2022 (Henry et al. 2023). Symptoms of Fusarium wilt of strawberry, typically controlled by the FW1 gene in resistant cultivars, were observed in a field planted with the resistant cultivar Portola. Testing revealed the presence of *F. oxysporum* f. sp. *fragariae* race 2, a previously unreported strain in California, which has since caused mortality in all tested, resistant cultivars (P. Henry, *personal communication*). This discovery indicates that the race 2 strain overcomes FW1

resistances, posing an immediate threat to strawberry production in Oxnard and the rest of the country. This survey was critical in identifying any additional sites of contamination and patterns of distribution of *F. oxysporum* f. sp. *fragariae* race 2 in the Oxnard district. As part of the survey, one new site of *F. oxysporum* f. sp. *fragariae* race 2 was identified and three known sites confirmed positive.

The study's comprehensive approach combined field observations, laboratory diagnostics, and statistical analyses to summarize the interactions between growing practices and pathogen incidence in strawberry production. This research aimed to provide insights into disease management strategies and inform growers' decisions in the Oxnard district. Upon conclusion of the Oxnard survey, the prevalence of pathogens affecting strawberry in all California growing districts has been quantified. The author suggests this survey be replicated at a 5- to 10-year interval to monitor trends and potential shifts in pathogen frequencies.

## 2.6 Acknowledgements

The authors thank the California Strawberry Commission for its funding and support of this project. The authors also thank the growers and ranch managers for allowing plant sampling and their continued support of the Cal Poly Strawberry Center. Kelly Ivors of Driscoll's and Andrew Molinar of the California Strawberry Commission assisted in facilitating sampling. Jason Sharrett of the California Strawberry Commission assisted with map visualization of strawberry fields.

# CHAPTER 3- EFFECT OF PLANTING DATE AND CHILL TREATMENT ON YIELD OF STRAWBERRY

## 3.1 Effect of planting date on strawberry yield

### 3.1.1 Abstract

Strawberries are typically planted at different times based on the area where they are grown and desired timing of yields. To determine the relationship between planting time and yield, two commonly grown cultivars ‘Monterey’ and ‘Fronteras’, were obtained as strawberry plug plants produced at North Carolina State University. These were planted at California Polytechnic State University San Luis Obispo at two-week intervals (26 Oct, 9 Nov, 23 Nov 2022). Yield assessments were taken twice-weekly beginning 13 Apr through 10 Aug 2023. Upon trial termination, the number of branch crowns in each plant was recorded. The earliest planting date, 26 Oct 2022, yielded the highest fruit weight and produced the highest number of branch crowns per plant in both cultivars.

### 3.1.2 Introduction

Strawberry cultivation is highly sensitive to the specific climate and growing conditions of each growing region, making the timing of planting a critical factor for timing and maximizing yield. In California, planting schedules vary across the state's three main production districts: Oxnard, Santa Maria, and Watsonville-Salinas. In Oxnard and Santa Maria, there are both fall and summer plantings (California Strawberry Commission 2024). In Oxnard, fall-planting takes place mid-September through October and summer-planting in late-June to mid-July (Pest Management Strategic Plan 2021). In Santa Maria, fall-planting takes place October to November and summer-planting in late-June to mid-July (Pest Management Strategic Plan 2021). In Watsonville-Salinas district, strawberries are exclusively planted in the fall from October to November (Pest

Management Strategic Plan 2021). Between all three districts and their respective planting seasons, strawberries are being produced year-round (Pest Management Strategic Plan 2021)

The success of strawberry production is not only influenced by regional climate but also by the specific cultivar grown. Different strawberry cultivars respond differently to planting dates, with factors such as temperature, day length, and soil conditions affecting their growth and fruit production (Anna et al. 2003). Understanding these cultivar-specific responses is essential for determining the optimal planting window, which can vary even within the same region and by specific marketing goals. This variability necessitates trials to determine the relationship between planting date and yield for each cultivar (Anna et al. 2003).

This trial was conducted at California Polytechnic State University San Luis Obispo to explore the relationship between planting date and yield for two commonly grown strawberry cultivars, 'Monterey' (29.5% of fall-planted and 5.3% of summer-planted acreage in California) and 'Fronteras' (13.2% of fall-planted acreage in California) (California Strawberry Commission 2024).

### 3.1.3 Materials and methods

The experiment was conducted in field 25 block 6 at the Cal Poly State University campus in San Luis Obispo, CA (35°18'16" N 120°40'32" W). The experimental area consisted of 3 beds, each 16.3 m long. Industry standard beds were used: 1.63 m. between bed centers; four lines of plants spaced 30 cm between plant lines and 41 cm between plants within a line. Black, 3 mil plastic mulch was used (TriCal, Inc., Hollister, CA). A split-plot design was used with planting date as the main plot and cultivar as the subplot. Plots were 0.98 m long (20 plants per plot) with a 0.15 m non-planted buffer between each plot. The four 'Monterey' and four 'Fronteras' plots were randomized on each bed. Plants were irrigated and fertilized via three lines

of 1.59 cm diameter, 0.15 mm (6 mil) thickness, 0.77 L/min per 100 m flow rate irrigation drip tape (The Toro Company, Bloomington, MN).

All daughter plants were sourced from North Carolina State University's nursery located at the Horticultural Field Laboratory in Raleigh, NC. Mother plants were grown in 15 cm pots and kept at 26 +/- 2°C to encourage stolon production. In year one, the daughter plants for the three planting dates were harvested and potted on 23 Sep, 7 Oct, and 21 Oct 2022. In year two, the daughter plants for the three planting dates were harvested and potted on 10 Oct, 24 Oct, and 2 Nov 2023. All daughter plants were rooted directly into 5 cm wells containing Sungro Professional Growing Mix (Agawam, MA, USA). After rooting, daughter plants were placed on a misting bench and received 30 s of water mist every 5 min. Trays were moved outside onto sprinkler pads 28 days after rooting for 2.5 to 3 days before they were wrapped in moist paper towels and shipped overnight to San Luis Obispo, CA.

Upon arrival in San Luis Obispo, all plugs were stored at 2°C for approximately 18 h. Approximately 3 h before planting, plugs were removed from cold storage and placed at room temperature. Strawberry plug plants were transplanted one bed at a time, with a single bed used for each of three different planting dates: 26 Oct, 9 Nov, 23 Nov 2022 and 1 Nov, 17 Nov, 30 Nov 2023. Plug plants were transplanted by hand into moist soil. Following transplanting, strawberry plants were monitored weekly for overall plant health. As the plants developed, flowers were removed through 26 Jan 2023 and 30 Jan 2024 to encourage root and plant growth. Stolons were removed throughout the season to encourage sexual reproduction.

The first harvest occurred when the first fully red fruit was produced. Each week thereafter, ripe fruit was harvested and weighed twice weekly. All fruit was counted and recorded as fruit weight and number of fruit. Any fruit that did not meet the USDA No.1 Grade of strawberries was separated, weighed, and recorded as cull weight and number of cull fruit. This includes fruit that had mold or decay, dirt, foreign matter, disease, insects or mechanical defects

(USDA 2024). Additionally, any fruit <2 cm diameter was considered non-marketable. Yield was calculated on a per-plant basis. The most common cause of fruit being unmarketable throughout the trial was fruit size, followed by mold decay. Throughout the season, the percent of cull fruit did not significantly vary between planting dates, therefore total fruit weight per plant was reported. Upon termination of the trial, the number of branch crowns per plant was recorded. Data was subjected to analysis of variance (1-way ANOVA) and Fisher's LSD mean separation was calculated using ARM version 2024.7.

### 3.1.4 Results

For 'Monterey' later plantings reduced season-long yields. At peak harvest (29 May 2023), a 2-week delay in planting reduced total strawberry yield in 'Monterey' by 37.8%. Furthermore, a 4-week delay in planting reduced total yield by 69.9%. Following peak harvest, total yield of the three planting dates was more similar (Fig. 6). For 'Fronteras' at peak harvest (29 May 2023), a 2-week delay in planting reduced total yield by 29.5%. Furthermore, a 4-week delay in planting reduced total yield by 51.4% (Fig. 7). Earlier planting resulted in the highest number of branch crowns per plant in both 'Monterey' ($p=0.0216$) and 'Fronteras' ($p=0.0391$) (Fig. 8 & 9). Later plantings resulted in a reduction in the number of branch crowns (Fig. 8 & 9). Plant mortality was not significantly impacted by planting date in either 'Monterey' ($p=0.4719$) or 'Fronteras' ($p=0.3022$) (Fig. 10 & 11).

In year two of this trial, there were not enough plug plants produced by North Carolina State University to fill the 20 plant plots. The plug plants that were received from North Carolina State University were highly variable in size and quality (Fig. 12). After more than half of the plots in the trial had less than 10 plants due to initial shortage and poor establishment, it was decided to conclude the trial on 20 May 2023. It was clear that the results would be heavily influenced by the initial plant quality, rather than the planting date.

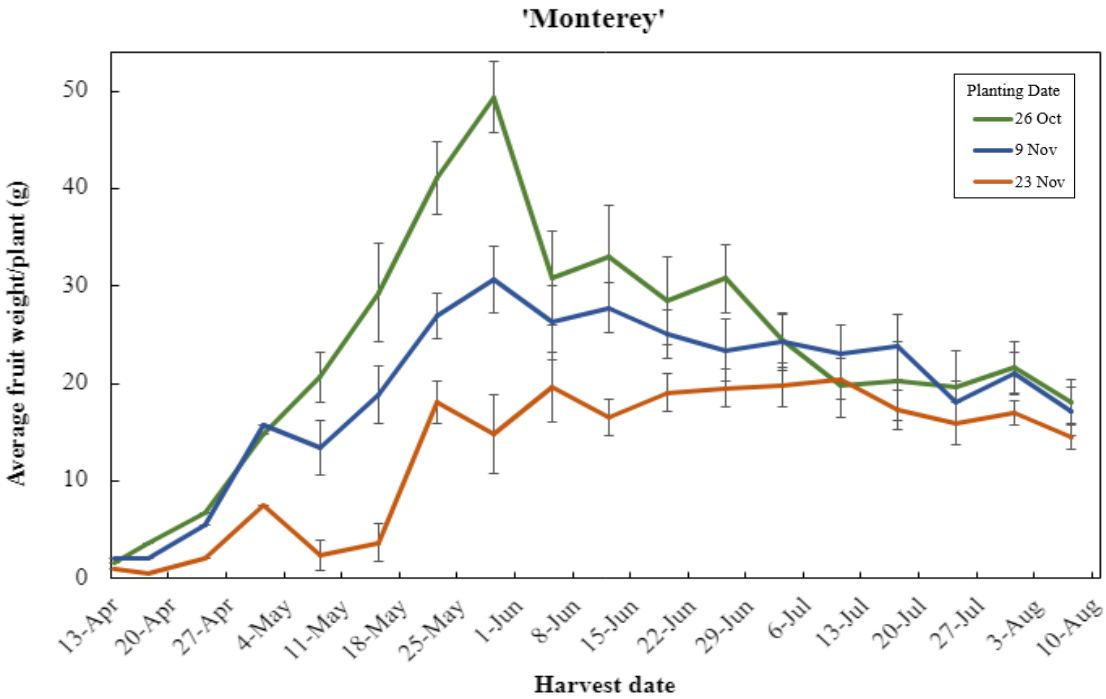


Figure 6. Weekly average total fruit weight per plant in 'Monterey' from 13 Apr to 10 Aug. Error bars represent standard error of the mean.

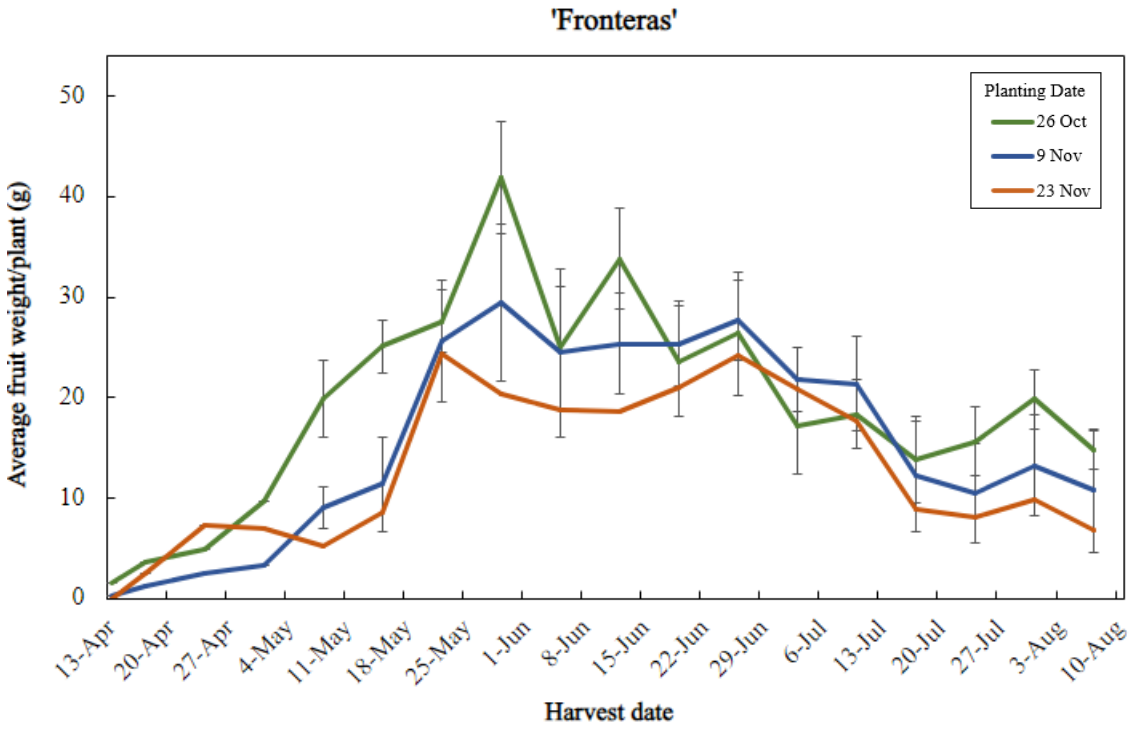


Figure 7. Weekly average total fruit weight per plant in 'Fronteras' from 13 Apr to 10 Aug. Error bars represent standard error of the mean.

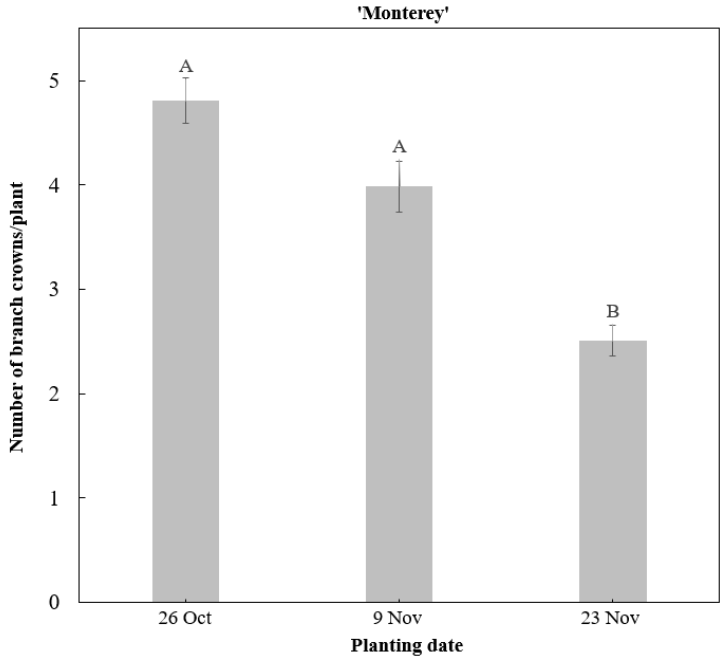


Figure 8. Number of branch crowns per plant in 'Monterey'. Error bars represent standard error of the mean. Treatments that do not share the same letter code are statistically different (α=0.05).

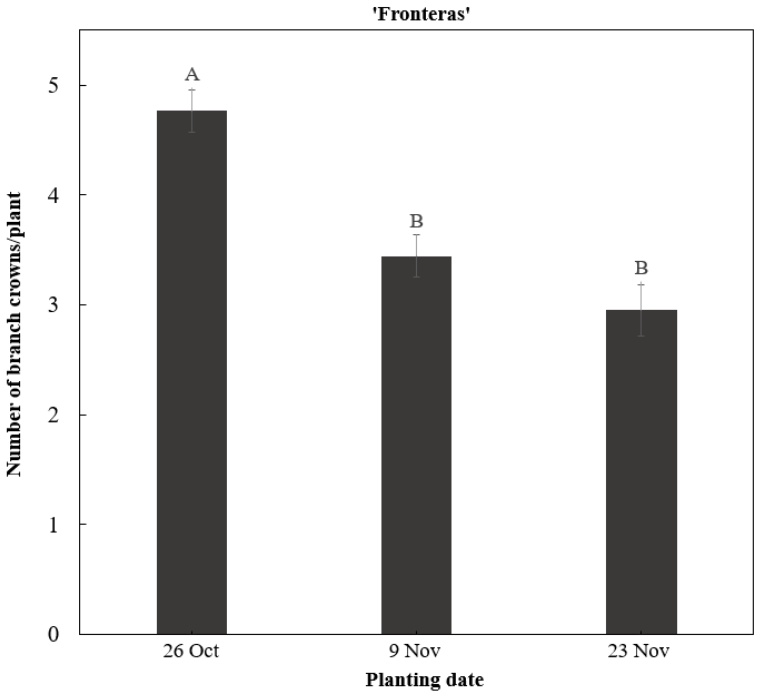


Figure 9. Number of branch crowns per plant in 'Fronteras'. Error bars represent standard error of the mean. Treatments that do not share the same letter code are statistically different (α=0.05).

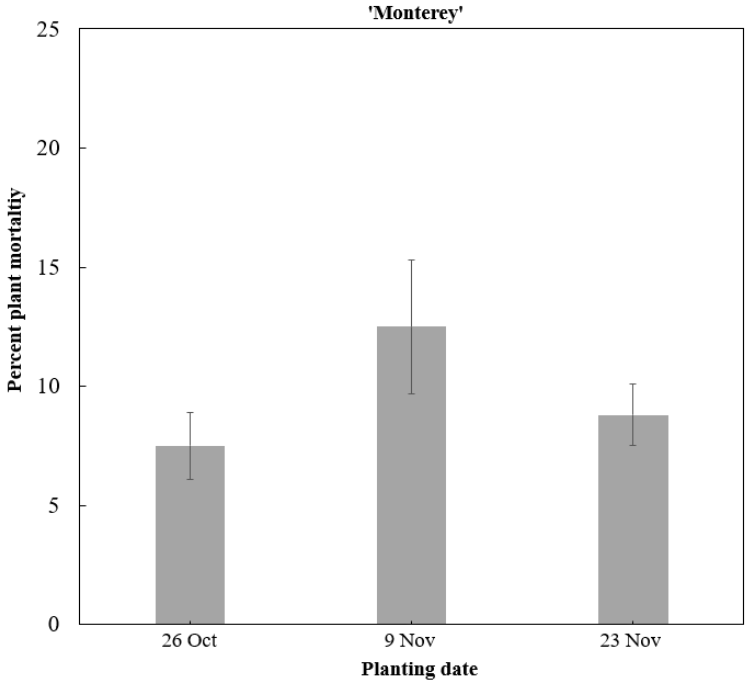


Figure 10. Percent plant mortality due to disease per 'Monterey' plot. Error bars represent standard error of the mean. No statistically significant difference between treatment (α=0.05).

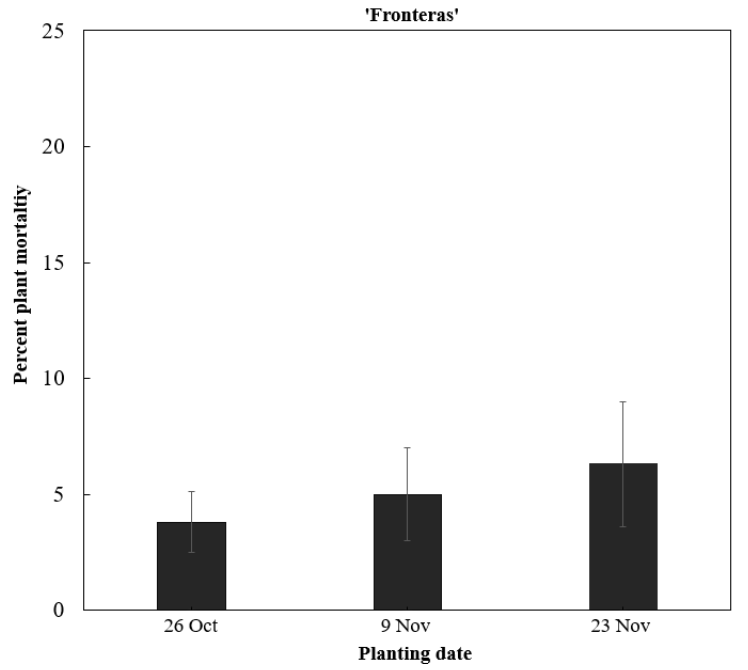


Figure 11. Percent plant mortality due to disease per 'Fronteras' plot. Error bars represent standard error of the mean. No statistically significant difference between treatment (α=0.05).

Figure 12. Strawberry plugs received in year two of the trial.

3.1.5 Discussion

In California strawberry production, approximately 70% of acreage is fall-planted (Holmes 2024). In Santa Maria, CA, fall-planted strawberries are typically planted from mid-October to early November to be harvested March through October (Pest Management Strategic Plan 2021). Exact planting date is often influenced by factors such as weather conditions, availability of labor, and preplant treatments; factors that typically vary annually. The results of this trial should be used and interpreted with these factors in mind.

In year one, earlier planting of both ‘Monterey’ and ‘Fronteras’ increased overall fruit weight per plant. There was also an increase in the number of branch crowns per plant. These are secondary crowns that develop from the main crown, and their number is typically influenced by the plant's growing conditions and overall health. Branch crowns in strawberries are an important indicator of plant size and vigor. A larger plant with more branch crowns suggests that it has had access to optimal conditions for vegetative growth (Strik et al. 2010). Plants with more branch crowns typically produce more fruit because each crown is capable of supporting flower trusses that develop into fruit-bearing structures (Durner et al. 2002). However, this relationship can be affected by other factors such as plant density and environmental conditions. While more crowns often lead to higher yields, excessively high crown numbers can result in smaller individual fruits

due to competition for resources within the plant (Heide et al. 2013). In this trial, quantity of branch crowns only had a positive impact on fruit yield.

The strawberry market may be another influence on a grower's decision to planting timing. Typically, the highest prices are seen earlier and later in the season when overall production is low, and there is less market competition (Guthman 2019). One key strategy is to avoid overlap between fall and summer plantings, which can saturate the market and drive prices down. By staggering plantings or selecting cultivars that mature at different times, growers can take advantage of these price fluctuations and avoid periods of oversupply (K. Ivors, *personal communication*). To create this stagger, growers of proprietary cultivars are often given strict planting timelines based on cultivar selection (K. Ivors, *personal communication*).

In year one of this trial, the process of producing high-quality strawberry plug plants in North Carolina, shipping to California, and planting the next day was feasible. However, the result of year two exposed the potential shortcomings of this process. When smaller plug plants were not well rooted in the substrate (Fig. 14), they became buried in loose soil throughout the shipping process, further depredating the plant quality. This greatly increased the amount of time it took to plant, a major drawback for growers that are planting hundreds of acres at a time. The reason for the shortage of plants and poor plant quality in the second year of the trial was due to employment changes at North Carolina State University.

### 3.1.6 Acknowledgements

The authors would like to thank the North Carolina State University Very Berry Lab, including Dr. Mark Hoffman, Dr. Ricardo Hernandez, and Michael Palmer, for their support in making this trial possible. In addition, thank you to Cal Poly Strawberry Center field manager Drew Summerfield and field research manager Kyle Blauer for their technical support. Thank you to the Strawberry Center undergraduates, in particular Anthony Bella, Sarah Herring, and Cassandra Martinez for their help with data collection. This trial was funded by USDA-NIFA

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## 3.2 Effect of chill treatment on strawberry yield

### 3.2.1 Abstract

This trial was conducted to determine the yield effect of a chill treatment on strawberry plug plants. 'Monterey' plug plants, sourced from North Carolina State University, received either 350 h of chill (i.e., exposure to 4°C) or no chill. Plugs were then shipped overnight to San Luis Obispo, CA and planting the following day (3 Nov 2024) in the field. Yield assessments were taken twice-weekly beginning 29 Mar 2024 through 3 Aug 2024. Upon trial termination, the number of branch crowns in each plant was recorded. The plants treated with 350 h of chill at 4°C yielded the most fruit as measured by weight per plant of season-long yield. There was not a statistically significant difference in the number of branch crowns or in plant mortality.

### 3.2.2 Introduction

Strawberry plants must be exposed to chill hours (below 7.2°C) pre-planting to maximize fruit production (Dunner 1999). The number of chill hours required varies among cultivars, but most cultivars need approximately 200 to 300 hours to ensure optimal performance (Strik et al. 2007). The effect of chilling hours on bareroot strawberry transplants has been heavily studied, including the development of yield prediction models (Tanino and Wang 2008). Much of this research has been focused in bareroot transplants given their exclusive use in California production.

California strawberry nurseries are located in the northern-California Sierra foothills where plants benefit from naturally occurring chill hours. Bareroot transplants can also receive supplemental chill if optimal quantity of chill hours is not received naturally at the nursery

(Holmes 2024). Plug plants, which are commonly used in east-coast and Canadian strawberry production, are often produced in controlled environments and receive artificial chill and light.

This trial aimed to determine the effect of exposing strawberry plugs to artificial chill on yield and number of branch crowns. In conducting this trial, stakeholders will be able to determine the feasibility of producing plugs in North Carolina, shipping to California, to then be planted and produce fruit in a standard California production field.

### 3.2.3 Materials and methods

The experiment was conducted in field 25 block 5 at the Cal Poly State University campus in San Luis Obispo, CA (35°30’44” N 120°67’63” W). The experimental area consisted of one, 20 m long bed. Industry standard beds were used: 1.63 m between bed centers; four lines of plants spaced 30 cm between plant lines and 41 cm between plants within a line. Black, 1.5 mil plastic mulch was used (TriCal, Inc., Hollister, CA). A complete-randomized block design was used. Plots were 1.3 m long (26 plants per plot) with a 0.15 m non-planted buffer between each plot. Treatments were replicated four times. Plants were irrigated and fertilized via three lines of 1.59 cm diameter, 0.15 mm (8 mil) thickness, 0.77 L/min per 100 m flow rate irrigation drip tape (The Toro Company, Bloomington, MN).

All ‘Monterey’ plug plants were sourced from North Carolina State University’s nursery located at the Horticultural Field Laboratory in Raleigh, NC. These plants were produced by rooting daughter plants directly into 5 cm wells containing Sungro Professional Growing Mix (Agawam, MA, USA) and grown for 28 days in a rooting chamber under 90-100% humidity at 27°C with a 18 h photoperiod. After 28 days of rooting, the plant population was split evenly and received either a chill or no chill treatment. Chill treated plug plants received 350 h of 4°C chilling while the no-chill treatment remained at 27°C. After 15 days, resulting plug plants were removed from chambers, wrapped in moist paper towels, and shipped overnight to San Luis Obispo, CA.

Upon arrival in San Luis Obispo, all plugs were stored at 2°C for approximately 18 h. Approximately 3 h before planting, plugs were removed from cold storage and placed at room temperature. Strawberry plug plants were transplanted on 3 Nov, 2023. Plug plants were transplanted by hand into moist soil. Following transplanting, strawberry plants were monitored weekly for overall plant health. As the plants developed, flowers were removed through 30 Jan 2024 to encourage root and plant growth. Stolons were removed throughout the season to encourage sexual reproduction.

The first harvest was recorded when the first fully red fruit was produced. Each week thereafter, ripe fruit was harvested and weighed twice weekly. All fruit was counted and recorded as fruit weight and number of fruit. Any fruit that did not meet the USDA No.1 Grade of strawberries was separated, weighed, and recorded as cull weight and number of cull fruit. This includes fruit that had mold or decay, dirt, foreign matter, disease, insects or mechanical defects (USDA, 2024). Additionally, any fruit <2 cm diameter was considered non-marketable. Data points for each plot were divided by the total number of plants in the plot. Upon termination of the trial, the number of branch crowns per plant was recorded. Data was subjected to analysis of variance (1-way ANOVA) and Fisher's LSD mean separation was calculated using ARM version 2024.7.

### 3.2.4 Results

There were significant differences between the yield of chill and no chill treatments on 9 out of 19 weeks of harvest (Fig. 13). The plants that received chill treatments consistently produced a higher average yield than those with no chill treatment throughout the season. The chill treated plants reached a peak in harvest on 31 May (42.6 g/plant), while the no chill plants peaked on 7 Jul (31.3 g/plant). Both treatments saw a consistent decline in yield following the peak. Chilled plants had a slightly higher number of branch crowns per plant than those not chilled ($p= 0.3997$) (Fig. 14). Chilled plots had statistically significant lower plant mortality than

unchilled plots (p= 0.0390) (Fig. 15). One replication of the no chill treatment was removed from statistical analysis due to its unusually low yield and high plant mortality (27.0%). This was a single plot that was planted at the end of the bed and an edge-effect was observed (Fig. 16) likely due to reduced irrigation and fertilization. In year two of this trial, the buffer between the end of trial area and the end of the bed will be planted to guard against any edge effects.

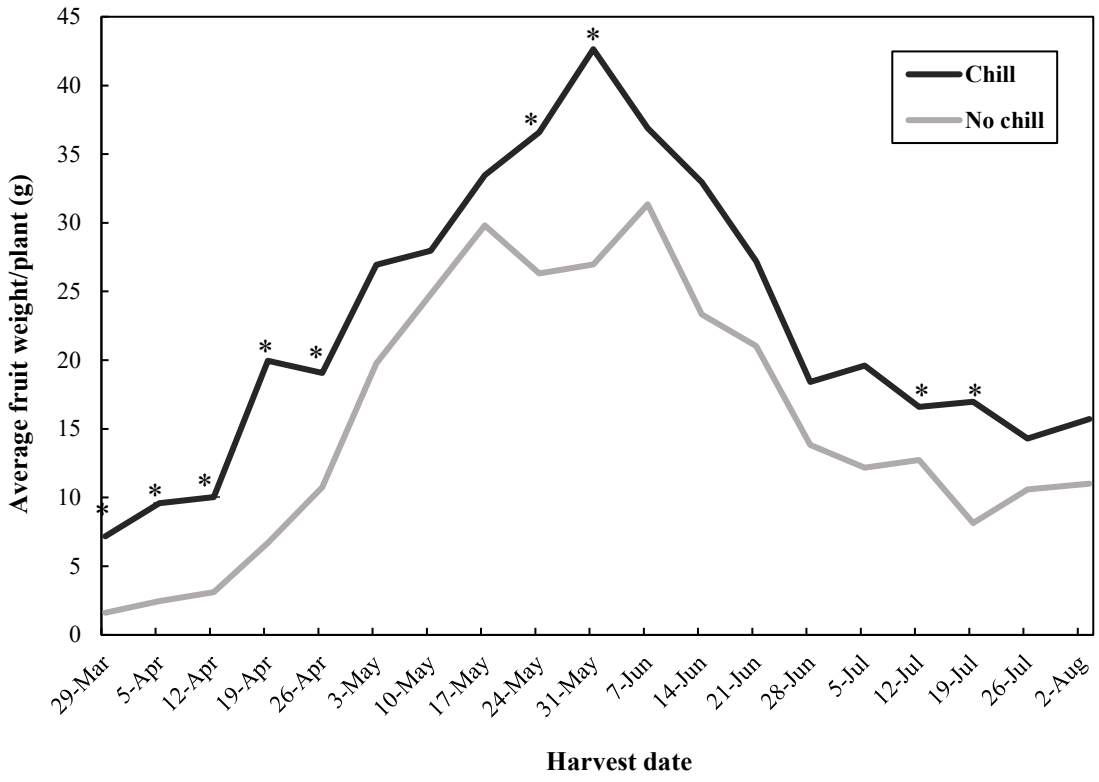


Figure 13. Weekly average total fruit weight per plant from 29 Apr to 3 Aug. Asterisk indicates a statistically significant difference in treatments (α=0.05) (n=4).

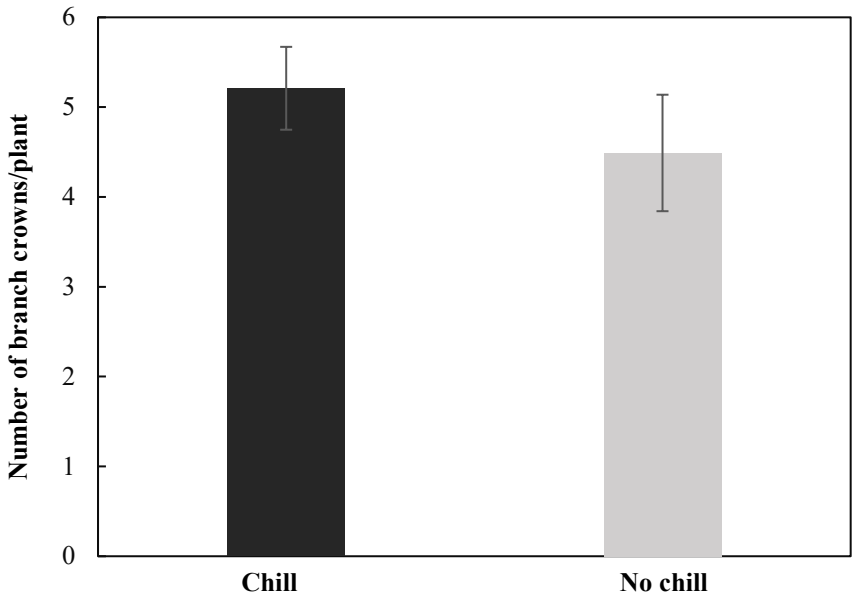


Figure 14. Number of branch crowns per plant. Error bars represent standard error of the mean. No statistically significant difference between treatment (α=0.05).

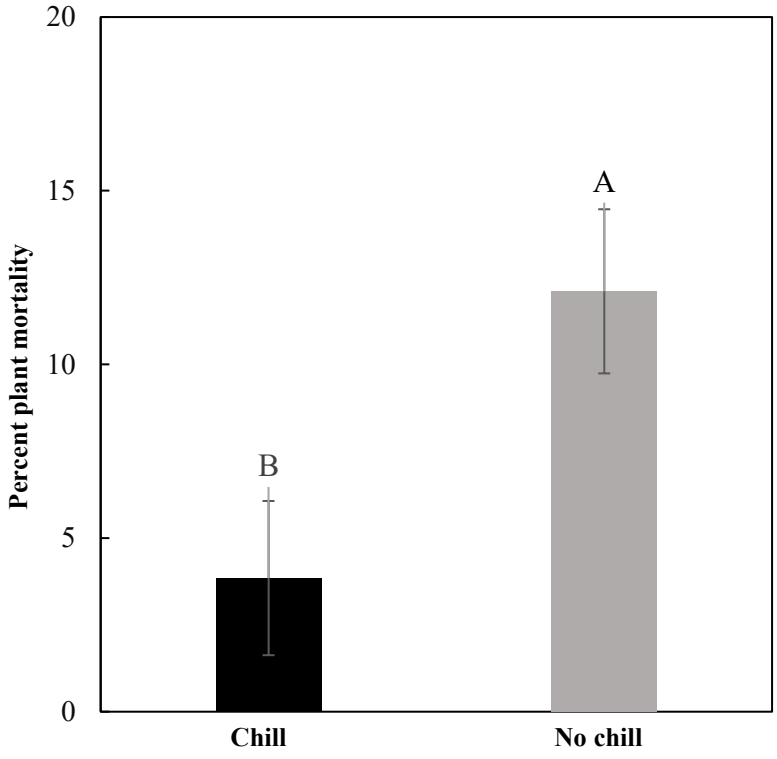


Figure 15. Percent plant mortality due disease per plot. Error bars represent standard error of the mean. Treatments that do not share the same letter code are statistically different (α=0.05).

Figure 16. Aerial photo of trial taken 6 Jun 2024. Plot removed from statistical analysis is boxed in red. Note the stunted plants at the right edge of the plot.

3.2.5 Discussion

Results indicated that the chilled plants consistently outperformed the non-chilled plants in terms of fruit weight. These findings underscore the importance of chilling in promoting strawberry yield, a standard in bareroot strawberry production. Areas for recommended future studies include additional treatments of ‘Monterey’ plug plants with variable total chill hours. Past studies have concluded that optimal chill hours are quite cultivar-dependent (Fletcher 1917), making this a key component for optimizing plant productivity for ‘Monterey’ plugs.

Plants treated with chill had lower plant mortality and higher number of branch crowns per plant, further supporting earlier findings that chilling promotes overall plant vigor, which is critical for successful field establishment (Bish et al. 2002). In future studies, the author would like to include a measure on the quantity of stolon production. This trial will be replicated in the 2024-2025 season and may include a third treatment of ‘Monterey’ bareroot transplants with natural chill as it occurred in a California nursery field.

The implications of this research extend to the logistics of producing and shipping strawberry plugs across large regions. By using artificial chilling, growers in locations like North Carolina could produce high-quality plugs that are conditioned to yield competitively in

California's strawberry production systems. The feasibility at a commercial scale has been explored by some California strawberry growers (Holmes, *personal communication*), however bareroot transplants remain the preferred type of planting stock in California production.

### 3.2.6 Acknowledgements

The authors would like to thank the North Carolina State University Very Berry Lab, including Dr. Ricardo Hernandez, Michael Palmer, and Ibraheem Olasupa for their support in making this trial possible. In addition, thank you to former Cal Poly Strawberry Center field managers Drew Summerfield and Lawson Orradre, as well as field research manager Kyle Blauer for their technical support. Thank you to Strawberry Center undergraduates, in particular Joseph Simard, Olivia Bruns, and Joel Derek Puckett, for their help with data collection. This trial was funded by USDA-NIFA through the Specialized Crop Research Initiative- Coordinated Agricultural Project, Grant no. 2021-51181-35857.

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