AN INTEGRATED APPROACH FOR CONTROLLING VERTICILLIUM WILT OF STRAWBERRY

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ABSTRACT

An Integrated Approach for Controlling Verticillium Wilt of Strawberry

Jack Thomas Koster

Strawberry (*Fragaria* × *ananassa,* Duch.) is an important crop in California, with more than 35,000 acres planted in 2018 resulting in a farm gate value of $3.1 billion. In 2020, California strawberry production accounted for more than 85% of national strawberry production and faces serious threats to production due to various soil-borne diseases. One such disease, Verticillium wilt, is caused by the fungal pathogen *Verticillium dahliae* and is commonly found in temperate zones around the world where strawberries are grown. Due to the phase-out of efficacious fumigants like methyl bromide, alternative disease management methods have become necessary to alleviate threats to production. Alternative fumigation practices such as crop termination have recently been investigated, and the integration of crop termination with bed fumigation and host resistance can play an integral role in control of Verticillium wilt.

A field trial was established at California Polytechnic State University, San Luis Obispo to examine the efficacy of integrative management solutions for control of Verticillium wilt of strawberry in a naturally infested field. The efficacy of sequential fumigation applications of crop termination and bed fumigation was examined. Further, the integration of a resistant cultivar was also implemented in hopes of further decreasing plant mortality and increasing yield. Different fumigant products such as metam potassium, metam sodium, and chloropicrin were used to assess their performance in different fumigation applications. Metam potassium and metam sodium were used for crop termination. When used for crop termination, both products delivered significant

reduction in soil inoculum density and adequate crop injury. Metam potassium, metam sodium, and chloropicrin were used for bed fumigation. All products reduced soil inoculum density. Lower plant mortality and higher yield resulted from sequential applications of crop termination and bed fumigation, with average plant mortality for non-treated control plots and sequentially fumigated plots being 67.2% and 24.1%, respectively. There were no significant increases in yield for plots bed fumigated and sequentially crop terminated and bed fumigated, but significant increases in yield for all plots treated versus the non-treated plots were found. The integration of a moderately resistant cultivar Valiant after the fumigation series showed lower mortality and higher yield versus a susceptible cultivar Seascape.

A two-year study was also conducted in order to evaluate host resistance to Verticillium wilt in 74 cultivars and elite breeding lines from five strawberry breeding programs. Genotypes were established in a field naturally infested with *V. dahliae* on the campus farm at California Polytechnic State University, San Luis Obispo. All five breeding programs had a wide range of susceptibility to Verticillium wilt, ranging from 1.5% to 100% mortality for both years of the trial. Twenty-three cultivars and elite breeding lines were common to both years of the trial; of these, five cultivars showed vastly different results between the two years. For example, ‘Monterey’ showed 78.8% mortality in 2021 and 11.5% mortality in 2022. This demonstrates the importance of evaluating host resistance over multiple years under different environmental conditions and field locations.

Keywords: *Fragaria* × *ananassa*, *Verticillium dahliae*, crop termination, bed fumigation, chloropicrin, metam potassium, metam sodium, soil inoculum, host resistance

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In loving memory and dedication to Nancy Lou, my WooWoo.

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CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

California grows the most strawberries of any state in the United States with an industry worth close to $3 billion, producing 68,000 pounds of fruit per acre (California Strawberry Commission 2022; NASS 2021). The California Strawberry Commission predicts a total of 38,026 planted acres for the 2022 growing season, 30,383 of which will be planted during the fall season (California Strawberry Commission 2022). The California strawberry growing region extends almost 500 miles, from the coastal areas of Santa Cruz County to southern areas near Orange and San Diego Counties (California Strawberry Commission 2018). Yields in California are much higher than yields in other strawberry growing regions within the United States due to the state's mild climate (Geisseler and Horwath 2016). This climate extends the window for growing strawberries from April to November (Hendrickson 1928). Contemporary production allows strawberries to be grown almost year-round. In the southern growing districts, the main production season is January to May, whereas in growing districts along the central coast, production mainly occurs from March to November (Geisseler and Horwath 2016).

The utilization of different strawberry cultivars facilitates this production. Day-neutral varieties are utilized in the Watsonville-Salinas areas where transplanting takes place in the fall (California Strawberry Commission 2018). The more southern growing regions near Santa Maria and Oxnard utilize warmer climates to produce a second crop called 'summer planting'. This second crop is typically planted in May or June to enable production during the winter months (California Strawberry Commission 2018). Within

California, strawberry cultivars from the University of California represent 58.4% of planted acreage, with cultivar 'Monterey' representing 33.1% of these cultivars. Proprietary cultivars, ones held by private companies, represent 36.6% of planted acreage (California Strawberry Commission 2022).

Strawberry yields in California increased steadily after World War II but skyrocketed in the mid-1960s mainly due to the use of soil fumigation and day-neutral cultivars (Bain and Hoos 1963). Although the mild, favorable climate has been beneficial towards yield, the long growing seasons common to California strawberry production provide a suitable environment for the development of various soilborne pathogens such as *Verticillium dahliae* (Dittmar et al. 2018). This pathogen has been a historical constraint to strawberry production in California, causing millions of dollars in losses annually (Wilhelm and Koch 1956). The practice of soil fumigation with methyl bromide began in the 1950s, specifically to control Verticillium wilt of strawberries (Wilhelm et al. 1961) and became the predominant management method. However, soilborne diseases have re-emerged after the phaseout of methyl bromide (Klose et al. 2007). Methyl bromide is considered a "Class I" ozone-depleting substance and was phased out by the United States in 2005 under the Montreal Protocol on Substances that Deplete the Ozone Layer (EPA 2020). California completed their phaseout in 2016 (Holmes et al. 2020) after being granted annual critical use exceptions since 2005. The industry's reliance on fumigation came hand-in-hand with investigations into genetic diversity of strawberry (*Fragaria* × *ananassa*) and other alternative practices such as crop termination and bed fumigation in order to combat threats to production.

The origin of the modern octoploid strawberry has a unique history rooted in interspecific hybridization between progenitor species (Edger et al. 2019). Originally described by Duchesne in 1766, historical strawberries had small fruit and were found in numerous and diverse habitats, from the sub-tropics to deserts (Hancock 1999). The history of events leading to the formation of the modern octoploid strawberry remains relatively misunderstood, with recent chromosome-scale assembly of the strawberry genome leading to new discoveries (Edger et al. 2019). Twenty-two wild species of *Fragaria* have been described, ranging from diploid to decaploid (Liston et al. 2014). Although the origin of the contemporary cultivated strawberry is unclear, the hybridization of *F. chiloensis* and *F. virginiana* became the most cultivated strawberry crop (Howard et al. 1992). The genome composition of the cultivated strawberry is octoploid and is among the most complex of any crop species; *Fragaria* × *ananassa* also is the youngest of contemporary crop species (Davis et al. 2006).

The plant pathogenic ascomycete genus *Verticillium* Kleb (Hypocreales: Plectosphaerellaceae) was described in 1816 by Nees von Esenbaeck (1817). Over 190 species are currently described (Zare et al. 2004) and there are seven major pathogenic species of *Verticillium*: *V. albo-atrum* (Reinke et Berth), *V. dahliae* (Kleb), *V. fungola* (Preuss, Hassebrauk), *V. nigrescens* (Pethybr), *V. nublium* (Pethybr), *V. theobromae* (Turc, Mas et. Hugh), and *V. tricorpus* (Isaac) (Pegg 1984). *V. albo-atrum* and *V. dahliae* are wide-spread throughout the world (Edger et al. 2019). *V. albo-atrum* was discovered in potatoes in Germany in 1879 (Reinke and Berthold 1879). *V. dahliae* was first described in 1931 and adversely affects strawberry production (Vining et al. 2015). Further, economic losses of over 50% have been reported in cotton (Friebertshauser and

DeVay 1982), lettuce (Atallah et al. 2011), olive (Jimenez-Diaz et al. 2012), potato (Rowe and Powelson 2002), and strawberries (Wilhelm and Paulus 1980).

Strawberries are susceptible to soilborne diseases such as Verticillium wilt (Wilhelm and Paulus 1980). *V. dahliae* causes vascular wilt and affects various high value agricultural crops such as lettuce and strawberries (Pegg and Brady 2002; Fig. 1-1). *V. dahliae* has a wide host range of ca. 200 different plants, including species of brassicas, cucurbits, and in the Solanaceae, making management with crop rotation strategies difficult (Bhat and Subbarao 1999). All strawberry cultivars are susceptible to various degrees; there are no natural octoploid cultivars exhibiting complete resistance to *V. dahliae* (Cockerton 2019).

1-1. An experimental plot of strawberry cultivar Laredo exhibiting symptoms of Verticillium wilt.

Advances in investigating genetic resistance in strawberry germplasm to soilborne pathogens have increased in the past 20 years, with work being done in both the public

and private sectors. Genetic resistance has been investigated for many different diseases, including anthracnose (*Colletotrichum acutatum*) (Salinas et al. 2019), gray mold (*Botrytis cinerea)* (Bestfleisch et al. 2015), Phytophthora crown and root rot (*Phytophthora cactorum)* (Mangandi et al. 2017), and Verticillium wilt (*V. dahliae*) (Pincot et al. 2020). Within California, the University of California (UC), Driscoll's Strawberry Associates Inc., Plant Sciences Inc., and Lassen Canyon Nursery have been investigating strawberry genetic diversity since the 1950s (Guthman 2019), and the UC program at Davis has released more than sixty cultivars (Nelson 2019). Advances in genetic resistance is not only limited to California production—the University of Florida has been investigating genetic resistance since 1948, for the state has 11,000 acres of strawberry production. Of those 11,000 acres, University of Florida's cultivars are planted on approximately 9,000 acres (University of Florida 2021).

This review will explore the modern strawberry and the threat of soilborne pathogens to the California strawberry industry, specifically focusing on *V. dahliae*, the causal agent of Verticillium wilt. The taxonomy, biogeography, host range, and life cycle will be included. Further, previous management methods and the eventual void left by methyl bromide will also be included. Fumigation practices such as bed fumigation and crop termination will be discussed, concluding with a summary of genetic resistance for Verticillium wilt.

1.2 The Strawberry

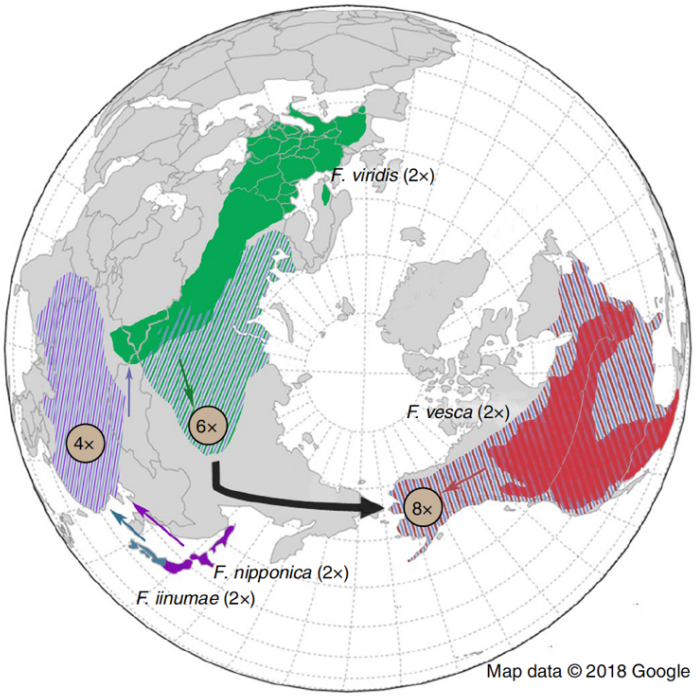
1.2.1 Biogeography of the Strawberry

Investigations into phylogenetic relationships between the modern octoploid strawberry and its extant progenitors reported mixed results due to the limited number of

molecular markers examined (Potter et al. 2000; Tennessen et al. 2014; Yang and Davis 2017). For example, non-coding nuclear chloroplast DNA (Potter et al. 2000) was used to examine progenitor roots whereas a multi-locus approach to examine progenitor roots was utilized by Yang and Davis (2017). Cytogenic data suggests the genome constitution is AAA'A'BBB'B (Bringhurst 1990) and A has been identified as *F. vesca* (Hancock et al. 1993). Previous studies suggested that the octoploid strawberry originated in Asia and moved across the Bering Strait to North America (Luby et al. 1992). More recently, a chromosome-scale assembly utilizing 19,302 nuclear genes was conducted to identify and trace diploid progenitor species for the modern octoploid strawberry and established the origin of the octoploid strawberry in North America (Edger et al. 2019).

Current research identified four progenitor species for the modern octoploid strawberry based on meiotic chromosome pairing. *F. vesca* and *F. iinumae* (Fedorova 1946) were established as two progenitor species (Tennessen et al. 2014) and are endemic to Japan (Fig. 1-2). The proximity to China places these two species geographically close to five other known tetraploid species also described (Edger et al. 2019). Another potential diploid progenitor species, *F. viridis*, is found in Europe and in Asia (Fig. 1-2). *F. viridis* partially overlaps with a sole hexaploid species, *F. moschata* (Edger et al. 2019), and may have led to the evolutionary intermediate between the wild diploid and octoploid species (Edger et al. 2019). A previous phylogenetic analysis supports the hypothesis that *F. viridis* is a possible parental contributor to *F. moschata* and the octoploid event (Lundberg 2011). *F. vesca* subsp. *bracheata* (Edger et al. 2019) is another potential progenitor species endemic to the western part of North America (Fig. 1-2), found as far south as Mexico and north to British Columbia (Edger et al. 2019).

There are two other subspecies, *F. vesca* subsp. *vesca* (Edger et al. 2019) and *F. vesca* subsp. *californica* (Edger et al. 2019), which are endemic to the Russian Far East and the coast of California, respectively. *F. vesca* subsp. *bracheata* was likely the maternal donor during the octoploid event based on the phylogenetic history of the plastid genome (Njuguna et al. 2013).



1-2. Geographic distributions of extant relatives of *Fragaria* × *ananassa*: diploid (2x), tetraploid (4x), hexaploid (6x) and the extant octoploid (8x). Research shows the origin of the modern octoploid strawberry is in North America. Excerpted from: Edger et al. 2019.

The modern octoploid strawberry is geographically restricted to the New World, being largely distributed around North America (Edger et al. 2019). There is the exception of *F. chiloensis* populations in Chile and the Hawaiian Islands (Johnson et al. 2020) but based upon the phylogenetic analysis reported by Edger et al. (2019), the modern octoploid strawberry originated in North America (Fig. 1-2). Thus, the hexaploid

ancestor to the modern octoploid strawberry crossed into North America from Asia and then hybridized with the native *F. vesca* subsp. *bracheata* about 1.1 million years ago (Njuguna et al. 2013). The occasional interspecific fertility adds credibility to the hypothesis that *F. chiloensis* and *F. virginiana* are the primary species for modern strawberry production (Hancock and Luby 1993).

1.2.2 Movement of the Strawberry from North America

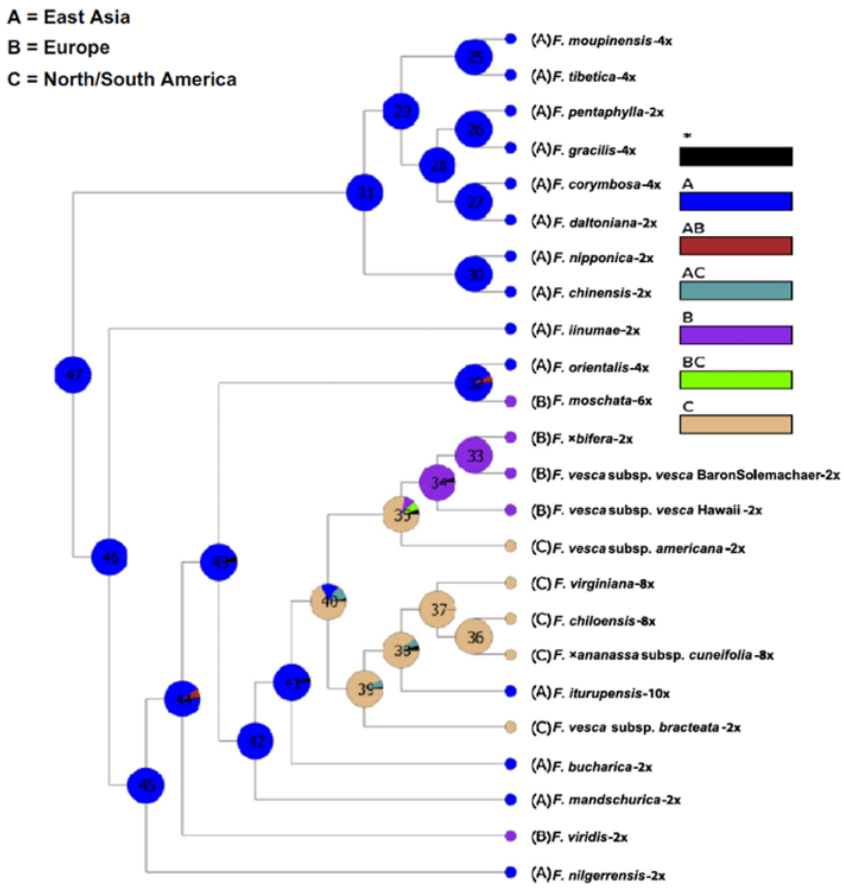
The modern strawberry migration from North America to Chile was likely aided by bird migration, and further separated into two distinct species: *F. chiloensis* and *F. virginiana* (Luby et al. 1992). There are isolated populations of *F. chiloensis* found in Hawaii, and researchers confirmed migratory birds as carriers of strawberry seeds (Hancock et al. 2021). Shorebirds such as Pacific Golden-plovers, Whimbrels, and Ruddy Turnstones all have migration patterns across North America and forage in habitats where *F. chiloensis* is found (Hancock and Prince 2021). The Whimbrel is the likely carrier of strawberry seeds to Chile, whereas the Pacific Golden-Plover is likely the carrier to Hawaii due to their unique wintering migrations (Andres et al. 2012). *F. chiloensis* and *F. virginiana* are completely interfertile and no significant differences between them have been identified in chloroplast DNA (Harrison et al. 1997).

As *F. chiloensis* plants were also moved from Chile to Europe by Spanish colonists (Darrow 1956; 1957), French spy Captain Amédée Frézier returned plants of *F. chiloensis* to France in the 1700s, but they were all female, limiting the diversity of the crop (Wilhelm and Sagen 1972). *F. chiloensis* plants are dioecious, differing from the modern *F.* × *ananassa* hermaphrodite (Hancock and Luby 1993). Yet, the offspring of *F. chiloensis* plants produced large and sweet fruit when planted adjacent to other

strawberry plants such as *F. moschata* and *F. virginiana* (Hancock et al. 1999). In 1766, Antoine Nicholas Duchesne determined that the plants producing large fruit were a cross between *F. chiloensis* and *F. virginiana* and named them *F.* × *ananassa* (Darrow 1956). For nearly a century, this original hybrid was disseminated and cultivated throughout Europe (Barnet 1826; Staudt 1962; 1989; 1999) and eventually found its way to North America in the 19th century (Fletcher 1917) where it became an important agricultural commodity.

1.2.3 Taxonomy of the Strawberry

The chloroplast donor of *F.* × *ananassa* originated from East Asia (Fig. 1-2), whereas the octoploid species originated from North/South America (Njuguna et al. 2013). *Fragaria* species were separated into three separate clades based on morphological characteristics (Fig. 1-3). Previous studies placed *F. vesca* in Clade A, *F. iinumae* in Clade B, and *F. nipponica* in Clade C (Potter et al. 2000). Recent investigations into the subgenome-dominance hypothesis suggest the frequent polyploid interactions between strawberry species had led to generally lower gene-expression over time (Freeling et al. 2012). The loss of genes can occur through deletion or recombination, and the loss of genes from subgenomes with lower gene expression will be favored over the loss of genes from genomes with higher expression rates (Bertioli 2019). Edger et al. (2019) recently identified *F. vesca* as the dominant subgenome in the modern octoploid strawberry. Genes retained by *F. vesca* include nucleotide-binding-site leucine-rich repeats (NBS-LRR), that are important for resistance against plant diseases such as *V. dahliae* (Edger et al. 2019).



1-3. Biogeographic relationship between *Fragaria* species. *F.* × *ananassa* is rooted in ancestry from Asia, Europe, and North/South America. Excerpted from: Njuguna et al. 2013.

1.3 Soilborne Pathogens—Threats to Strawberry Production

Within the past decade, worldwide strawberry production has faced the onset of increased soilborne pathogen incidence. Most recently, reduction in yields due to these diseases have caused alarm (López-Aranda et al. 2012) and causal agents have been identified. In Spain, *Macrophomina phaseolina*, the causal agent of charcoal rot (Avilés et al. 2008); *Fusarium oxysporum* f. sp. *fragariae*, the causal agent of Fusarium wilt (Arroyo et al. 2009); and *Phytophthora cactorum*, the causal agent of crown and root rot (dos Santos et al. 2002) have recently emerged. In California, growers are faced with the

same problems. Macrophomina crown rot (Koike 2008) and Fusarium wilt (Koike et al. 2009) have also recently emerged. These two pathogens have further been found in Argentina and Australia (Koike et al. 2009; Hutton et al. 2013), Iran (Sharifi and Mahdav 2012), South Korea, and Japan (Koike et al. 2009). Verticillium wilt once was the primary threat to strawberry production in California, but the phaseout of methyl bromide has led to the emergence of Macrophomina crown rot and Fusarium wilt throughout all growing districts (Holmes et al. 2020).

Soilborne pathogens include fungi, bacteria, oomycetes, and nematodes. These pathogens cause plant disease with soil inoculum (Koike et al. 2009) and are difficult to control. Propagules of these pathogens can survive in soil for years, dependent on temperature, light, water, and nutrient availability (Velasquez et al. 2018). In the case of fungi, these pathogens can be considered biotrophic, necrotrophic, or hemi-biotrophic. Biotrophic fungi live and reproduce on living plant tissues, whereas necrotrophs do so by killing plant cells and obtaining nutrients from dead or decaying matter. Hemi-biotrophic pathogens can do both; this makes control for these pathogens a multi-faceted concept. Fungi enter their hosts with hyphae, which extend either into or between plant cell walls (Amil-Ruiz et al. 2011). There are four major soilborne diseases of strawberry, all of which are caused by fungi, except for *P. cactorum* which is an Oomycete: Macrophomina crown rot, Fusarium wilt, Verticillium wilt, and Phytophthora crown and root rot (*P. cactorum*) (Koike et al. 2013). These pathogens manifest similar above ground symptoms such as poor growth, crown discoloration, older leaf death and stunting, and plant death, but possess some differences (Table 1). In the case of *V. dahliae*, this pathogen does not always cause crown discoloration and it does not cause the roots to soften, darken, and

loose integrity (Koike et al. 2013). In the case of *P. cactorum*, it causes coordinated collapse of leaves (Table 1).

Table 1-1. Symptoms associated with five common soilborne pathogens of strawberry production (Adapted from: Koike et al. 2003).

Symptoms caused by pathogens	*M. phaseolina*	*F. oxysporum* f. sp. *fragariae*	*V. dahliae*	*P. cactorum*
Poor growth	Yes	Yes	Yes	Yes
Stunting	Yes	Yes	Yes	Yes
Wilting, mostly older leaves	Yes	Yes	Yes	No
Wilting, all leaves at once	No	No	No	Yes
Plant collapse	Yes	Yes	Yes	Yes
Crown Discoloration	Yes	Yes	No	Yes
Soft, dark, rotted roots	No	No	No	Yes

1.4 Verticillium Wilt of Strawberry

Verticillium wilt of strawberry is caused by the fungal pathogen *Verticillium dahliae* and all strawberry cultivars are susceptible; there are no natural octoploid cultivars exhibiting complete resistance (Cockerton 2019). *V. dahliae* is an ascomycete plant pathogen and causes vascular wilt (Inderbitzin and Subbarao 2014). The genus *Verticillium* was established in 1817 by Nees von Esenbaeck (1817) and the first plant-pathogenic species (*V. albo-atrum*) was discovered in potatoes around Germany in 1879 (Reinke and Berthold 1879). *V. dahliae* was first described in 1931 and has been an

obstacle to the strawberry industry for many decades (Vining et al. 2015). *V. dahliae* has a wide host range of ca. 200 different species, including the Brassicaceae, the Cucurbitaceae, and the Solanaceae, which makes it difficult to manage with crop rotation strategies (Bhat and Subbaro 1999). Crop losses due to Verticillium wilt have been diverse and abundant—economic losses of over 50% have been found in cotton (Friebertshauser and DeVay 1982), lettuce (Atallah et al. 2011), olive (Jimenez-Diaz et al. 2012), potato (Rowe and Powelson 2002), and strawberry (Wilhelm and Paulus 1980). Verticillium wilt occurs most frequently in temperate regions (Hawksworth and Talboys 1970) and is rare in the subtropics (Pegg and Brady 2002). Along the temperate regions of California's central coast, the dominant use of land in the Salinas and Pajaro Valleys has been the cultural practice of crop rotation with lettuce and strawberry crops, which continues to be a main factor of economic stability. Growers often integrate broccoli plantings to reduce *V. dahliae* incidence (Lloyd 2020) (Fig. 1-4). This rotation strategy between lettuce and strawberries has allowed growers to conserve costs of fumigation through the strawberry crop, and then benefit from the residual fumigation effects when rotating back to lettuce production (Fig. 1-4; Chellemi et al. 2017).

1-4. A cropping system west of Salinas, CA. The proximity of strawberry to other host crops like cauliflower (left picture) and lettuce (right picture) exemplifies difficulty with crop rotation strategies since strawberry are far more susceptible to *Verticillium dahliae* than cauliflower and lettuce. Longer growing seasons with strawberry show how other *V. dahliae*-susceptible crops are often integrated in the same growing area. Pictures taken 21 March 2022 and 16 July 2022 by J. T. Koster.

Yet, this recent shift in lettuce production practices, including the phaseout of methyl bromide, exacerbated existing lettuce diseases such as Verticillium wilt (Wu et al. 2011); in strawberry production, losses of up to 75% have been documented in the absence of soil fumigation (Wilhelm and Paulus 1980). Verticillium wilt is especially a problem in organic strawberry production, and yields are often dramatically reduced relative to conventional production. Symptoms of Verticillium wilt include leaves turning yellow and drooping, eventually turning brown and desiccating (Subbarao and Kabir 2007). Since *V. dahliae* can survive in the soil for up to 14 years (Schanathorst 1981), problems have arisen that necessitate the examination of alternative methods of control.

1.4.1 Biogeography of Verticillium

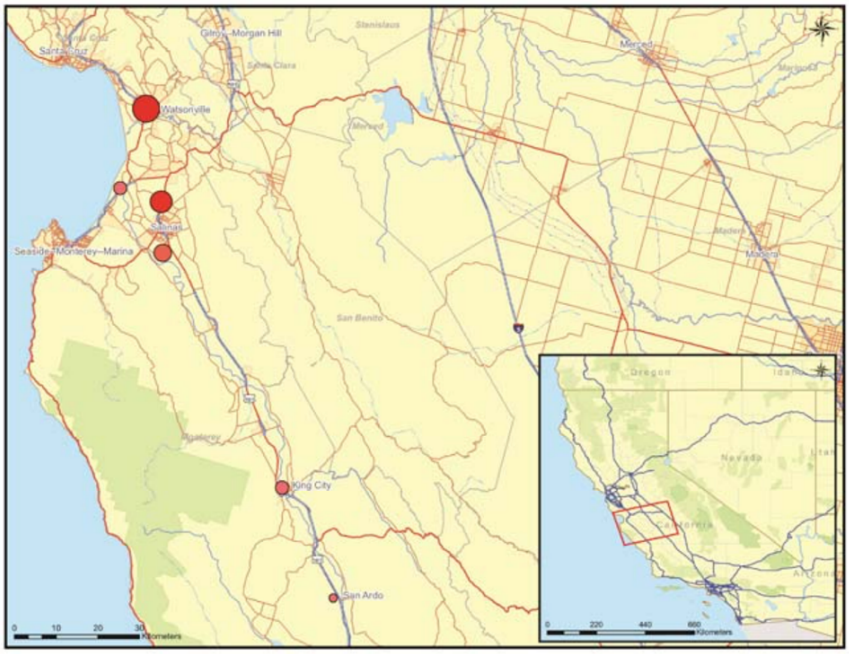
V. dahliae is among the older genera of filamentous fungi that was first discovered on hollyhock (*Alcea rosea* L.) (Anonymous 2010; USDA-NRCS 2013) in 1817 by Nees von Esenbach. *V. dahliae* occurs on the California central coast and its introduction, establishment, and disease status are intwined with the history of agriculture

in the region (Subbarao et al. 1997). Crop rotation between lettuce and strawberry is a common practice due to market needs and the ability to utilize residual fumigation (Chellemi et al. 1994). Lettuce was introduced to Monterey County in the 1920s (Geisseler and Horwath 2016) and the strawberry was introduced ca. 1940. Between 1950 and 1960 the California state average strawberry yield was no higher than five to six tons per acre (Hawksworth and Talboys 1970). In 1984, *V. dahliae* was identified as a contributor to the low yields (Pegg 1984). Researchers have noted the presence of *V. dahliae* along the northern parts of California's central coast but have not seen disease incidence due to *V. dahliae* in the southwestern Arizona lettuce growing areas (Attalah et al. 2011). There is noticeable presence of *V. dahliae* on the Greek island of Crete (Ligoxigakis et al. 2002) and northern Italy (Garibaldi et al. 2007). These geographic areas have similar climates to that of the central coast of California, where cool, moderate temperatures are common. *V. dahliae* thrives in temperate, cool weather and occurs most frequently in temperate regions (Hawksworth and Talboys 1970) and is rare in the subtropics (Pegg and Brady 2002).

In 1994, fields on a single farm in Watsonville, CA reported a loss of their entire lettuce crop due to an unknown disease (Subbarao et al. 1997). The loss was originally blamed on herbicide damage, but after the completion of Koch's Postulates, researchers confirmed that *V. dahliae* was the causal agent (Subbarao et al. 1997). This incident in Watsonville was the first reported case of *V. dahliae* in lettuce (Subbarao et al. 1997). Lettuce can withstand greater amounts of *V. dahliae* inoculum density in the soil (49-99 CFU/g; Wu and Subbarao 2014) compared to that of strawberry (1.9 CFU/g; Harris and

Yang 1996). The introduction of *V. dahliae* appeared to have increased over time, ultimately overwhelming the more resistant lettuce crops (Subbarao et al. 1997).

The discovery of *V. dahliae* causing losses in lettuce and strawberry crops led to observations of other fields in the Salinas and Pajaro Valleys with the disease (Attalah et al. 2011). Between 1995 and 2011, there were over 150 fields with reported incidence of Verticillium wilt, accounting for 2,720 acres (Attalah et al. 2011). In 1999 *V. dahliae* was discovered on the north side of the Salinas Valley (Fig. 1-5). By 2006, *V. dahliae* was reported 100 km south of Salinas in San Ardo, CA (Attalah et al. 2011).



1-5. Distribution of *Verticillium dahliae* along California's Salinas and Pajaro Valleys. Size and intensity of the circles are proportional to the number of fields affected. Excerpted from: Attalah et al. 2011.

Dispersion of *V. dahliae* at the local level is accomplished through microsclerotia, the asexual overwintering structure (Schanathorst 1981). Wind, water, farm machinery, infected seeds, and plant debris aid the dispersal of microsclerotia (Howard 1984). *V. dahliae* transport also is facilitated by humans, with the movement of asymptomatic,

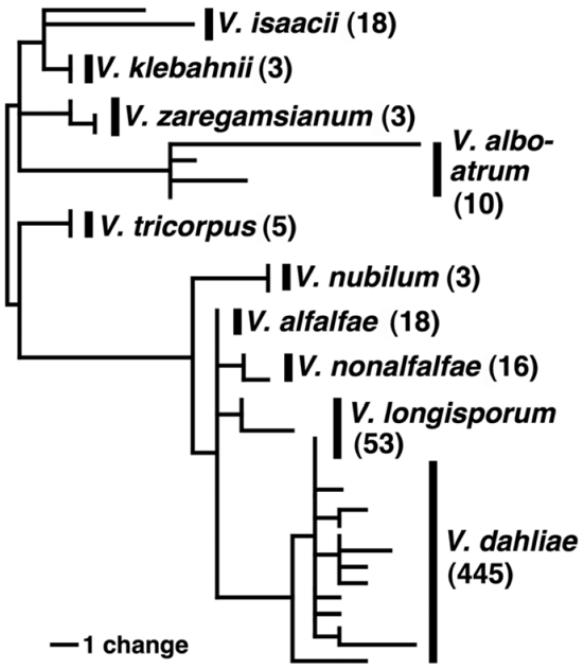
infected host plants (Thanassoulopoulos 1993). In olive and cotton, wind can carry plant debris farther distances, and transmission through infected seed has also been documented (Duressa et al. 2012). Little is known about the spread of *V. dahliae* to the central coast of California, much less its global spread. The human-aided movement of *V. dahliae* to California through infected spinach seeds is the consensus among researchers (Attalah et al. 2010; Subbarao 2002).

1.4.2 Taxonomy and History of Verticillium

The confusion surrounding *Verticillium* systematics and evolution has impeded management (Inderbitzin et al. 2011), for knowledge of the biology and genetics of a disease is the cornerstone for effective, applicable disease management (Inderbitzin et al. 2011; Inderbitzin and Subbarao 2014). The first plant-pathogenic *Verticillium* species was described in 1879 as *V. albo-atrum* (Reinke and Berthold 1879). All species in the genus share the characteristic conidiophore comprised of narrow, flask-shaped, conidia that are assembled in whorls along the main axis (Inderbitzin et al. 2011). The genus *Verticillium* was first divided into different clades (Gams 1971). DNA sequencing eventually was used to identify and transfer related species to other genera (Gams and Zare 2001). In 2005, researchers were cognizant to avoid changing names of major *Verticillium* species to decrease confusion, and through a conservative approach redefined the genus with *V. dahliae* as the type species (Gams et al. 2005). *Verticillium* species that are in the same clade as *V. dahliae* are now referred to as *Verticillium* sensu stricto (Zare et al. 2007).

The longest-lasting controversy surrounding *Verticillium* taxonomy is the differentiation between *V. dahliae* and *V. albo-atrum* (Klebahn 1913). Original

differentiation depended on microsclerotia morphology, and the results were independently confirmed in regions such as Australia (Ludbrook 1933) and Europe (Van der Meer 1925). *V. dahliae* and *V. albo-atrum* were controversially considered as separate species (Isaac 1949; Robinson et al. 1957). This controversy eventually became resolved with Inderbitzen and Subbarao (2014) confirming them as two species based on ITS phylogeny (Fig. 1-6). Future research with multiple genetic regions regarding the identification of *Verticillium* species needs to be conducted and the inclusion of more isolates will aid taxonomic clarification (Inderbitzin et al. 2011). Such taxonomic clarification may also assist researchers to develop taxonomic keys to species using morphological differences and colony formation patterns on agar media (Inderbitzin et al. 2011).



1-6. Phylogenetic tree of species within the genus Verticillium based on ITS sequences from GenBank. The number of ITS sequences for each species are given in parenthesis. Excerpted from: Inderbitzin and Subbarao 2014.

1.5 Management Methods and Current Void

California's unique climate favors strawberry production, yet it also favors unwanted pathogens. Due to depletion of the ozone (Gemmill et al. 2013), the utilization of methyl bromide became less tolerant within the agricultural community and the public. Further, a 14% reduction in gross revenue was estimated for those using alternative methods to methyl bromide (USEPA 2009b). Therefore, the loss of methyl bromide as a management strategy necessitates examinations into possible alternatives, including the use of steam, solarization, and anaerobic soil disinfestation (ASD). The concept of anaerobic soil disinfestation and soil solarization had been previously investigated (Goud et al. 2004; Momma 2008) and these treatments became suitable for strawberry production, in some cases reducing *V. dahliae* in soil by 80-90% (Goud et al. 2004). Further, warm climates make soil solarization easily accessible to some growers, yet along the central coast of California, fog and lower summer soil temperatures render this practice unsuitable (Samtani et al. 2012). Therefore, the use of steam has been investigated, including its effects on pathogen suppression, yield, and rhizosphere microbial communities. These alternatives to fumigation are important for growers in organic settings or whose fields are within 35 m or less from schools and neighborhoods (Fennimore et al. 2014).

1.5.1 Pest Control with Steam

The investigation into steam as a possible pest control measure was first accomplished in 1893 (Johnson 1946) and has been used for decades to control soilborne pests in greenhouse soils and potting mixes (Holmes et al. 2020). Since then, multiple variables have been examined to access its efficacy as a control measure. An advantage

to the use of steam as an alternative is it lacks negative environmental and human health effects. Studies have reported that the use of steam has little to no lasting effect on soil quality or microbial communities (Norberg et al. 2001; Roux-Michollet et al. 2008). In contrast, studies have reported that there is a more significant change to soil microbe density after the use of steam (Tanaka et al. 2003; Yamamoto et al. 2008). These differences have been attributed to the duration of application and organic matter composition (Fennimore and Goodhue 2016). Effective steam disinfestation treatments for most pests such as pathogens, weeds, and insects require conditions that are heated to 65°C or greater for at least 30 minutes (Baker and Roistacher 1957; Fennimore and Goodhue 2016). Further, thermal depth curves (Bega and Smith 1962; van Koot and Wiertz 1947), soil moisture factors (Gay et al. 2010), and linear relationships between application length and pathogen death (Pullman et al. 1981) have also been investigated. Subbarao and Hubbard (1996) reported that constant temperatures of 35°C for 45 days reduced *V. dahliae* microsclerotia density by 70%, although this was not done in a strawberry crop setting.

Steam distribution in the soil is mainly accomplished by conduction, and the amount of steam needed to raise the temperature of soil to a certain threshold depends on soil factors such as texture, soil type, and moisture (Fennimore and Goodhue 2016; Minuto et al. 2003). There are also two different kinds of steam generators. Traditional steam boilers (Baker and Roistacher 1957) inject steam under pressure with use of a pressure valve containing water. Fire steam generators (Hoffman et al. 2015) utilize propane that acts as a heat source to warm water through a fueled flame. More recently, investigations into mobile steam applicators have been examined in strawberry

(Fennimore et al. 2014). This study reported adequate control of *Pythium* spp. and *M. phaseolina*, yet its effect on *V. dahliae* populations have not been examined in strawberry production. Although the use of steam as a pest control measure has promise, large-scale applications are hindered by fuel costs, speed, and labor (Melander and Jørgensen 2005). The increased adoption of steam disinfestation will be dependent on its cost and on fumigant availability (Hoffman et al. 2015). Adapting this practice into a production setting is challenging due to the need for expensive specialized machinery and the time and space required for treatment (Holmes et al. 2020). Therefore, other alternatives to fumigation such as soil solarization have been investigated, including its synergistic effects with steam (Samtani et al. 2012).

1.5.2 Pest Control with Soil Solarization

Soil solarization can be characterized as a passive approach to pest control. The use of soil solarization has been examined within the United States in states such as Arizona, California, Florida, North Carolina, Texas, and Virginia (Chellemi et al. 1994; Hartz et al. 1993; Samtani et al. 2017) and these studies report mixed effects. Soil solarization is accomplished by covering moist soil with clear, polyethylene tarp and allowing solar radiation to heat soil to temperatures lethal for soilborne pathogens (Porras et al. 2003). This technique has also shown effectiveness against weeds, bacteria, and nematodes (DeVay 1991; Katan 1981), and tolerance towards some saprophytic and antagonistic microbes such as *Bacillus* spp. and *Trichoderma* spp. (Stapleton and DeVay 1982; Munnecke and Van Gundy 1979). Further, this pest management technique has been effective against populations of *V. dahliae* at depths of 1.2 m (Ashworth and Gaona 1982). This management practice has shown mixed results against *V. dahliae*, with a

study in Spain reporting reduced populations of *V. dahliae* in the top 0.2 m of soil for an 8-week period (Lopez-Escudaro and Blanco-Lopez 2001). Further, if the soil temperature is maintained at 40-50°C for at least 4 to 6 weeks, *V. dahliae* populations can be eradicated (Katan 1983).

A study conducted in California showed that soil solarization returned similar yields compared to methyl bromide/chloropicrin treated plots, while only certain treatments controlled *V. dahliae* to a depth of 0.25 m (Samtani et al. 2012). This study also reported the synergistic effects between soil solarization and steam returned mixed results, with economic analyses showing net returns were less for crop treated with methyl bromide/chloropicrin. There are ways to conserve costs, such as using 25 μm polyethylene instead of 100 μm (Pullman et al. 1981) but its long-term effects are still being examined due to the long survivorship of *Verticillium* microsclerotia in soil (Schanathorst 1981). Repeated applications would be necessary, thus weakening the economic viability of this strategy (Samtani et al. 2012). For root rot pathogens such as *Phytophthora fragariae, Pythium* spp*., Rhizoctonia,* and *Cylindrocarpon* spp., adequate control can be maintained for at least two months following solarization (Pinkerton et al. 2002). The addition of soil solarization to an integrated pest management technique is complimentary towards other pest management programs (Chellemi et al. 1997) and its effectiveness towards *V. dahliae* has been further investigated in other crops.

The efficacy of solarization in pathogen management varies with different climate patterns. In Greece, *V. dahliae* causes severe losses to artichoke, and soil solarization has been shown to reduce natural populations of the pathogen and spare yield loss (Tjamos and Paplomatas 1988). Within the United States, soil solarization has reduced *V. dahliae*

populations in potato (Davis and Sorenson 1986) and in Italy, soil solarization reduced *V. dahliae* populations in eggplant by 40% (Tamietti and Valentino 2001). Although effective, this efficacy is largely influenced by weather. Cloud cover and lower temperatures reduce solar radiation and leads to less-effective management (Chellemi et al. 1994). Along California's central coast, coastal maritime layers that encroach the coast during the summer reduce the potential efficacy of solarization. Yet, together with biofumigation (Stapleton and DeVay 1986) or even the incorporation of *Brassica* residues such as cabbage or broccoli (Koron et al. 2014; Souza and Solarização 1994), the performance of soil solarization can be improved. Furthermore, there are alternatives to soil solarization that includes the utilization of anaerobic microbes.

1.5.3 Pest Control with Anaerobic Soil Disinfestation

Anaerobic soil disinfestation (ASD) is a non-chemical alternative to pest management. The suppression of soilborne pathogens under anaerobic conditions was first documented in 1983 (Cook and Baker 1983) and was investigated further in Japan with asparagus and onion (Blok et al. 2000; Shinmura 2000). ASD is implemented by introducing organic material, flooding the soil through irrigation, and covering the soil with gas impermeable plastic film (Hewavitharana et al. 2014). The potential applicability of ASD for pest control has been well documented (Hewavitharana et al. 2014; Momma et al. 2013, Shennan et al. 2011), however ASD alone as a non-fumigant practice is insufficient to adequately control disease (Zavatta et al. 2021).

In apple, the suppression of *Pythium* spp. using ASD has been documented (Hewavitharana et al. 2014); alternatively in strawberry, Shennan et al. (2011) first investigated ASD in California strawberry production but found challenge in the amount

of water needed to create anaerobic conditions. Further, ASD treatments with rice bran resulted in high incidence of Fusarium wilt compared to standard soil fumigation with Pic-Clor 60 (Mazzola et al. 2018). Yield increases in strawberry have been documented after ASD treatments, yet it did result in complete disease control (Subbarao et al. 2007; Zavatta et al. 2021). More investigations into ASD for control of Verticillium wilt are required to ensure consistency and applicability for long-term crop rotation strategies. Main factors hindering adoption of this practice include cost and the separation of numerous optimum factors such as soil pH, temperature, and treatment duration (Holmes et al. 2020).

1.5.4 Pest Control with Biological Agents

Biological control for pest management is a non-chemical alternative to pest control. Several rhizobacteria and their ability to suppress fungal diseases has been well documented (Emmert and Handelsman 1999; Whipps 1997). In strawberry, greenhouse trials utilizing *Serratia plymuthica* as an antagonistic fungal bacterium have reported decreases in disease incidence (Kurze et al. 2001). Beneficial root-associated bacteria such as *S. plymuthica* produce hydrogen cyanide in the root zone, which has shown natural inhibition of plant disease (Raaijmakers et al. 2002). The utilization of Pseudomonad bacteria to suppress *V. dahliae* has been examined, yet the addition of bacteria to the rhizosphere did not decrease *V. dahliae* fungal populations in a field setting (DeCoste et al. 2010). Field studies examining the efficacy of biological control agents to control *V. dahliae* in strawberry have reported inconsistent results (Berg et al. 2000; Mercado-Blanco et al. 2004). For example, a strain of *Bacillus subtilis* showed suppression of both *V. dahliae* and *Fusarium oxysporum* f. sp. *fragariae* (Zhang et al.

2012) but a study in 2006 reported no significant reduction in plant protection utilizing *Bacillus* spp. (Tahmatsidou et al. 2006). More studies are needed to examine biological control agents and their ability to suppress fungal pathogens and disease incidence rates.

1.6 Bed Fumigation

Preplant soil fumigation has widely been used by strawberry growers and in annual production systems, becoming a contemporary pest management practice. Broad spectrum fumigants such as methyl bromide, 1,3-dichloropropene, chloropicrin, metam potassium, and metam sodium have been used to control pests such as root pathogens, weeds, and nematodes (Chammoro et al. 2016). A synergistic effect between methyl bromide and chloropicrin made applications effective (Wilhelm and Paulus 1980) yet the phaseout of methyl bromide has left chloropicrin as a primarily used fumigant. Heavy limitations have been put on fumigant use, including the phaseout of methyl bromide and the introduction of township caps in some growing areas (Carpenter et al. 2001). In recent years, soil fumigation with mixtures of chloropicrin and 1,3-dichloropropene have filled the void left by methyl bromide, yet this shift has led to the emergence of new soilborne pathogens (Koike et al. 1994).

1.6.1 Fumigation with Chloropicrin

Chloropicrin (trichloronitromethane) was first introduced to control *V. dahliae* in California and has strong fungicidal and nematocidal properties (Wilhelm and Paulus 1980). Due to the more efficacious properties of methyl bromide, chloropicrin was pushed to the side, yet in the 1960s growers started to mix chloropicrin with methyl bromide at various ratios (Wilhelm 1966). The synergistic activity of these two fumigants

showed excellent control of *V. dahliae* in soil (Wilhelm et al. 1961) and the practice became standard for California strawberry growers.

In 1997, chloropicrin as a stand-alone fumigant was investigated. Broadcast fumigation with chloropicrin at 140 liters/A presented 94-96% similar yields to those fumigated with a mixture of chloropicrin and methyl bromide (Duniway et al. 1997). Further, the effect of the fumigation did not diminish, even with rotation with lettuce. Klose et al. (2007) demonstrated that application of InLine (1,3-dichloropromene 61% and chloropicrin 33%) at 1,636 mol kg^{-1} was sufficient at killing >90% of major weed seeds, yet an application of 2,735 mol kg^{-1} was needed to kill 90% of *V. dahliae* in the soil. Due to varying concentrations of pathogens in the soil, *V. dahliae* may require the simultaneous or sequential application of another fumigant. These findings were confirmed by Slusarski and Spotti (2016) as to levels of *V. dahliae* in pepper cultivation. They concluded that chloropicrin application rates of 30 and 40 gm^{-2} provided acceptable control of *V. dahliae*. Moreover, the average reduction in pathogen density reached about 86% and was higher than the efficacy of drip-applied chloropicrin used at similar rates. Although these findings strengthen the effectiveness of chloropicrin as a stand-alone fumigant, studies conducted along California's central coast show inconsistencies. In one study, bed fumigation with chloropicrin at 90.6 or 113.3 liters/A provided yields 12% less than in the standard methyl bromide/chloropicrin control (Larson 1998). A survey conducted in 1999 showed an average yield loss of 9.6% with use of chloropicrin as a stand-alone fumigant (Shaw and Larson 1999). Chloropicrin's effectiveness as a stand-alone fumigant provides use for the control of pests, yet the application of chloropicrin with metam sodium in a field setting demonstrated adequate

control of *V. dahliae* with comparable yields to the application of methyl bromide with chloropicrin (Trout and Ajwa 1999; 2004). This multitactical application of chloropicrin, together with other fumigants, has shown promise in the control of soilborne pathogens.

1.6.2 Fumigation with Metam Sodium and Metam Potassium

Metam sodium (sodium N-methyl dithiocarbamate, VAPAM) has been used since the 1950s and is an effective soil fumigant due to its rapid degradation into methyl isothiocyanate (MITC; Kreutzer 1963). MITC is the active agent of metam sodium in soil, and provides significant control against fungi, Oomycota, nematodes, and weeds (Kreutzer 1963). Use of metam sodium has been limited since it is not very mobile in soil. The utilization of a drip application that facilitates the movement of the fumigant has been examined, yet results show that an application of 113.3 liters/A gave about half of the yield increase as compared to the standard fumigation with methyl bromide and chloropicrin (Shaw and Larson 1999; Trout and Ajwa 1999). Further, metam sodium has not shown effectiveness when mixed with other fumigants such as chloropicrin. In 2000, one study showed that shank applications of chloropicrin at 113.3 liters/A when followed two weeks later with metam sodium via drip did not provide strawberry yields equivalent to the standard methyl bromide/chloropicrin application (Larson and Shaw 2000). MITC can also be found in another product such as metam potassium.

Metam potassium (potassium N-methyldithiocarbamate, KPAM) is a commonly used soil fumigant, especially in the Florida strawberry production industry. The use of metam potassium requires the consideration of proper replanting intervals. Desaeger et al. (2008) demonstrated that it took approximately three to four weeks for the fumigant to

dissipate from the soil bed. Further, applications of metam potassium have shown to be efficacious at lower labeled rates.

When examining the efficacy of metam potassium to reduce soilborne pathogens, Chamorro et al. (2016) demonstrated that the application of metam potassium had similar effects to that of methyl bromide in reducing pathogen survivability. An application rate of 226.8 liters/A was adequate in killing *M. phaseolina*. When applied in a drip irrigation method, it provided statistically similar reduction rates compared to that of methyl bromide. This is important because due to the high efficacy of methyl bromide, it is the standard by which all new fumigants are measured.

1.7 Crop Termination

Crop termination, or crop kill, is used to reduce pathogen inoculum build-up and dissemination by rapidly killing infected strawberry plants at the end of a season using herbicides or soil fumigants (Holmes et al. 2020). This practice has also been utilized by growers to kill weeds and facilitate plastic removal (Palhano et al. 2018). The utilization of paraquat in later method does not provide suppression for soilborne diseases like Verticillium wilt (Noling 2006). The use of soil fumigants such as metam sodium and metam potassium are alternatives to traditional termination with paraquat since it provides broad spectrum control of pests and limits worker exposure to pesticides (Khatri et al. 2018). However, published literature on crop termination for strawberry is limited.

1.7.1 Termination with AITC

The utilization of crop termination by Chellemi et al. (2017) was accomplished using allyl-isothiocyanate (AITC, Dominus) with an application rate of 62.7 liters/A through a drip system. Use of AITC-derived products is beneficial since it

occurs naturally in crops such as broccoli and Brussels sprouts and is associated with suppression of Verticillium wilt (Harborne and Baxter 1993). Both crops are commonly found along the central coast of California. In one study, crisp head lettuce was grown following broccoli, and reintroduction of this crop led to an increase in microsclerotia density from <1.0 to >8.06 g^{-1} of soil in the area subject to rotation only. In the area receiving AITC crop-termination, microsclerotia remained below 1.0 g^{-1} of soil (Chellemi et al. 2017). Since AITC-derived products occur in the Brassicaceae family as a natural defense component, AITC as a stand-alone fumigant can replace expensive summer cover cropping and reduce pathogen survivability in the soil (Baggio et al. 2018). Multitactical applications of AITC has also been examined.

Rather than applications as stand-alone fumigants, investigation into the multitactical approach has shown that AITC as a stand-alone treatment leads to inconsistent pathogen control (Hoffmann et al. 2020). Yet, AITC combined with higher rates of chloropicrin is a promising way to reduce pathogen populations and increase yield. Hoffmann et al. (2020) concluded that chloropicrin and AITC application controls Verticillium wilt when applied through a drip irrigation system. AITC in combination with chloropicrin resulted in 0 microsclerotia per gram of soil, whereas AITC in both high and low rates of application did not provide as much reduction in pathogen density, resulting in 21.0 and 1.0 microsclerotia per gram, respectively. The soil characteristics of the Central and Southern coasts of California do not demonstrate consistent efficacy with a standalone treatment of AITC (Hoffmann et al. 2020). Yet, if AITC is combined with another fumigant, such as chloropicrin, the results show sufficient pathogen control.

1.7.2 Termination with Metam Sodium and Metam Potassium

The use of metam sodium in a crop termination setting to control *M. phaseolina* was conducted in Florida with a series of high and low rates of the fumigant (Khatri et al. 2018). Rates between 78.9 and 473.5 liters/A provided 100% crop mortality at 14 days after fumigation (DAF) with 99 to 100% control of *M. phaseolina* inoculum at a depth of 0.8 m (Khatri et al. 2018). Rates between 26.3 and 131.5 liters/A provided >96% crop mortality at 14 DAF and *M. phaseolina* inoculum was 100% controlled across all beds (Khatri et al. 2018).

The use of metam potassium as a crop-terminating chemistry was also examined. An application of metam potassium at a rate of 26.3 liters/A is adequate to kill strawberry plants and the movement of the fumigant throughout the bed can be facilitated with high application rates of roughly 161.9 liters/A (Khatri et al. 2020). For example, plant injury 7 days after drip irrigation of metam potassium at rates of 26.3 to 131.5 liters/A showed <94% crop injury at 7 days after application. Further, all rates resulted in >90% crop injury at 14 days after application. These results contradict the general belief that metam potassium cannot control pests and pathogens across the entire bed due to limited movement. Owing to the high-water solubility of metam potassium, it is well-suited for drip-irrigation (Chamorro et al. 2016). This is important since drip irrigation delivers the fumigant near the plant roots.

1.8 Host Resistance

Host resistance plays an important role in an integrated management system and is considered the most effective and sustainable control method for crown and root diseases in strawberry (MacKenzie et al. 2006; Shaw et al. 2005). The search for non-

chemical disease management alternatives came to the forefront after the phaseout of methyl bromide (Holmes et al. 2020). Investigations into Verticillium wilt resistance have been successful in other hosts such as lettuce (Hayes et al. 2011) and tomato (Fradin et al. 2009), where race-specific resistant (R)-genes have been discovered. Yet, R-genes conferring resistance in strawberry are yet to be identified (Vining et al. 2015).

Efforts into resistance screening for *V. dahliae* in strawberry show high variation in germplasm resistance (Holmes et al. 2016; Shaw et al. 1997) and the high frequency of moderately to highly susceptible cultivars around the world exemplify challenges of genetic resistance investigations in strawberry (Govorova and Govorov 1997; Pincot et al. 2020). Heirloom cultivars and wild ecotypes may serve as strong novel sources for resistance to Verticillium wilt (Pincot et al. 2020), but the harboring of undesirable alleles (Mundt 2018; Poland and Rutkoski 2016) deliver uncertainty. A significant technical challenge faces investigations into Verticillium wilt resistance in strawberry, and current rating of available cultivars and breeding lines are necessary to integrate resistance into industry practices.

1.9 Conclusion and Objectives

An integrated approach to pathogen control is required for both contemporary and future agricultural production regions around the globe. In strawberry, the phaseout of methyl bromide has necessitated investigations into both chemical and non-chemical management methods. Standard fumigation practices such as bed fumigation and alternative fumigation practices such as crop termination may play an important role in a grower's pathogen management regimen. Host resistance will certainly become an

important tool for strawberry production as the uncertainty faced with fumigation applications highlights the importance of investigations into host resistance.

In field settings where high disease incidence due to *V. dahliae* have been identified, terminating the crop may decrease the amount of pathogen going back into the soil and help decrease soil inoculum. Pre-plant bed fumigation may also help decrease soil inoculum, where the utilization of a resistant cultivar may also help decrease disease incidence and increase yields. The integration of these management methods has been examined.

My first objective was to evaluate integrating a standard-practice fumigation application method, bed fumigation, with an end-of-season crop termination fumigation application. Understanding the pathogen life cycle is imperative and I can target stages in the cycle where I can inhibit pathogen reproduction and survival. I aim to target the production of microsclerotia in the crop tissue by employing a crop termination. The goal with crop termination is to eradicate pathogen microsclerotia and cease reproduction in the infected crop tissue. For residual microsclerotia in the soil, I employed bed fumigation to lower the pathogen propagules and reduce future disease. The integration of a resistant cultivar after the fumigation series was also examined, and disease incidence rates, mortality, and yield differences were analyzed. Two replicated field trials were established to determine the efficacy of soil inoculum reduction, the degree of injury induced on the crop due to termination, as well as the odds of *V. dahliae* survival in the terminated crop. The efficacy of different fumigants and the presence or absence of significant differences were also investigated.

My second objective was to determine the susceptibility of strawberry cultivars and elite breeding lines to Verticillium wilt. A two-year, replicated field trial was conducted to evaluate 74 genotypes for their resistance to Verticillium wilt. These results, along with results from other research, will help breeders advance characterizing resistance genes and help growers utilize publicly available information.

CHAPTER 2: EVALUATING THE INTEGRATION OF CROP TERMINATION, BED FUMIGATION, AND HOST RESISTANCE IN MANAGING VERTICILLIUM WILT OF STRAWBERRY

2.1 Abstract

Crop termination using metam potassium and metam sodium is being investigated as part of an integrative pest management solution to control Verticillium wilt of strawberry, caused by *Verticillium dahliae*. A field trial was conducted to assess the efficacy of crop termination, bed fumigation, and the integration of a resistant cultivar to alleviate the threat *V. dahliae* poses to production. Crop termination with metam potassium and metam sodium significantly reduced soil inoculum and pathogen survival in crop tissue, compared to non-treated controls. Furthermore, the sequential applications of crop termination and bed fumigation decreased soil inoculum of *V. dahliae* but did not attain the level below the disease threshold of 1.9 CFU/g of soil for strawberry. A significant reduction in plant mortality and an increase in yield was found for all treatments versus the non-treated control. Plots that were sequentially subjected to crop termination and bed fumigation resulted in lower plant mortality and higher yield in comparison to plots that were only subjected to either crop termination or bed fumigation. The sequential applications of crop termination at end of season and bed fumigation at the beginning of the season could provide adequate reduction of Verticillium wilt in fields that are heavily infested with *V. dahliae*.

2.2 Introduction

Strawberry (*Fragaria* × *ananassa,* Duch.) is an important crop in California, with more than 35,000 acres planted in 2018 resulting in a farm gate value of $3.1 billion (CDFA 2018). In 2020, California strawberry production accounted for more than 85% of national strawberry production, with main production occurring in fumigated soils due to a variety of soil-borne diseases that affects strawberry production (Njoroge et al. 2009). Within the state, 12.1% of total acreage is in organic systems (California Strawberry Commission 2022). Pre-plant fumigation has been a predominant method for managing soilborne pathogens since the 1950s. Predictably, diseases caused by soilborne pathogens have been become numerous after the phaseout of methyl bromide (MeBr; Klose et al. 2007). Increasing restrictions and regulations on fumigants due to perceptions of harm to public health and the environment (Gemmill et al. 2013; UNEP 2006) necessitates investigations into alternative control methods. Since the phaseout of MeBr, strawberry growers have transitioned to alternative broad-spectrum fumigants. These fumigants have shortcomings due to their intrinsic chemical properties that limit movement through the soil (both lateral and vertical) and into lignified tissues (Baggio et al. 2018; Chamorro et al. 2016; Porter et al. 2004). With this, management strategies to reduce inoculum in the soil and in plant tissues and debris necessitates investigation.

Verticillium wilt of strawberry, caused by *Verticillium dahliae* Kleb. (Wilhelm 1955) was first described in California in 1931 (Thomas 1931) and affects many high value cropping systems in the world, such as lettuce, strawberry, and tomato (Bhat and Subbarao 1999; Lopez-Escudero and Mercado-Blanco 2011). *V. dahliae* can survive in soil as microsclerotia, and these structures can persist for up to 14 years without a host

(Wilhelm 1955). These dark-colored, resistant, structures can also survive in strawberry crowns, and be released into the soil upon decomposition (Baggio et al. 2021). Due to the wide host range of *V. dahliae*, certain crop rotation strategies enable the pathogen to increase (Mihail 1992). Upon contact with strawberry roots, the *V. dahliae* microsclerotia germinate and colonize the xylem, resulting in plant collapse and death (Beckman and Roberts 1995). Control of Verticillium wilt in strawberry is important for the continued economic viability of the California strawberry industry due to its impact on plant health, yield, and revenues (Zavatta et al. 2021).

Due to the loss of MeBr as a disease control tool, as well as the long-term survival of *V. dahliae* in soil, chemical and nonchemical approaches for disease management should be investigated. Crop termination, end-of-season crop burn down or crop kill, has been utilized by strawberry growers at end-of-season to kill weeds and facilitate plastic removal (Palhano et al. 2018). The utilization of paraquat in this method does not provide control of soilborne pathogens like *V. dahliae* due to its lack of fungicidal properties (Noling 2006). The use of soil fumigants such as metam sodium (sodium N-methyldithiocarbamate, VAPAM) and metam potassium (potassium N-methyldithiocarbamate, KPAM) are alternatives to traditional termination with paraquat since it provides broad spectrum control of pathogens while also killing the crop (Khatri et al. 2020). In strawberry, crop termination has been investigated, although the use of crop termination combined with bed fumigation in a strawberry system to control *V. dahliae* is yet to be investigated. Crop termination was utilized by Chellemi et al. (2017) using allyl-isothiocyanate (AITC, Dominus) through a drip system. Allyl-isothiocyanate occurs naturally in crops such as broccoli and Brussels sprouts and is associated with

suppression of Verticillium wilt (Harborne and Baxter 1993). Further, AITC combined with higher rates of chloropicrin is a promising way to reduce pathogen populations and increase yield. Hoffmann et al. (2020) concluded that a chloropicrin and AITC application controls Verticillium wilt when applied via drip irrigation.

The utilization of metam products like metam potassium in a crop termination application has been adequate to kill strawberry plants and reduce *M. phaseolina* inoculum. The movement of the fumigant throughout the bed can also be facilitated with high application rates (Khatri et al. 2020). These results contradict the general belief that metam potassium cannot control pests and pathogens across the entire bed due to limited movement (Chamorro et al. 2016). Owing to the high-water solubility of metam potassium, it is well-suited for drip-irrigation (Chamorro et al. 2016). When examining the efficacy of metam potassium to reduce soilborne pathogens, Chamorro et al. (2016) demonstrated that the application of metam potassium had statistically similar pathogen reduction rates to that of methyl bromide in reducing *M. phaseolina* survivability when applied in a drip irrigation method.

MITC is the active ingredient of metam sodium in soil, and provides significant control against fungi, oomycetes, nematodes, and weeds (Kreutzer 1963). Metam sodium has been used since the 1950s due to its rapid degradation into methyl isothiocyanate (MITC; Kreutzer 1963). Use of metam sodium has been limited since it is not very mobile in soil. Application via drip irrigation facilitates the movement of the fumigant, yet results show that an application of 113.3 liters/A gives about half of the yield increase as compared to fumigation with methyl bromide and chloropicrin (Shaw and Larson 1999; Trout and Ajwa 1999). Further, metam sodium has not shown effectiveness when

mixed with other fumigants such as chloropicrin (Larson and Shaw 2000) but it has shown the ability to suppress *M. phaseolina* at a depth of 8 cm in a strawberry bed compared to control plots (Khatri et al. 2018).

Preplant soil fumigation, specifically bed fumigation, has widely been used by strawberry growers and in annual production systems, becoming a common disease management practice. Broad spectrum fumigants such as methyl bromide, 1,3-dichloropropene, chloropicrin, metam potassium, and metam sodium have been used to control root pathogens, weeds, and nematodes (Chammoro et al. 2016). A synergistic effect between methyl bromide and chloropicrin made applications highly effective (Wilhelm and Paulus 1980) yet the phaseout of methyl bromide has left chloropicrin as a stand-alone fumigant. In recent years, soil fumigation with mixtures of chloropicrin and 1,3-dichloropropene have partially filled the void left by methyl bromide, yet this shift has led to increased incidence of soilborne pathogens (Koike et al. 1994).

Intelligent integration of non-chemical approaches for disease management is necessary to sustain strawberry production in California (Holmes et al. 2020). Investigations into genetic resistance came to the forefront and became more attractive after the phaseout of MeBr (Miles et al. 2018). Resistance to Verticillium wilt in strawberry is under complex control of multiple genes (Antanaviciute et al. 2015) and a wide range of susceptibility has been recorded among 90 different strawberry cultivars and elite breeding lines (Holmes et al. 2016). Other investigations into host resistance showed positive correlation between fruit firmness and resistance to Verticillium wilt (Bringhurst et al. 1968) and slowed progress in breeding for resistance. Investigations into Verticillium wilt resistance has been successful in other hosts such as lettuce (Hayes

et al. 2011) and tomato (Fradin et al. 2009), where race-specific resistant (R)-genes have been discovered. Yet, R-genes conferring resistance to Verticillium wilt in strawberry are yet to be identified (Vining et al. 2015).

Therefore, the main objective of this study was to evaluate the effectiveness of crop termination, bed fumigation, and the integration of genetic resistance in reducing inoculum of *V. dahliae* in soil and lowering future disease incidence. The objectives were (i) to assess the efficacy of crop termination alone, bed fumigation alone, and the sequential applications of these methods to reduce *V. dahliae* soil inoculum and lower future disease incidence, (ii) to compare the efficacy of different fumigant chemicals, (iii) to determine the survival of *V. dahliae* in terminated crops as well as the degree of crop injury due to termination, and (iv) to evaluate the integration of a resistant cultivar after fumigation applications to lower future disease incidence.

2.3 Materials and Methods

2.3.1 Crop Termination and Bed Fumigation

To assess the efficacy of crop termination alone and sequential bed fumigation on *V. dahliae*, a field trial was established during the 2020-2021 growing season at California Polytechnic State University, San Luis Obispo, field 25, block 6 (35°30’ N, 120°67’ W). Soil in the trial site, characterized as clay loam (36% sand, 26% silt, 38% clay), had a pH of 7.2, electrical conductivity of 2.2 dS/m, cation exchange capacity of 20.5 meq/100g, and an organic matter composition of 2.9% (A & L Western Agricultural Laboratories, Modesto, CA). Prior to planting, lettuce (cv. Black Seeded Simpson) was broadcast in June 2020 to increase pathogen inoculum and the ground was disked horizontally and vertically and beds shaped in September 2020. A total of 12 industry

standard beds were used. Each bed measured 1.1 m between row centers, 0.3 m high, and 90 m in length. Plants were separated by 0.2 m. Beds were formed on 14 October 2020. Bare-root strawberry transplants of cultivar Seascape were established on 2 November 2020 in four rows of plants per bed and three lines of 0.5 mm drip-tape spaced 0.4 m apart and at a depth of 1.5 cm (Toro Ag, El Cajon, CA). A total of 465 plants were established in each bed. Irrigation, fertilization, and the management of other pests were conducted according to industry standards. For crop termination, fumigants metam potassium (AMVAC, Newport Beach, CA) and metam sodium (AMVAC, Newport Beach, CA) were utilized whereas for bed fumigation metam potassium, metam sodium, and chloropicrin (Tri-Clor EC, TriCal, Hollister, CA) were utilized (Table 2-1). There were more plots terminated with metam potassium than metam sodium since plots assigned chloropicrin could not be terminated with this chemistry and thus were assigned metam potassium.

To assess the integration of a resistant genotype after the fumigation series, subplots were divided into sub-sub plots and randomly assigned either 'Seascape' (susceptible) or 'Valiant' (resistant). A total of 78 plants were established in each sub-sub plot.

Soil inoculum density of *V. dahliae* was determined seven days before transplanting. A total of ten soil samples were collected per bed in a "zig-zag" pattern at 0.3 m depth using a 0.03 m soil-core, with samples separated by 3 m. Soil samples were pooled, air-dried for two weeks, pulverized with a mortar and pestle, and 0.1 g of soil in ten replicates were plated on a semiselective media, Sorenson's NP-10 (Sorensen et al. 1991), with use of an Andersen sampler (Andersen 1958). Colony forming units (CFU)

were counted using a dissecting microscope at 10 × (Olympus, Tokyo, Japan) magnification after dark incubation for two weeks at ambient temperature.

The experiment was a split-split plot design with a whole plot treatment of fumigant and a subplot treatment of application method. Each bed consisted of three plots (30 m per plot) with 210 plants per plot. Each subplot was characterized as either receiving only crop termination, only bed fumigation, or both. Three non-treated control beds were included. The description of the applications and treatments are provided in Table 2-1. Crop termination was applied 238 days after transplanting on 28 June 2021 when the crop experienced 5% mortality and 45% symptom expression. Bed fumigation was applied on 18 October 2021, 112 days after termination and 15 days before transplanting of the next strawberry crop.

Following crop termination, plant injury was visually assessed 7 and 14 days after fumigation (DAF). A total of 100 plants from the middle 15 m of each subplot were visually assessed on a 0-4 scale (Table 2-3) where 0 = no mortality and 4 = 100% mortality. Further, *V. dahliae* survival in the terminated crop was examined at 7 and 14 DAF. Petiole and crown tissue of a total of ten plants from the middle 15 m of each subplot were sampled, surface disinfected, and plated on NP-10 semi-selective medium, incubated in darkness at ambient temperature for 10-14 days and observed under a dissecting microscope for *V. dahliae* microsclerotia. Infection of both petiole and crown tissue was recorded.

Soil was sampled 10 days after crop termination and 14 days after bed fumigation from each subplot and within the middle 15 m of the plot near the outer and inner drip

lines. Control beds were also sampled from the middle 15 m of the bed. Soil inoculum density of *V. dahliae* was determined as mentioned previously.

2.3.2 V. dahliae Molecular Identification

A total of 15 isolates of putative *V. dahliae* were selected for species identification with molecular techniques. Genomic DNA extraction was performed on the isolates following the protocol of Bok and Keller (2012) with modifications. The ITS region was PCR amplified with primers ITS1 and ITS4 (White et al. 1990) following the thermal cycles: an initial denaturation at 95°C for 3 min, followed by 36 cycles of 95°C for 30 s, 54°C for 45 s, and 72°C for 45 s, and a final extension at 72°C for 5 minutes. PCR products were then purified with ExoSAP-IT Express (Applied Biosystems) and sequenced at MCLAB (San Francisco, CA). Sequences were aligned using ClustalW in BioEdit v7.0.5.3 (Hall 1999) and the ITS region of the 15 isolates appeared to be identical. The sequence was searched with BLAST in NCBI for species identification. Then the ITS region of one isolate was deposited in GenBank with the accession number is OP131837. Based on BLASTn searching results, the ITS region of the 15 isolates obtained in this study was identical with isolates identified as *V. dahliae* in GenBank.

2.3.3 Yield Analysis

Plots were harvested during peak yield by picking all ripe fruit twice each week during a three-week period (23 May 2022 to 9 June 2022). Fruit was harvested, placed in trays, and weighed to measure the total amount of fruit picked. Following, fruit was then sorted into marketable and unmarketable and weighed. Marketable fruit was defined as ripe fruit large enough (> 21 mm) to meet market acceptance, and free of disease, bruising or misshapen.

2.3.4 Performance of Resistant and Susceptible Genotypes after Fumigation

Strawberry cultivars Seascape (susceptible) and Valiant (resistant) were established after the fumigation series to assess performance against *V. dahliae*. Mortality assessments were conducted bi-weekly following transplanting on 2 November 2021. Symptomatic plants were uprooted, and petioles plated on a semi-selective medium, Sorensen's NP-10 (Sorensen et al. 1991). Final mortality counts were conducted on 27 July 2022, 267 days after transplanting.

2.3.5 Statistical Analyses

Infection of crown and petiole tissue of *V. dahliae* after termination was analyzed using Kruskal-Wallis with a post-hoc Dunn test. Soil inoculum density and plant injury data was analyzed with Kruskal-Wallis test, followed by a nonparametric analysis with treatment means separated using the pairwise statement with Wilcoxon signed-rank test adjustment at $P = 0.05$. Pathogen survival in crop tissue data was analyzed with a multilevel logistic regression. Marketable yield was subject to a split-split plot analysis with an agricolae package. Following, a least significant difference (LSD) post-hoc test at $P = 0.05$ was conducted. All data analyses were conducted using R (version 1.2.5003, R Core Team, Vienna, Austria).

2.4 Results

2.4.1 Pathogen Density in Soil after Crop Termination.

The overall pre-plant soil inoculum density of *V. dahliae* was determined to be 7.0 ± 2.4 CFU/g. Following crop termination, soil inoculum density significantly decreased in beds treated versus those that were not (Fig. 2-1; $X^2 = 19.5$, df = 2, $P \leq 0.001$). In control beds, inoculum density increased from 7.0 ± 2.4 CFU/g to 15.0 ± 2.7

CFU/g. In plots terminated, inoculum density decreased from 7.8 ± 2.4 CFU/g to 5.1 ± 0.7 CFU/g. Furthermore, there was no significant in pathogen density for plots treated with metam potassium and metam sodium ($P = 0.73$); there was significant difference in pathogen densirt between plots treated with metam potassium and the control ($P \leq 0.001$) as well as metam sodium and the control ($P \leq 0.001$). There was no significant difference in pathogen density between the outer and inner plant rows (Fig. 2-2; $P = 0.91$).

2.4.2 Crop Injury Due to Fumigant

The overall average plant injury rate for 7 and 14 days after fumigation (DAF) was 77% and 89.4%, respectively. There was no significant difference in the efficacy of metam potassium and metam sodium on crop injury 7 DAF ($X^2 = 0.24$, df = 1, $P = 0.62$). After 14 days, the most effective fumigant was metam potassium in terms of inducing injury to the crop (Table 2-2; $X^2 = 8.31$, df = 1, $P = 0.0039$).

2.4.3 Pathogen Survival in Fumigated Plants

With all other predictors held constant, the log-odds of *V. dahliae* survival are four times less in crops terminated with metam potassium and metam sodium than crops not terminated (Fig. 2-2). In the unterminated crop, there is no significant difference between the likelihood of *V. dahliae* survival in crown versus petiole ($\beta = 0.20$, $Z = 0.34$, $P = 0.73$). In crop terminated with metam potassium, there is a significant difference between likelihood of *V. dahliae* survival in crown and petiole, with the pathogen more likely to survive in the petiole ($\beta = 1.36$, $Z = 0.52$, $P = 0.00$). In crop terminated with metam sodium, the same results apply, i.e. *V. dahliae* is more likely to survive in the petiole than the crown ($\beta = 1.66$, $Z = 0.61$, $P = 0.00$). Over time, there was a significant difference in *V. dahliae* survival at 7 DAF and 14 DAF, with 14 DAF having a

significantly lower level of *V. dahliae* survival ($\beta = 2.0$, $Z = 0.49$, $P < 0.001$). There was no significant difference between the efficacy of the two fumigants over time ($\beta = 0.57$, $Z = 0.41$, $P = 0.17$). There was a significant difference in *V. dahliae* survival in crop tissue over time, with the pathogen more likely to survive in the petiole than the crown ($\beta = 1.17$, $Z = 0.44$, $P = 0.00$).

2.4.4 Pathogen Density in Soil after Bed Fumigation

Soil inoculum density did not significantly decrease in beds treated versus the control (Fig. 2-4, 2-5; $\beta = 0.88$, $Z = 0.50$, $P = 0.07$). During the fumigation period, the inoculum density in control plots increased from 15.0 ± 2.7 CFU/g before the crop termination treatment to 19.0 ± 4.9 CFU/g after the bed fumigation treatment (Fig. 2-3). In plots only crop terminated, inoculum density increased from 5.1 ± 0.7 CFU/g after the crop termination treatment to 8.2 ± 0.8 CFU/g after the bed fumigation treatment. In plots only bed fumigated, inoculum density decreased from 12.1 ± 1.4 CFU/g before the bed fumigation treatment to 8.9 ± 0.2 CFU/g after the bed fumigation treatment. In plots sequentially terminated and fumigated, inoculum density increased from 5.4 ± 0.3 CFU/g after the crop termination treatment to 6.4 ± 0.7 CFU/g after the bed fumigation treatment (Fig. 2-4, 2-5). Bed fumigation with chloropicrin resulted in significantly lower inoculum densities than bed fumigation with metam potassium or metam sodium ($\beta = -0.82$, $Z = 0.36$, $P = 0.02$). There was a significant difference in the pathogen inoculum distribution after bed fumigation; the outer plant lines resulted in a lower pathogen density than the inner lines (Fig. 2-4; $X^2 = 5.14$, $df = 1$, $P = 0.02$).

2.4.5 Yield Analysis

2.4.5.1 Year 1

The mean yield for beds planted to ‘Seascape’ before any fumigation treatment was 17.4 kg over the 3-week harvest assessment. A total of 465 plants were established in each bed, with an average yield of 0.04 kg per plant.

2.4.5.2 Year 2

Significant increases in yield were found for all treatments versus the non-treated control (Fig. 2-8; $F = 33.7$, $df = 3$, $P < 0.001$). In the non-treated control plots, ‘Seascape’ produced a total of 63.4 kg of fruit per plot, versus ‘Valiant’ which produced a total of 49.4 kg of fruit per plot. Each plot consisted of 78 plants. In plots that were only crop terminated, ‘Seascape’ produced a total of 109.0 kg of fruit per plot, versus ‘Valiant’ which produced a total of 98.9 kg of fruit per plot (Fig. 2-8). Within plots only crop terminated, no significant difference in total yield was found between the plots crop terminated with metam potassium and plots crop terminated with metam sodium ($P = 0.40$) (Fig. 2-9). Within plots only bed fumigated, ‘Seascape’ produced a total of 99.8 kg of fruit per plot, versus ‘Valiant’ which produced a total of 121.1 kg of fruit per plot (Fig. 2-10). Within plots only bed fumigated, no significant differences in yield were found between plots bed fumigated with metam potassium, plots fumigated with metam sodium, and plots bed fumigated with chloropicrin ($P = 0.88$). Furthermore, plots that were subject to both crop termination and bed fumigation that were planted to ‘Seascape’ produced 129.2 kg of fruit per plot versus plots that were planted to ‘Valiant’ which produced 133.7 kg of fruit per plot (Fig. 2-8).

Within plots sequentially crop terminated and bed fumigated, significant differences were found between fumigants applied. In plots that were subject to crop termination with metam potassium and bed fumigated with chloropicrin, yield was significantly higher versus plots that were subjected to crop termination with metam sodium and a bed fumigation with metam sodium ($P = 0.03$). Furthermore, plots that were subjected to crop termination with metam potassium and bed fumigated with metam potassium were not significantly different versus plots crop terminated with metam potassium and fumigated with chloropicrin ($P = 0.08$). These results also apply to plots that were subject to crop termination with metam sodium and bed fumigated with metam sodium; no significant differences were found ($P = 0.15$) (Fig. 2-11).

2.4.6 Plant Mortality Assessment

Significant decreases in plant mortality were found in treated plots versus non-treated control plots ($F = 22.4$, $df = 3$, $P < 0.001$). Within non-treated control plots, average plant mortality was 67.2% (Fig. 2-12). Within plots subjected to crop termination with metam potassium and metam sodium, average plant mortality was 35.9% and 39.3%, respectively (Fig. 2-13). There was no significant difference in plant mortality for plots crop terminated with metam potassium and metam sodium ($P = 0.55$).

Within plots subjected to only bed fumigation, average plant mortality was 28.2%. There were no significant differences between plots bed fumigated with metam sodium, metam potassium, and chloropicrin ($P = 0.40$).

Within plots subjected to both crop termination and bed fumigation, average plant mortality was 24.1% (Fig. 2-12). Plots that were crop terminated with metam potassium and bed fumigated with metam potassium had average plant mortality of 30.6%. Plots

that were crop terminated with metam potassium and bed fumigated with chloropicrin had average plant mortality of 15.4%. Plots that were crop terminated with metam sodium and bed fumigated with metam sodium had average plant mortality of 26.5% (Fig. 2-13). No significant differences in plant mortality were found between plots sequentially crop terminated with metam potassium and bed fumigated with metam potassium, plots sequentially crop terminated with metam potassium and bed fumigated with chloropicrin, and plots sequentially crop terminated with metam sodium and bed fumigated with metam sodium ($P = 0.15$).

2.4.7 Performance of Resistant and Susceptible Genotypes after Fumigation

No significant differences in plant mortality between 'Seascape' and 'Valiant' were found across all treatments ($F = 1.0$, $df = 4$, $P = 0.42$). Within non-treated control plots, 'Seascape' and 'Valiant' had average plant mortality of 54.7% and 65.3%, respectively (Fig. 2-12). Within plots that were only subjected to crop termination, 'Seascape' and 'Valiant' had average plant mortality of 34.9% and 39.2%, respectively. Within plots that were subjected to only bed fumigation, 'Seascape' and 'Valiant' had average plant mortality of 29.8% and 26.6%, respectively. Within plots subjected to both crop termination and bed fumigation, 'Seascape' and 'Valiant' had average plant mortality of 21.1% and 27.2%, respectively (Fig. 2-12).

2.5 Discussion

Khatri et al. (2020) established that metam products such as metam potassium when applied via drip irrigation in a crop termination application provided adequate crop kill, suppression of *M. phaseolina* at bed center, and control of various weeds such as purple nutsedge. Furthermore, Chamorro et al. (2016) demonstrated that the application

of metam potassium had similar effects to that of methyl bromide in reducing pathogen survivability yet was not effective against *M. phaseolina* inoculum at the edge of the bed. Investigations into crop termination as a disease-suppressive tool has limited application; thus, our study investigating the integration of crop termination, bed fumigation, and host resistance demonstrates that an integrative approach to soilborne disease control is necessary in cropping systems faced with high inoculum density and disease pressure. This study demonstrates that sequential fumigation applications of crop termination and bed fumigation does not result in complete eradication of *V. dahliae* from the soil but does increase yield and lower mortality compared to non-treated control plots. Furthermore, corroborating previous studies mentioned above, our study illustrates adequate eradication of pathogen survival structures in crops subject to termination and could provide reduction in pathogen survival structures being released into soil upon plant decomposition.

Although crop termination with metam potassium and metam sodium significantly reduced *V. dahliae* density in the soil, it was insufficient in reducing levels to below the disease threshold for strawberry. Further, the remaining propagules that were not eradicated between applications increased, and bed fumigation was not sufficient to eradicate the remaining population. These findings are consistent with work reported by Baggio et al. (2021); remaining populations of *M. phaseolina* in soil also increased after crop termination and bed fumigation was not sufficient to completely eradicate the pathogen. The lack of full control of *V. dahliae* could be attributed to the degree to which the crop was infested at the time of crop termination. In our study, 45% of our crop showed symptom expression before the termination, with 5% of the crop

being completely dead. Since plants that are completely necrotic and dead do not actively uptake fumigants, *V. dahliae* propagules inhabiting the crop tissue was thus not eradicated and was released into the soil upon decomposition of the plant material. In plots that were not subject to crop termination, we see that inoculum density increased before bed fumigation, but inoculum levels were then reduced with bed fumigation to comparable levels in plots that were subject to only crop termination. These oscillations in pathogen density can be attributed to redistribution of microsclerotia in the soil profile due to tillage practices, which corresponds with findings from Chellemi et al. (2017).

For plots that were subject to both crop termination and bed fumigation, inoculum levels were somewhat controlled, but not to a level that is below the threshold for strawberry. The overall pre-plant inoculum density for *V. dahliae* in the experimental plots was 7.0 ± 2.4 CFU/g and at the end of the fumigation series, plots that were sequentially fumigated resulted in an inoculum density of 6.44 ± 0.73 CFU/g. These findings show *V. dahliae* inoculum densities remaining somewhat constant even with sequential crop termination and bed fumigation. In this study, the integration of other disease management practices like crop rotation were not implemented. In addition to crop termination, crop rotation with broccoli and the applications of crab/feather meal has shown to bring microsclerotia densities down to 1.0 CFU/g (Chellemi et al. 2017) and thus fumigation practices alone may not be suitable for adequate control of *V. dahliae*. In comparison to the non-treated control plots, inoculum density rises drastically (19.0 ± 4.9 CFU/g), but statistical analyses show no significant differences compared to fumigated plots. Plots terminated with metam sodium and then fumigated with metam sodium resulted in a significantly higher inoculum density of *V. dahliae* than plots terminated

with metam potassium and fumigated with chloropicrin which shows a possible synergistic effect of metam potassium and chloropicrin. A synergistic effect between methyl bromide and chloropicrin has been reported (Wilhelm et al. 1961; Wilhelm and Paulus 1980) and these findings show synergistic effects between chloropicrin and other fumigant products may occur.

Plots that were subjected to only bed fumigation showed different inoculum densities in the outer plant rows in comparison to the inner plant rows (Fig. 2-4), but these levels were not significantly different from each other. Candole et al. (2007) showed that in sandy soil beds, highest metam product fumigant concentrations were found 20 cm below the centrally located drip tape emitter and was lowest at 30 cm away from the emitter. Our findings correspond with these studies, for our inoculum density was higher away from the centrally placed drip tape even with our clay loam soil type. Although, our study utilized three lines of drip tape per bed providing greater fumigant dispersal than two lines. Second, after crop termination, experimental beds were tilled by hand, plastic and drip were also laid by hand, and this could explain the higher inoculum densities in the outer plant rows; while we were cognizant to not move soil from the furrows to the beds, this is a plausible reason for the higher inoculum levels in the outer plant rows. The difference in inoculum density in the outer plant rows was found across all fumigant types, and no statistical differences were identified. Thus, we conclude that the fumigants were not uniformly distributed, and the result of hand tillage could have led to increased inoculum density in the outer plant rows.

Plots bed fumigated with chloropicrin resulted in significantly lower *V. dahliae* inoculum density than metam potassium, but there was no significant difference between

chloropicrin and metam sodium. There was no significant difference between metam potassium and metam sodium in decreasing soil inoculum in a bed fumigation setting, which suggested that these two fumigants had similar chemistries and abilities to suppress pathogen populations. Chloropicrin has been shown to provide effective control of *V. dahliae* in strawberry systems when applied via drip irrigation (Wilhelm and Koch 1956) and our study also illustrates that chloropicrin provides adequate control. Metam products have been shown effective in control of *M. phaseolina* (Baggio et al. 2021; Khatri et al. 2020) and our study is the first to illustrate adequate control of *V. dahliae* with metam products.

Significant differences in visual plant injury assessments were found at 14 DAF, but not at 7 DAF. At 14 DAF, crops terminated with metam potassium had significantly higher plant injury levels compared to crops terminated with metam sodium. It is known that as the plants are subject to the fumigant and lack of irrigation over time, injury is suspected to be higher at 14 DAF. Khatri et al. (2020) demonstrated 100% crop injury at 14 DAF with high fumigant rates of metam potassium (195-975 kg ha) and >90% crop injury at 14 DAF with lower fumigant rates (65-325 kg ha). For our trial, metam potassium was applied at 439.6 kg/ha and metam sodium was applied at 523.8 kg/ha. These rates were based upon the labelled rates for a crop termination application, in accordance with local and state regulations. Our study also demonstrates that fumigant rates between 400-600 kg/ha were adequate to provide >90% crop injury at 14 DAF. Although economic analyses have not been conducted, identifying sufficient rates of fumigant inputs in future research is an important consideration for growers, for if less

fumigant is needed to provide crop injury, this could result in less of an economic burden to growers as well as the environment.

Although visual assessments of crop injury are adequate for evaluating the effects of fumigants in a crop termination application, whether the pathogen survived inside the terminated crop tissue is also something that needs to be examined. Our study suggests that the odds of pathogen survival at 14 DAF is roughly two to three times lower compared to 7 DAF. This is an important consideration, for growers need to be cognizant of re-plant intervals and the allowance for the fumigants to terminate the crop accordingly. The survival of *V. dahliae* in the terminated crop tissue correlates visually to assessments of plant injury, both at 7 DAF and 14 DAF. We find that pathogen survival is less likely at 14 DAF, and that the pathogen is more likely to survive in the petioles of the terminated crops in comparison to the crowns. As the plants actively uptake the fumigants, we assume that the crop tissue would be destroyed and translocation of the fumigants to other parts of the plant such as the petioles and leaves may be hindered. Therefore, survival of *V. dahliae* in the petiole may lead to an increase in inoculum for the next season. The integration of other disease management techniques like crop rotation may be able to alleviate inoculum pressure (Chellemi et al. 2017) before strawberry is planted again. These findings further illustrate that fumigation alone may not be sufficient for complete control of *V. dahliae*.

It is known that Verticillium wilt symptoms develop rather quickly within infected strawberry plants, as the pathogen produces conidia that move upwards in the xylem of the plant tissue after one or more cortical infections (Gordon and Subbarao 2008). As the plant declines, *V. dahliae* can grow more extensively in the water

conducting tissue and produce microsclerotia in the decomposing crop residue. Since the fumigants applied in a crop termination setting relies on the translocation through water conducting tissues, crops with more severe infections or increased symptoms could have less movement of the fumigants through the plant tissues. As mentioned, the crop in our field showed 5% mortality and 45% symptom expression before being subjected to termination; terminating the crop at lower symptom expression rates could lead to more plant injury and less pathogen survival in plant tissue. The timing of crop termination is important for growers due to monetary concerns, for the grower is not likely to kill the crop prematurely for the sake of pathogen control. Along the California Central Coast, peak harvest occurs in May to July (Bolda et al. 2021) and the timing of crop termination with peak harvest would be a big concern for growers. Growers prefer to wait until end-of-season for crop termination. This waiting period could increase inoculum and give pathogen momentum for successive seasons.

Dependent on the cultivar being used in the cropping system, disease symptoms and infection may be slower to develop in less susceptible cultivars (Gordon and Subbarao 2008) compared to more resistant cultivars. ‘Seascape’ was used in this study due to its high susceptibility to *V. dahliae* (California Strawberry Commission 2018), and infection could happen more quickly compared to other more resistant cultivars. This factor also plays a role in the efficacy of crop termination, for more resistant cultivars could show less disease progression compared to susceptible cultivars. Examining the efficacy of termination in other cultivars besides ‘Seascape’, especially in highly used cultivars like ‘Monterey’ (California Strawberry Commission 2018) could lead to differences in pathogen survival and the efficacy of termination to suppress

pathogen reproduction. ‘Valiant’ has been shown to be relatively resistant to *V. dahliae* (California Strawberry Commission 2018), but our study illustrates otherwise. Due to the lack of a fully resistant cultivar to *V. dahliae* (Vining et al. 2015), the integration of host resistance and fumigation does not seem to be an adequate option to lower mortality and increase yield. Further investigations are needed for the discovery of resistant cultivars to *V. dahliae*.

Although sequential applications of crop termination and bed fumigation resulted in decreases in inoculum density, sufficient reduction of *V. dahliae* from the soil was not achieved. One would expect that lower inoculum levels would lead to an increase in yield and lower mortality rates. Our yield analysis before fumigation was applied is not comparable to yield found after the fumigation series. We established our crop in four rows per bed with ‘Seascape’ and at the end of the fumigation series we established our crop in two rows per bed with ‘Seascape’ and ‘Valiant’. The decrease in plant rows was due to the loss of shoulders from the beds after hand-tillage. Thus, yield before the fumigation series is not comparable with yield after the fumigation series.

The yield data analysis shows that similar yields can be achieved with only a bed fumigation in comparison to subjecting the cropping system to both crop termination and bed fumigation. The economical aspect of this is important; growers could save costs if numerous fumigation series are not effective. A crop termination application alone does not result in comparable yields to sequentially fumigated plots; we hypothesize that crop termination plays a role in decreasing inoculum pressure, whereas bed fumigation helps eradicate surviving structures that may lead to crop mortality. During the first season, a sequential fumigation application may not show notable

benefits, but our study shows that four-times less inoculum goes back into the soil following crop termination. This could result in the gradual decrease of inoculum over time, and the integration of other disease management techniques may assist this decrease in *V. dahliae* inoculum and reduce future disease in strawberry systems.

Comparable yields between 'Seascape' (susceptible) and 'Valiant' were achieved across all treatments. Cultivars inherently have yield differences, not just due to resistance or susceptibility to a specific pathogen. 'Valiant' has been described to provide higher yields in low-input and organic production systems as well as producing fruit earlier than other cultivars like 'Monterey' and 'Cabrillo' (Knapp et al. 2021). The ability to produce fruit earlier may lead to increases in yield before *V. dahliae* development and could play a role in alleviating the negatives of disease pressure in organic fields infested with *V. dahliae*. 'Seascape' is shown to produce fruit three months after planting (Bringhurst and Voth 1991) and may produce more or less fruit than 'Valiant' dependent on the timing of harvest. For our study, peak harvest was conducted at the same time for 'Valiant' and 'Seascape' and peak harvest times and yield potential may be different, thus comparison of yields may be limited due to physiological factors controlled by cultivar genetics. In non-treated control beds, 'Valiant' resulted in lower yields compared to 'Seascape' and the same applies for plots subjected to only crop termination. We expect that a moderately resistant cultivar like 'Valiant' would result in higher yields, yet that was not the case. For example, 'Valiant' produces much bigger fruit compared to 'Seascape' and if the crop dies, this would result in a lower total fruit weight. In plots that were subject to only bed fumigation and sequential applications of crop termination and bed fumigation, 'Valiant' resulted in

higher yields compared to ‘Seascape’. This could be due to lower pathogen pressure, for inoculum densities were higher at the planting interval in plots subjected to only crop termination. The efficacy of integration of a resistant cultivar on increased yield should be further assessed to evaluate the performance in a cropping system infested with *V. dahliae*.

Disease incidence levels for plots only crop terminated correspond with Baggio et al. (2021) where failure to sufficiently reduce *V. dahliae* from soil resulted in continued disease. Strawberry is extremely sensitive and vulnerable to infection by *V. dahliae*, with levels as low as 1-2 CFU/g of soil being sufficient for plant infection (Harris and Yang 1996). Since sufficient reduction of *V. dahliae* was not achieved, we see that even low levels of inoculum correspond to Verticillium wilt development, which suggests a very low inoculum-infection threshold as reported by Hartz et al. (1987). For example, in plots sequentially crop terminated and bed fumigated, inoculum density was found to be 6.4 ± 0.7 CFU/g (Fig. 2-4) and average plant mortality was found to be below 20% (Fig. 2-12). At this time, no correlation between inoculum density and mortality has been examined, yet further investigations into these correlations is needed to fully understand the interactions between inoculum density and the integration of resistant cultivars.

Proper management of Verticillium wilt can possibly be achieved with the reduction of *V. dahliae* populations in strawberry cropping systems. This study focused not only on the adequacy of inoculum reduction in the soil but reducing the build-up of *V. dahliae* survival structures before plant incorporation. Further, the applications of an end-of-season fumigation practice like crop termination, together with a pre-plant fumigation

practice like bed fumigation could aid growers in decreasing plant mortality and increasing yield in cropping systems with high disease pressure. The integration of resistant cultivars also reduces mortality, yet further investigations are needed to examine the selection of cultivars after fumigation applications. With focus on reducing inoculum already present, as well as preventing inoculum build-up due to successive strawberry crops, integrating different approaches for control of Verticillium wilt would also provide favorable conditions for plant success.

Table 2-1. Applications and treatments applied to soil by drip irrigation during the 2020-2021 and 2021-2022 growing seasons in San Luis Obispo, CA.

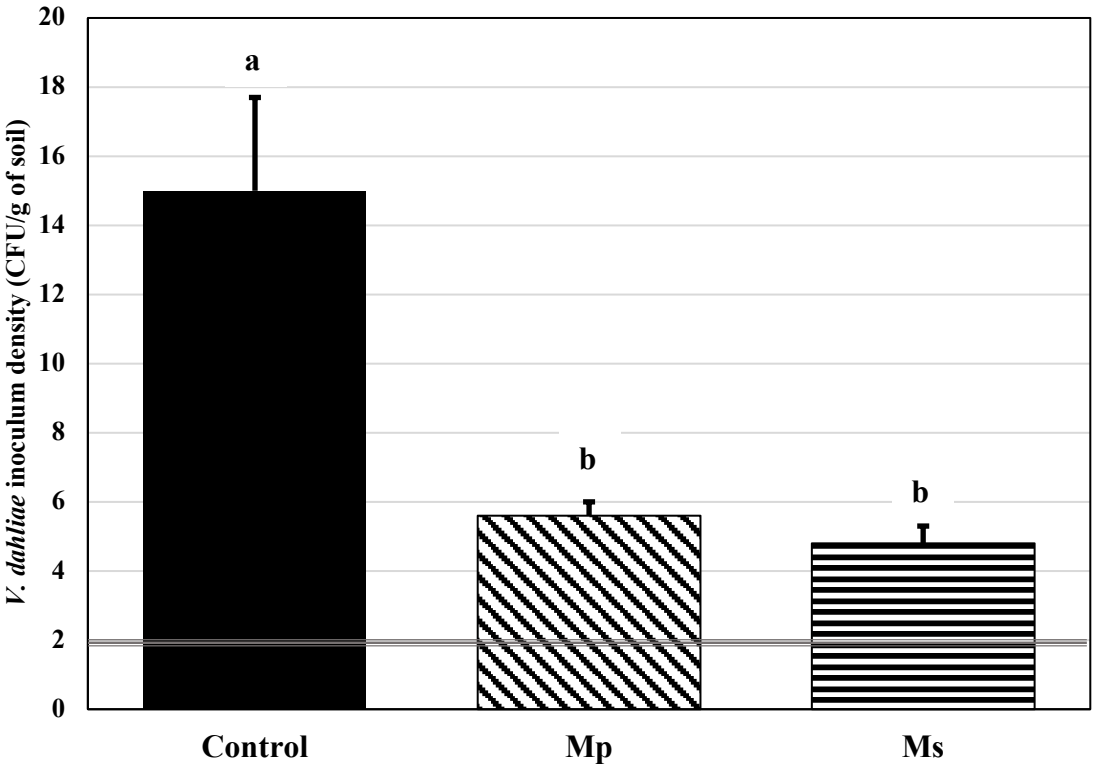
Application	Treatment	Active Ingredient(s)	Rate (L/ha)
crop termination (CT)	metam potassium	potassium *N*-methyldithiocarbamate (KPAM)	439.6
	metam sodium	sodium methylthiocarbamate (VAPAM)	523.8
bed fumigation (BF)	metam potassium	potassium *N*-methyldithiocarbamate	580.0
	metam sodium	sodium methylthiocarbamate	701.6
	chloropicrin	chloropicrin (Tri-Clor)	203.0
crop termination + bed fumigation (CT + BF)	metam potassium + metam potassium	potassium *N*-methyldithiocarbamate + potassium *N*-methyldithiocarbamate	439.6 + 580.0
	metam sodium + metam sodium	sodium methylthiocarbamate + sodium methylthiocarbamate	523.8 + 701.6
	metam potassium + chloropicrin	potassium *N*-methyldithiocarbamate + chloropicrin	439.6 + 203.0

Table 2-2. Fumigant efficacy against *V. dahliae* in host tissue 7 and 14 days after fumigation (DAF). % positive indicates samples showing *V. dahliae* miscrosclerotia on a semi-selective medium, NP-10.

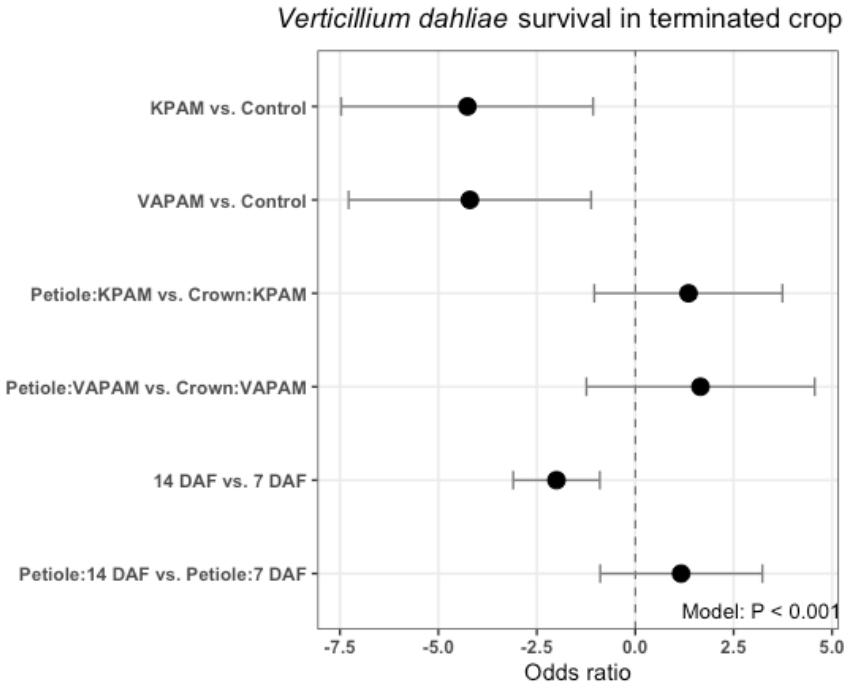
	Overall (% positive)		Metam potassium (% positive)		Metam sodium (% positive)	
	Petioles	*Crowns*	*Petioles*	*Crowns*	*Petioles*	*Crowns*
7 DAF	30.8	7.5	28.8	7.2	34.7	7.5
14 DAF	19.7	1.7	20.7	2.2	17.7	0.7

Table 2-3. Crop injury visual evaluation rubric following crop termination treatments.

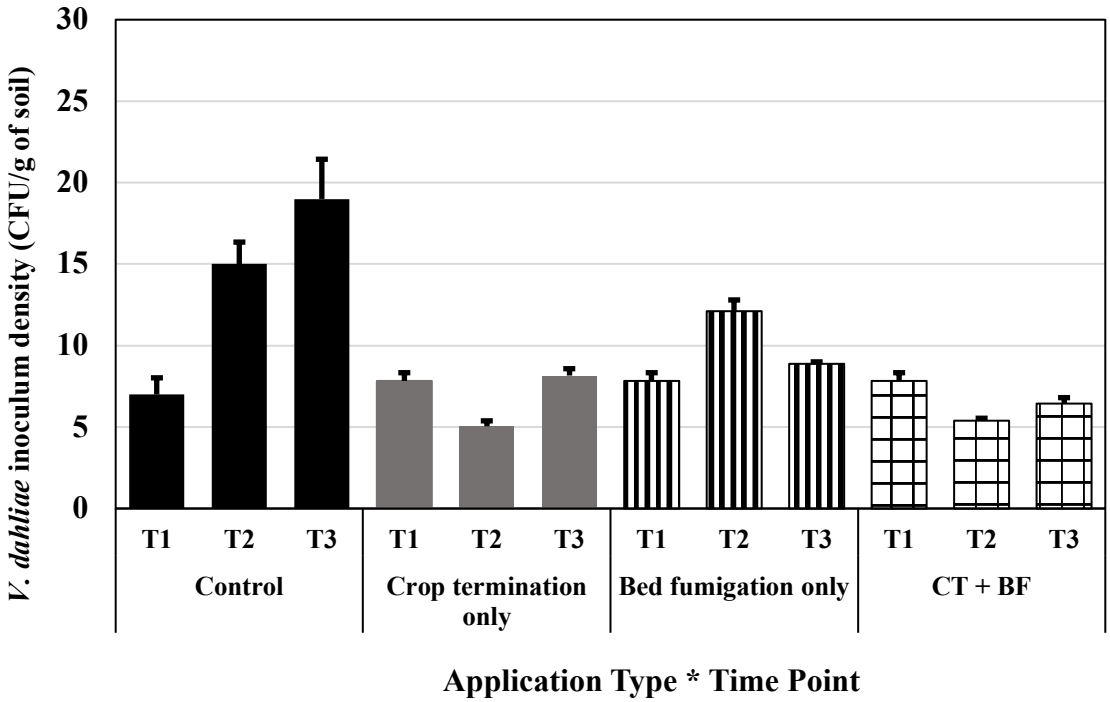
Score	Description
0	0% mortality, no wilting, no necrotic plant parts
1	25% mortality, minimal wilting, half of plant parts not necrotic
2	50% mortality, complete wilting, half of plant parts necrotic
3	75% mortality, complete wilting, plant parts necrotic except apical meristem
4	100% mortality, complete wilting, all plant parts necrotic



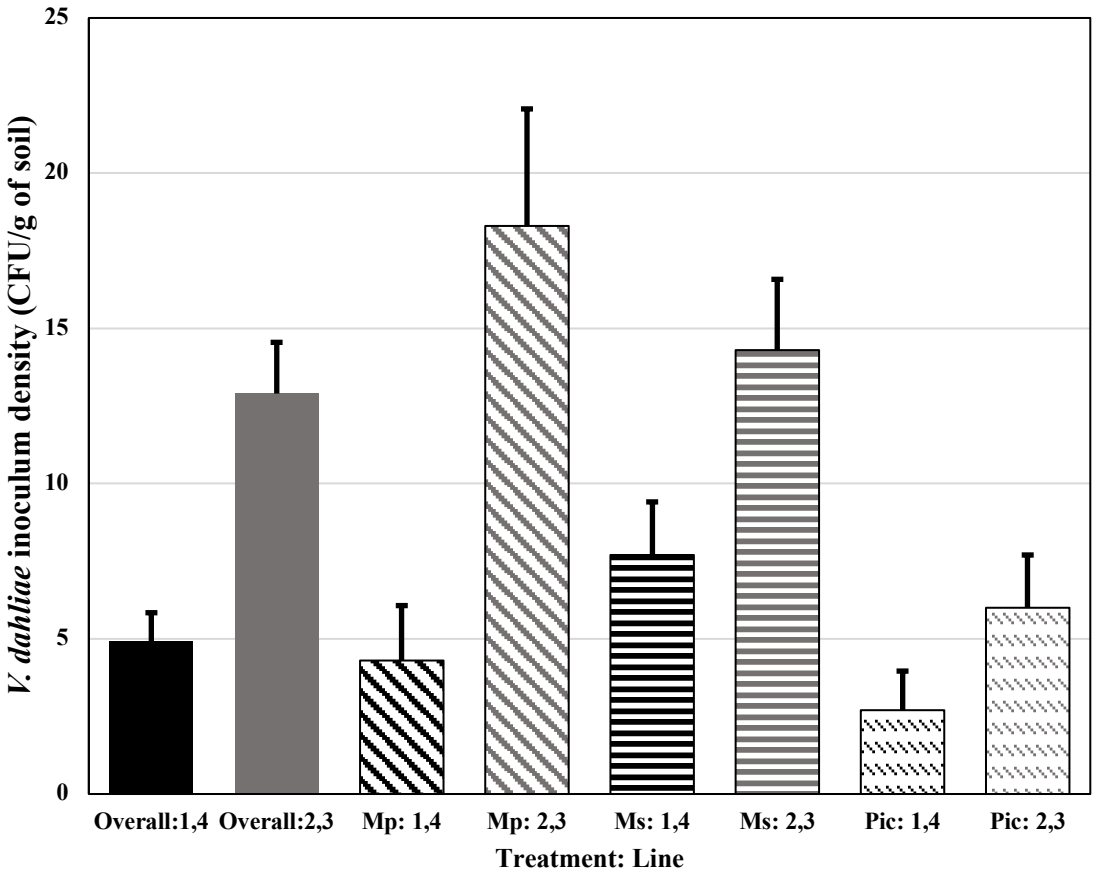
2-1. Soil inoculum density of *V. dahliae* after crop termination. Horizontal gray line denotes minimum disease threshold for strawberry (2.0 CFU/g). Control = no treatment; Mp = metam potassium; Ms = metam sodium. The letters denote Kruskal-Wallis, Wilcoxin signed-ranks ($P < 0.001$). Error bars represent standard error of the means.



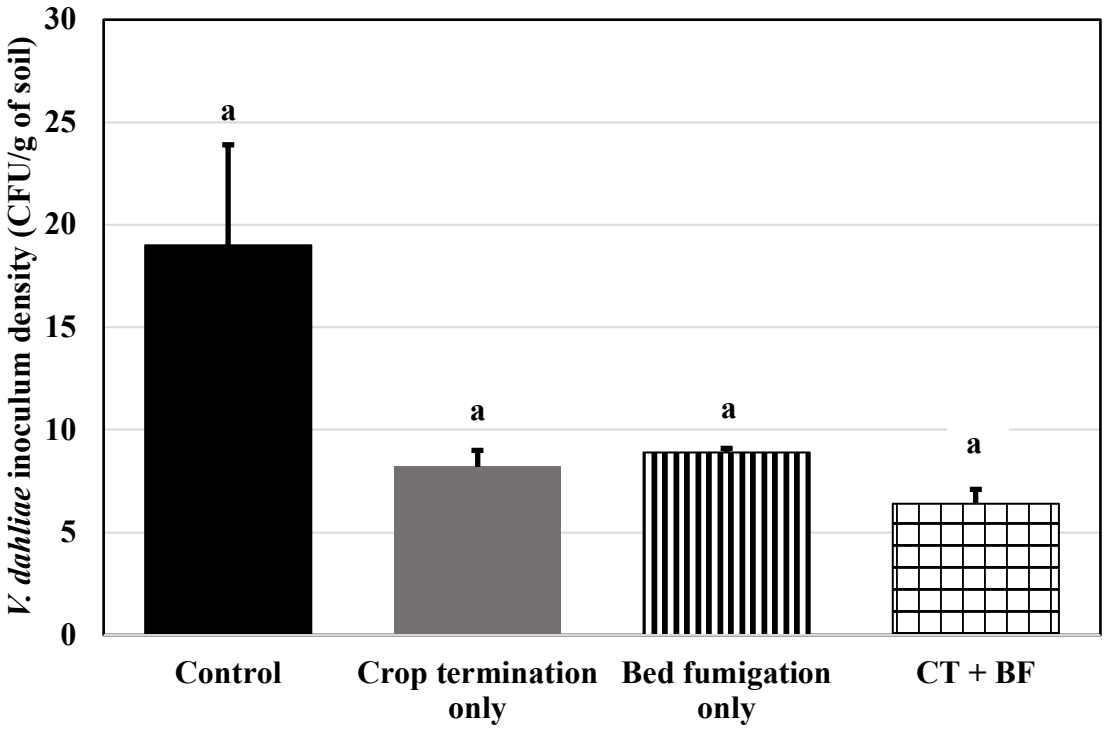
2-2. The log-odds ratio of *V. dahliae* survival in terminated crop. With all other predictors held constant, the odds ratio represents the likelihood of pathogen survival after a particular exposure (crop termination). Kruskal-Wallis, Wilcoxin signed-ranks ($P < 0.001$).



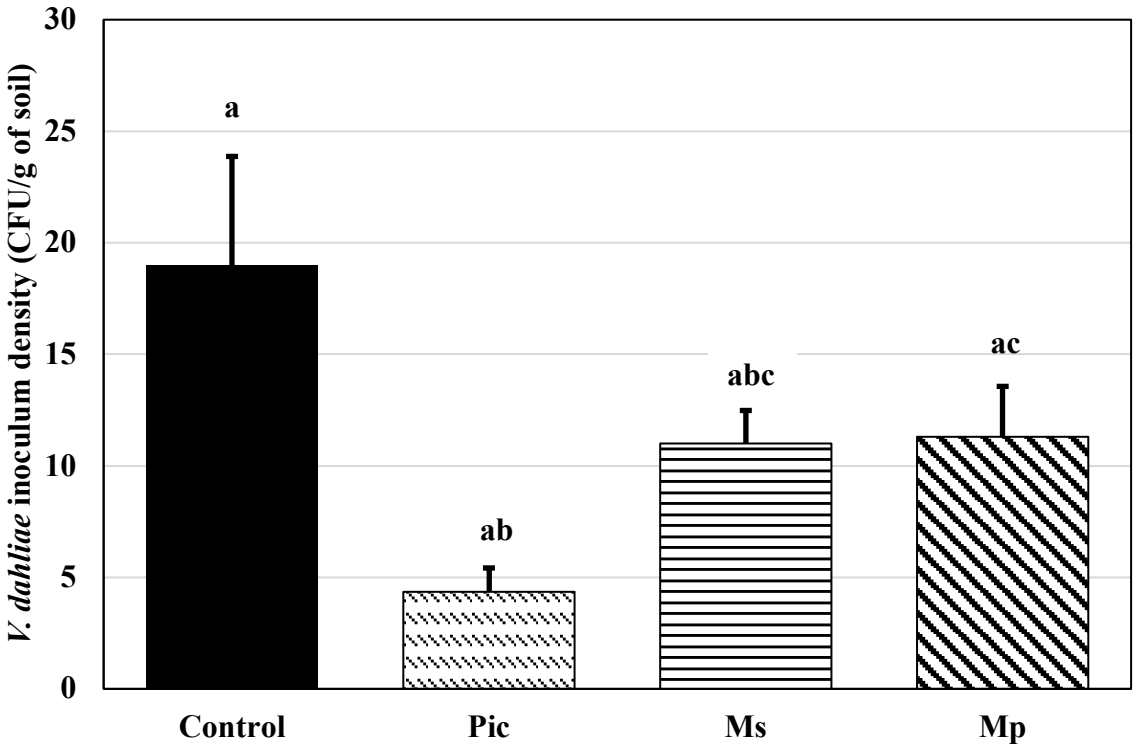
2-3. Soil inoculum density of *V. dahliae* at a pre-plant (T1), post crop termination (T2), and post bed fumigation (T3). Error bars represent standard error of the means.



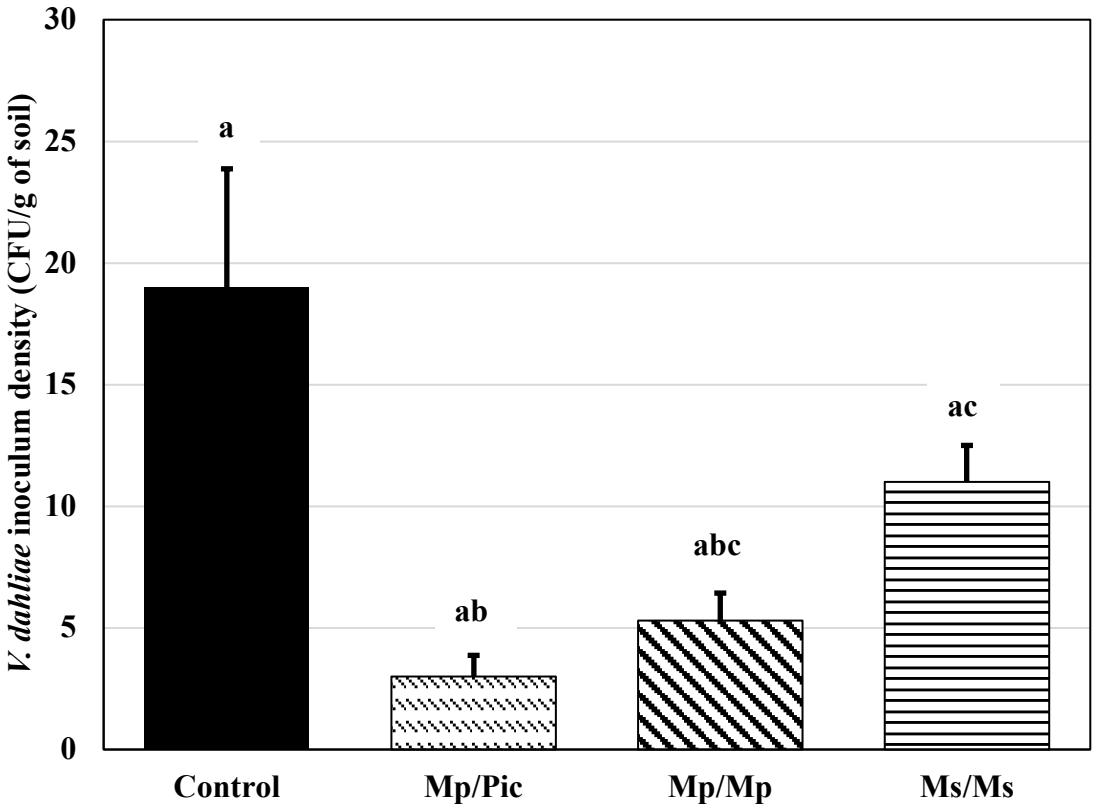
2-4. Inoculum density of *V. dahliae* by drip irrigation line following bed fumigation. There was no significant difference between inoculum density in the outer and inner plant rows ($P = 0.91$). 1,4 = outer plant lines; 2,3 = inner plant lines. Overall = all treatments; Mp = metam potassium; Ms = metam sodium; Pic = chloropicrin. Error bars represent standard error of the means.



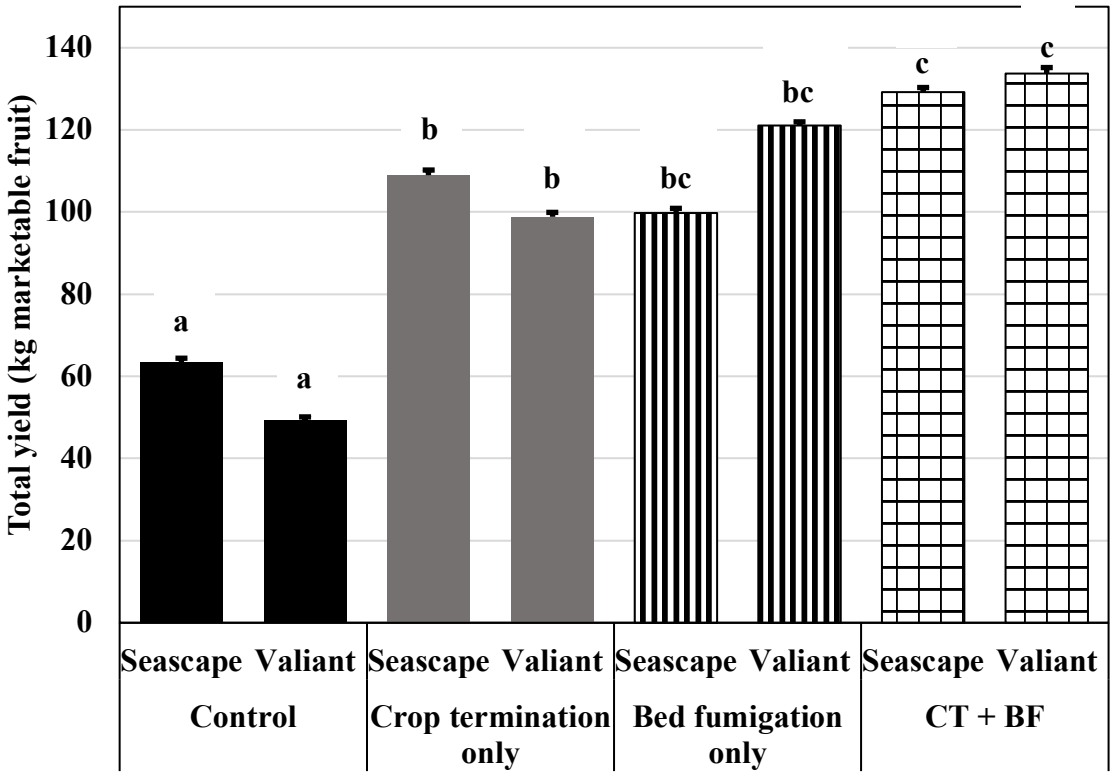
2-5. Soil inoculum density of *V. dahliae* for all treatments at the conclusion of the fumigation series. Negative Binomial Regression (α= 0.05). Control = no treatment. Error bars represent standard error of the means.



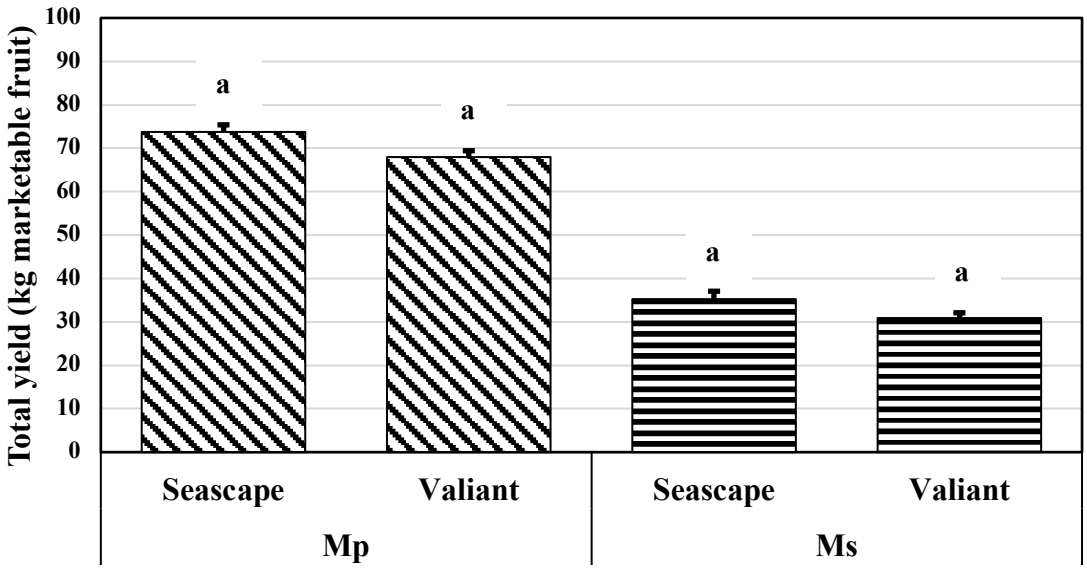
2-6. Soil inoculum density of *V. dahliae* for plots only bed fumigated. Control = no treatment; Pic = chloropicrin; Ms = metam sodium; Mp = metam potassium. Negative Binomial Regression (α= 0.05). Error bars represent standard error of the means.



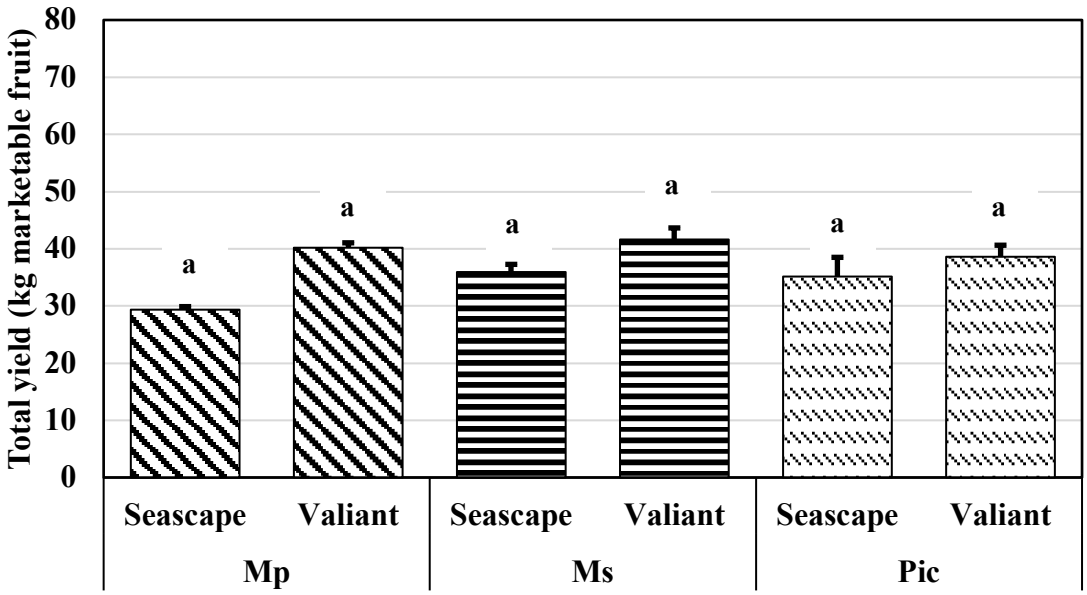
2-7. Plots sequentially terminated and fumigated. Control = no treatment; Mp/Pic = metam potassium followed by chloropicrin; Mp/Mp = metam potassium followed by metam potassium; Ms/Ms = metam sodium followed by metam sodium. Negative Binomial Regression (α= 0.05). Error bars represent standard error of the means.



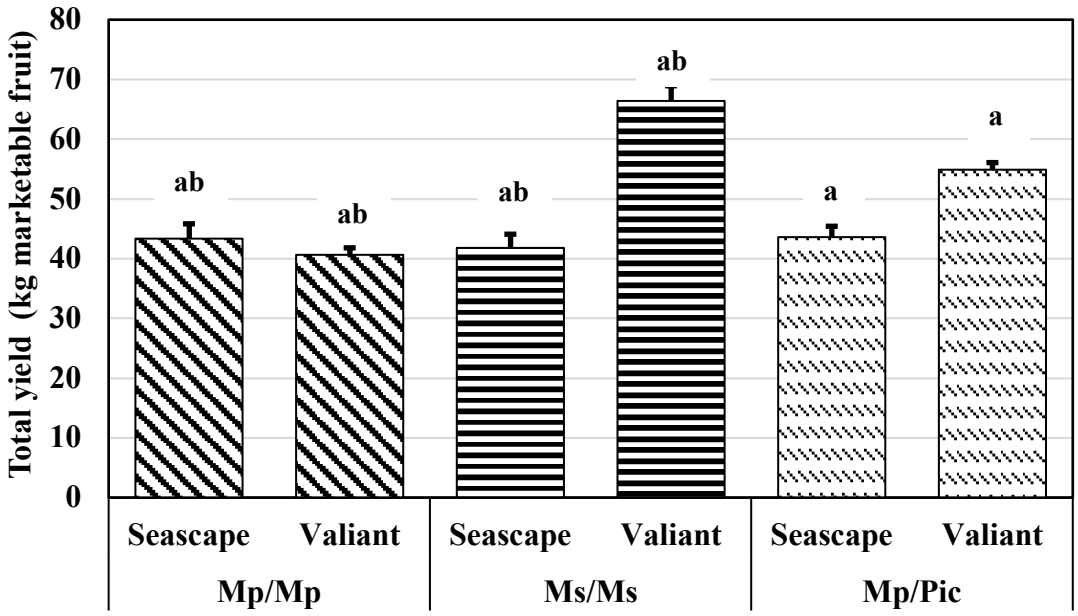
2-8. Total marketable yield per plot following a 3-week peak harvest assessment. Cultivars Seascape (susceptible) and Valiant (resistant) were established to assess performance against *V. dahliae.* Control = no treatment; CT + BF = crop termination + bed fumigation. ANOVA ($P = 0.05$). Error bars represent standard error of the means.



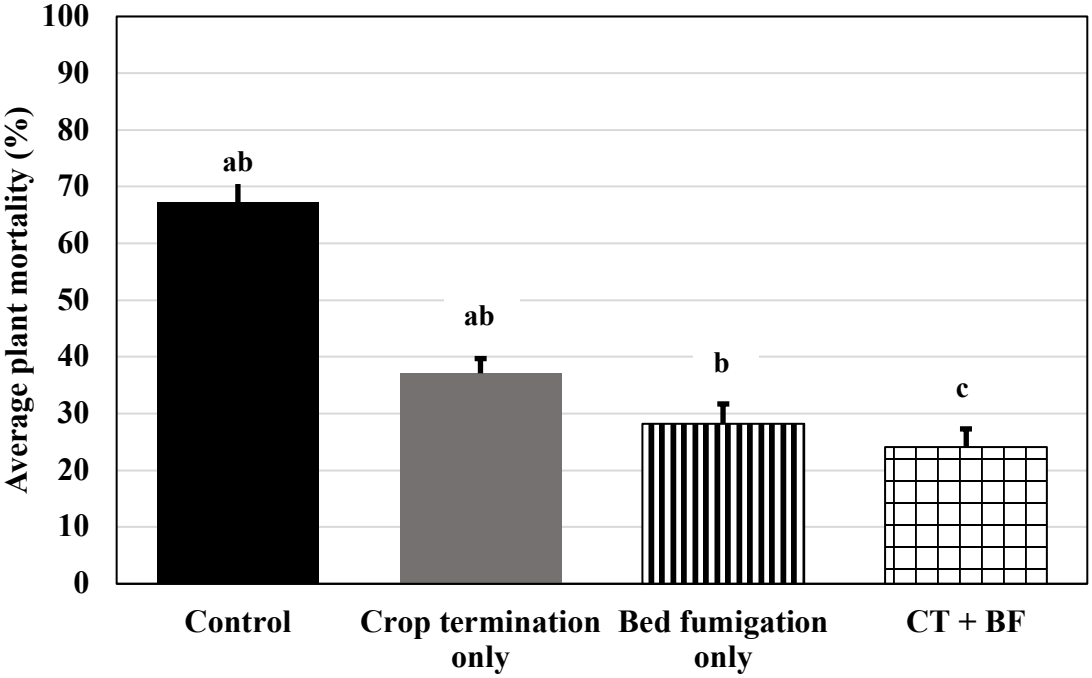
2-9. Total marketable fruit per plot following a 3-week peak harvest assessment for plots only crop terminated. Fumigants metam potassium (Mp) and metam sodium (Ms) were utilized and cultivars Seascape (susceptible) and Valiant (resistant) were established. ANOVA ($P = 0.05$). Error bars represent standard error of the means.



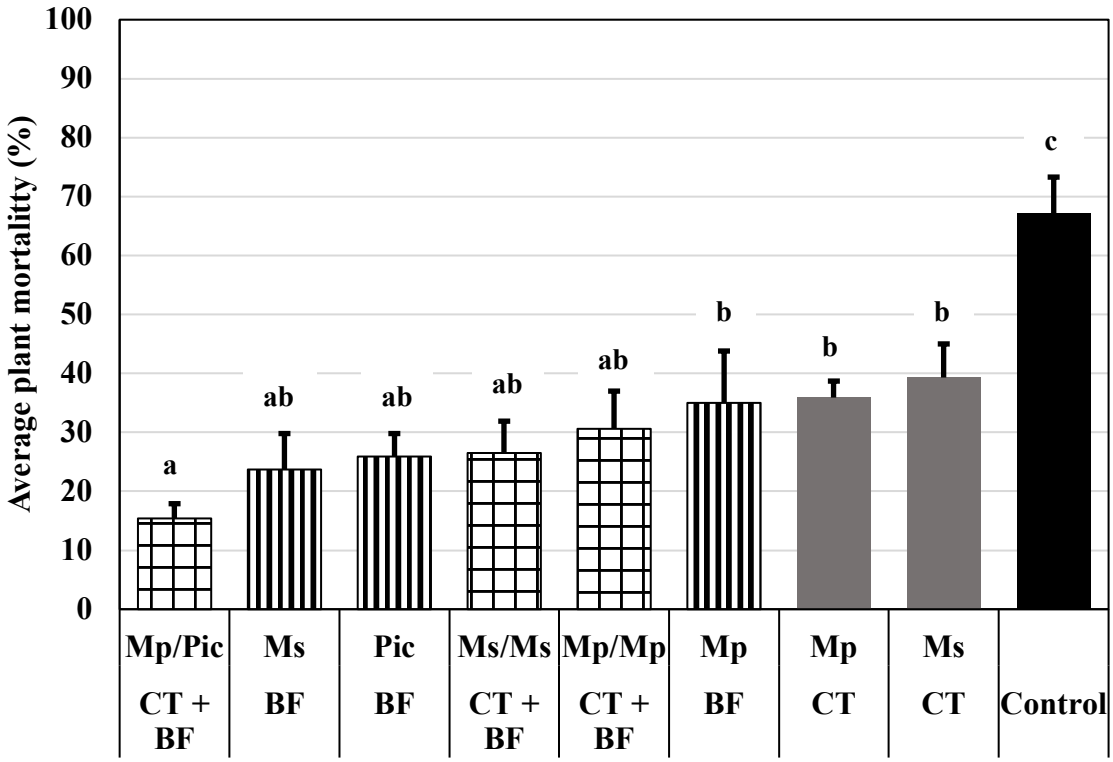
2-10. Total marketable fruit following a 3-week peak harvest assessment for plots only bed fumigated. Fumigants metam potassium (Mp), metam sodium (Ms), and chloropicrin (Pic) were utilized and cultivars Seascape (susceptible) and Valiant (resistant) were established. ANOVA ($P = 0.05$). Error bars represent standard error of the means.



2-11. Total marketable fruit following a 3-week peak harvest assessment for plots sequentially terminated and fumigated. Fumigants metam potassium (Mp), metam sodium (Ms), were utilized for the termination, where Mp, Ms, and chloropicrin (Pic) were utilized for the bed fumigation. Cultivars Seascape (susceptible) and Valiant (resistant) were established to assess performance. ANOVA ($P = 0.05$). Error bars represent standard error of the means.



2-12. Average plant mortality across all treatments 267 days after transplanting. Control = no treatment; CT + BF= crop termination + bed fumigation. ANOVA ($P = 0.05$). Error bars represent standard error of the means.



2-13. Average plant mortality due to *V. dahliae* per treatment, application, and fumigant type. CT = crop termination only, BF = bed fumigation only, CT + BF = crop termination + bed fumigation. Mp/Pic = metam potassium/chloropicrin, Ms = metam sodium, Pic = chloropicrin, Ms/Ms = metam sodium/metam sodium, Mp/Mp = metam potassium/metam potassium, Mp = metam potassium, Ms = metam sodium. Control = no treatment. ANOVA ($P = 0.05$). Error bars represent standard error of the means.

CHAPTER 3: EVALUATING HOST RESISTANCE TO VERTICILLIUM WILT IN 74 STRAWBERRY CULTIVARS AND ELITE BREEDING LINES

3.1 Abstract

Strawberry (*Fragaria* × *ananassa,* Duch.) is an economically important crop in the state of California, with production often hindered by soilborne pathogens such as *Verticillium dahliae* Kleb., the causal agent of Verticillium wilt. The reduction in use of fumigants such as methyl bromide for control of soilborne diseases has exacerbated disease occurrence and has led to increased crop loss. Due to the efficacy of fumigants in controlling soilborne pathogens, examinations into strawberry breeding for disease resistance was of lower priority than other horticultural traits like fruit size, firmness, and taste. The contemporary shift away from fumigation as a disease management practice necessitates investigations into breeding for resistance to *V. dahliae* in strawberry. Resistance to soilborne diseases varies by genotype. Thus, management of the disease should include use of cultivars with higher levels of disease resistance. A total of 74 strawberry cultivars and elite breeding lines were evaluated for susceptibility to Verticillium wilt in field soil naturally infested with *V. dahliae* over a two-year period from 2020-2022. Plant mortality due to *V. dahliae* was evaluated over time and genotypes varied in their response to this disease. During the 2020-2021 trial period, plant mortality varied from 13% to 100%, with an average mortality of 52.3% across all genotypes. During the 2021-2022 trial, plant mortality varied from 1.5% to 84.1%, with an average mortality of 35.8% across all genotypes. For the twenty-three cultivars common to both years of the trial, average plant mortality was 81.7% and 44.1% for year 1 and year 2 of the trial, respectively. No genotype was found to be fully resistant to *V.*

dahliae; and these findings will aid breeding programs in evaluating and validating resistance to Verticillium wilt under field conditions with natural inoculum.

3.2 Introduction

Strawberry (*Fragaria* × *ananassa,* Duch.) is an important crop in California, with more than 35,000 acres planted in 2018 with a farm gate value of $3.1 billion (CDFA 2018). California strawberry production accounts for more than 85% of national strawberry production (Njoroge et al. 2009). Advances in investigating genetic resistance in strawberry germplasm to soilborne pathogens have increased in the past twenty years, with strawberry breeding being done in both the public and private sectors. Genetic resistance has been investigated for many different diseases, including anthracnose (caused by *Colletotrichum acutatum*) (Salinas et al. 2019), crown and root rot (caused by *Phytophthora cactorum)* (Mangandi et al. 2017), Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *fragariae*) (Pincot et al. 2018), gray mold (caused by *Botrytis cinerea)* (Bestfleisch et al. 2015), and Verticillium wilt (caused by *Verticillium dahliae*) (Pincot et al. 2020). Within California, the University of California, Davis, Driscoll's Strawberry Associates Inc., Plant Sciences Inc., and Lassen Canyon Nursery have been investigating strawberry genetic diversity since the 1950s (Guthman 2019), and today the UC strawberry breeding program at Davis has released more than sixty cultivars (Nelson 2019). Strawberry varieties from the University of California represent 58.4% of planted acreage, with cultivar 'Monterey' representing 33.1% of these varieties. Proprietary cultivars represent 36.6% of planted acreage (California Strawberry Commission 2022).

Verticillium wilt of strawberry is caused by the fungal pathogen *Verticillium dahliae* and all strawberry cultivars are susceptible—there are no natural octoploid cultivars exhibiting complete resistance (Cockerton 2019). Symptoms of Verticillium wilt

have been reported, including leaves turning yellow and drooping, eventually turning brown and desiccating (Subbarao and Kabir 2007). Losses of up to 75% in strawberry have documented in the absence of soil fumigation (Wilhelm and Paulus 1980).

Resistance to Verticillium wilt in strawberry is under complex control of multiple genes (Antanaviciute et al. 2015) and a wide range of susceptibility has been recorded among 90 different strawberry cultivars and elite breeding lines (Holmes et al. 2016). Other investigations into host resistance showed small positive correlations between fruit firmness and resistance to Verticillium wilt (Bringhurst et al. 1968; Shaw et al. 1996) and slowed progress in breeding for resistance. Investigations into Verticillium wilt resistance has been successful in other hosts such as lettuce (Hayes et al. 2011) and tomato (Fradin et al. 2009), where race-specific resistant (R)-genes have been discovered. R-genes conferring resistance to Verticillium wilt in strawberry are yet to be identified (Vining et al. 2015), nor have large-effect quantitative trait loci (Antanaviciute et al. 2015; Cockerton 2019).

Investigations into host plant resistance to Verticillium wilt is becoming more important due to the phaseout of efficacious fumigants like methyl bromide. In order to determine susceptibility to Verticillium wilt, a replicated field trial over two consecutive seasons was established to assess 74 strawberry cultivars and elite breeding lines. Of these, 23 cultivars and elite breeding lines were common to both years. Further, evaluation of germplasms over several years and under different weather conditions were also examined.

3.3 Materials and Methods

3.3.1 Strawberry Cultivars and Elite Breeding Lines

Cumulatively, 74 strawberry cultivars and elite breeding lines were evaluated in field experiments conducted in 2020-21 and 2021-22. A total of 51 strawberry cultivars and elite breeding lines each were included each year. Twenty-three of the 51 cultivars and breeding lines were evaluated both years (Table 3-1). Strawberry genotypes were provided by five public and private breeding programs: Driscoll's Inc. (Watsonville, CA), Lassen Canyon Nursery (Redding, CA), Planasa (Red Bluff, CA), Plant Sciences, Inc. (Watsonville, CA), and the University of California, Davis (Davis, CA). Genotypes provided by Planasa were only evaluated during year two of the trial (Table 3-1).

3.3.2 Experimental Site and Layout

The trials for both years of the study (2020-2021, 2021-2022) were conducted at Field 25, Block 6 (35°30' N, 120°67' W) on the California Polytechnic State University Campus in San Luis Obispo, CA. The soil at the trial site was characterized as clay loam (36% sand, 26% silt, 38% clay) with a pH of 7.2, electrical conductivity of 2.2 dS/m, cation exchange capacity of 20.5 meq/100g, and an organic matter composition of 2.9% (A & L Western Agricultural Laboratories, Modesto, CA). The experimental plot has decades of history of *V. dahliae* susceptible row and vegetable crop production (broccoli, cole crops, and lettuce) and lettuce (cv. Black Seeded Simpson) was broadcast planted in June 2020 to increase *V. dahliae* inoculum prior to transplanting. The control area of the field for year 1 and year 2 was broadcast fumigated using Tri-Clor EC (94% chloropicrin at 208.2 liters/treated A) on 16 October 2020 and 11 September 2021, respectively.

Raised beds (160 cm center to center, 114 cm wide and 30 cm tall) were formed prior to year one of the trial and after disking of the previous lettuce crop. Raised beds were formed at same dimensions prior to year two of the trial and after disking of the previous strawberry crop. Three lines of drip irrigation (low-flow, 1.2 liter/min/30.4 m at 55 kPa, with 20 cm spacing on emitters; Tri-Cal®, Hollister, CA) were buried at a depth of 1.5 cm and spaced 40 cm apart. Beds were covered with polyethylene mulch, which was 84 inches wide, 1.1 mil thick, and completely black. Four planting rows were established 25 cm apart and strawberry plants were spaced 35 cm apart within the plant row. Bareroot strawberry transplants were planted by hand on 02 November 2020 and 02 November 2021, respectively.

3.3.3 Soil Inoculum Density

Soil inoculum density of *V. dahliae* was determined seven days before transplanting for both year one and year two of the trial. A total of ten soil samples were collected per bed in a "zig-zag" pattern at 0.3 m depth using a 0.03 m soil-core, with samples separated by 3 m. Soil samples were pooled, air-dried for two weeks, pulverized with a mortar and pestle, and 0.1 g of soil was plated on a semi-selective medium, Sorenson's NP-10 (Sorensen et al. 1991), in ten replicates with use of an Anderson sampler (Anderson 1958). Colony forming units (CFU) were counted using a dissecting microscope after dark incubation at ambient temperature for two weeks.

3.4 Experimental Design

A total of fifteen beds contained seventeen plots approximately 1.8 m long. Plots were subject to a randomized complete block design (RCBD) with four block replicates and one fumigated control replicate. Each plot consisted of 20 strawberry plants. All 51

strawberry cultivars and breeding lines for a single replicate were randomly assigned a plot and established on three individual beds.

3.4.1 Mortality Assessments and Area Under the Disease Progress Curve (AUDPC)

Plant mortality assessments were visually conducted bi-weekly. Year one assessments began two weeks after planting on 16 November 2020 and concluded on 26 July 2021. Year two assessments also began two weeks after planting on 16 November 2021 and concluded on 18 July 2022. Plants exhibiting symptoms (50% foliar necrosis) were sampled and plated on Acidified Potato Dextrose Agar (ADPA), P_{10}ARP (Erwin and Ribeiro 1996) and Sorensen's NP-10 medium (Sorensen et al. 1991). Percent mortality due to *V. dahliae* was counted for each 20-plant plot.

The area under the disease progress curve (AUDPC) is a quantitative summary of disease intensity over time, used to calculate average disease incidence between individual time points (Madden et al. 2007). AUDPC was calculated using the following formula:

$$AUDPC = \sum_{i=1}^{N_i-1} \frac{(y_{i+}\, y_{i+1})}{2} \times (t_{i-1} - t_i)$$

N represents the total number of observations, y_i represents the percent mortality at observation number i and t_i is the number of days from date of establishment. Relative area under the disease progress curve (rAUDPC) was calculated to determine the proportion of maximum disease severity over each year. To calculate rAUDPC, AUDPC values were divided by the maximum AUDPC over the length of the season.

3.4.2 Data Analysis

To evaluate the single effect of cultivar on AUDPC, a standard sum of squares analysis of variance (ANOVA) was performed (JMP® pro version 16 SAS Institute, Cary, NC). Beforehand, AUDPC was calculated in Excel using mortality assessments for each cultivar and elite breeding line. Both years were analyzed separately. The relative area under the disease progress curve (rAUDPC) was calculated detailing the proportion of maximum possible disease severity over each growing season (year). AUDPC values were divided by the maximum AUDPC possible over the course of each season. Maximum AUDPC scores were calculated by utilizing the maximum mortality count (i.e. 20/20 plants) on each assessment date. These calculations show values ranging from 0.0 to 1.0, with 0.0 indicating complete resistance with no disease severity over time and 1.0 signaling complete susceptibility with 100% disease severity over time. Significant differences between genotypes and elite breeding lines were established using the F-test with a 5% level of probability ($\alpha = 0.05$). Following this assessment, a Bonferroni (Dunn's) t test was utilized as a multiple comparisons test.

3.5 Results

3.5.1 Percent Mortality by Cultivar and Elite Breeding Line

Over 1,000 plants from the experimental plots were sampled during year 1 of the trial, showing extremely low incidence of *M. phaseolina* being the causal agent of plant mortality (<1.2%). *M. phaseolina* was not detected in any plant samples during year two of the trial. These mortalities were adjusted for under baseline plant counts and were not included in mortality assessments. Furthermore, during the first two months of observations during both years, plant mortality due to oomycete pathogens of *Pythium*

spp. and *Phytophthora* spp. were found, but these mortalities were few and adjusted for under baseline plant counts and were not included in mortality assessments.

3.5.1.1 Year 1 (2020-2021)

The pre-plant inoculum level for *Verticillium* spp. microsclerotia in year 1 of the study was 7.0 CFU/g soil. Symptoms due to *V. dahliae* were first assessed two weeks after planting in November 2020, with mortality first being seen in early March 2021. A majority of plant mortality was observed at the end of May 2021 and early June 2021 (Fig. 3-1).

For all genotypes evaluated, average plant mortality was 52.3%. Statistically significant effects on strawberry cultivars and elite breeding lines were observed ($F = 5.79$, $P < 0.0001$) at the end of the assessment period in July 2021. Average mortality for each genotype was variable, ranging from 13.0% to 100.0% (Fig. 3-2). Five genotypes showing the highest mortality levels were: BG-4.352, PEP-12.6007, BG-10.3181, Casmalia, and PS-10.116 with 100.0, 98.8, 97.5, 96.3, and 96.3 percent mortality, respectively (Fig. 3-2). These genotypes with the highest average mortality were significantly different from each other (Fig. 3-3; $F = 5.79$, $P = < 0.0001$). Five genotypes showing the lowest mortality were: Xareni, 152X18, Fronteras, Pomona, and 17EDN019 with 13.0, 37.1, 47.3, 49.2, and 50.0 percent mortality, respectively (Fig. 3-2). These genotypes with the lowest average mortality were not significantly different from each other (Fig. 3-3; $F = 5.79$, $P < 0.001$).

All individual breeding programs showed a wide range of variability in year one of the trial. Variability among blocks was detected, with standard error ranging from 0 to 17.6. A total of twelve genotypes had standard error values over 10.0. Genotypes

provided by the University of California had mortality percentages ranging from 13.0% (17EDN019) to 93.4% (Warrior) (Table 3-2; Table 3-3). Genotypes provided by Driscoll's Inc., had mortality percentages ranging from 37.1% (Pomona) to 96.3% (Casmalia) (Table 3-2). Genotypes provided by Plant Sciences, Inc., had mortality percentages ranging from 64.0% (BG-9.3128) to 100.0% (BG-4.352) (Table 3-2; Table 3-3). Finally, genotypes provided by Lassen Canyon Nursery had mortality percentages ranging from 47.5% (152X18) to 90.0% (078X01) (Table 3-2; Table 3-3). The average daily temperature for year one of the study was 21.2°C (Fig. 3-4).

3.5.1.2 Year 2 (2021-2022)

The pre-plant inoculum level for *Verticillium* spp. microsclerotia in year 2 of the study was 10.0 CFU/g soil. Symptoms due to *V. dahliae* were first assessed two weeks after planting in November 2021, with mortality first detected in early April 2022. Most plant mortality was observed at the end of May 2021 and June 2022 (Fig. 3-1).

For all genotypes evaluated, average plant mortality was 35.8%. Statistically significant effects on strawberry cultivars and elite breeding lines were observed ($F = 5.79$, $P < 0.001$) at the end of the assessment period in July 2022. Average mortality for each genotype was variable, ranging from 1.5% to 81.4% (Table 3-2; Table 3-4). Five genotypes showing the highest mortality levels were: BG-6.3024, BG-9.3147, BG-4.367, UCD_Warrior, and UCD_Victor with 84.1, 80.7, 70.5, 69.6, and 66.7 percent mortality, respectively (Fig. 3-5). These five genotypes with the highest average mortality were significantly different (Fig. 3-6; $F = 5.49$, $P < 0.001$). Five genotypes showing the lowest mortality were: 17C242P023, 17C138P021, Mojo, UCD_Moxie, and Sierra with 1.5, 2.8, 2.9, 7.6, and 8.9 percent mortality, respectively (Fig. 3-5).

All individual breeding programs showed a wide range of variability in year two of the trial. Variability among blocks was seen with standard error ranging from 1.2 to 18.3. A total of twenty genotypes had standard error values over 10.0. Genotypes provided by the University of California had mortality percentages ranging from 1.5% (17C242P023) to 69.5% (Warrior) (Fig. 3-5). Genotypes provided by Driscoll's Inc., had mortality percentages ranging from 23.5% (Mariposa) to 54.1% (Maverick) (Fig. 3-5). Genotypes provided by Plant Sciences, Inc., had mortality percentages ranging from 13.6% (BG-9.3128) to 84.1% (BG-6.3024) (Fig. 3-5). Genotypes provided by Lassen Canyon had mortality percentages ranging from 8.9% (Sierra) to 44.7% (Camila) (Fig. 3-5). Finally, genotypes provided by Planasa had mortality percentages ranging from 11.3% (15-105R) to 65.8% (18-145R) (Fig. 3-5). Twenty-three genotypes were common to both years of the trial, and these genotypes showed an average plant mortality of 81.7% in 2021 and 44.1% in 2022. The average daily temperature for year two of the study was found to be 21.4°C, nearly identical to year one (Fig. 3-7).

3.5.2 Cultivars Evaluated During Both Years of the Trial

A total of twenty-three cultivars were evaluated during both years of the trial (Table 3-1, 3-2; Fig. 3-8). The University of California, Davis, Lassen Canyon Nursery, Driscoll's, and Plant Sciences provided eight, one, three, and eleven genotypes for both years of the trial, respectively. Of these twenty-three cultivars, all resulted in lower mortality in the second year of the trial, besides cultivar BG-6.3024 which resulted in a 3.2% higher mortality during year 2 of the trial (Fig. 3-8). A total of five cultivars common to both years of the trial resulted in different mortality percentages higher than

50%. BG-9.3128, 16C029P012, Monterey, PEP-12.6010, and BG-4.352 had a 50.4%, 53.3%, 67.3%, 67.5%, and 65.9% lower mortality during year 2 of the trial, respectively.

3.5.3 AUDPC by Cultivar

3.5.3.1 Year 1 (2020-2021)

Statistically significant effects were observed at the end of the trial period on 26 July 2021 ($F = 7.21$, $P < 0.0001$). The average AUDPC for all 51 cultivars was 526.0, with the total possible AUDPC maximum value of 25,200 for the 266 days of assessment. Average AUDPC ranged from 55.5 to 1194.5 (Table 3-6). Of all genotypes, five strawberry genotypes with the highest rAUDPC values were: BG-9.3147, BG-10.3181, BG-4.352, BG-10.3169, and PEP-12.6007 with 0.05, 0.04, 0.04, 0.04, and 0.04, respectively (Table 3-6). A total of four strawberry genotypes resulted in an rAUDPC value of zero: 17EDN019, 16C100P023, Xareni, and 152X18. Within all breeding programs, a small range of rAUDPC values were seen.

3.5.3.2 Year 2 (2021-2022)

Statistically significant effects on AUDPC were observed at the end of the trial period on 18 July 2022 ($F = 3.48$, $P < 0.0001$). The average AUDPC for all 51 cultivars was 247.5, with the total possible AUDPC maximum value of 24,400 for the 258 days of assessment. Average AUDPC ranged from 14.0 to 601.8 (Table 3-7). Of all genotypes, five strawberry genotypes with the highest rAUDPC values were: BG-9.3147, BG-6.3024, 18-145R, UCD_Warrior, and Maverick with 0.03, 0.03, 0.02, 0.02, and 0.02, respectively (Table 3-7). These cultivars with the highest rAUDPC values were not significantly different from each other ($F = 1.45$, $P = 0.21$). A total of 10 strawberry genotypes resulted in an rAUDPC value of zero: Mojo, 17C138P021, 17C242P023,

002Y03, UCD_Moxie, BG-9.3128, 16C029P012, 15-105R, Monterey, and Sierra. Within all breeding programs, a small range of rAUDPC values were seen.

3.6 Discussion

This study explored host resistance among 51 strawberry genotypes in each of two years in a field naturally infested with *V. dahliae*. Of these genotypes, none exhibited complete resistance to *V. dahliae* which corresponds with Cockerton (2019). These genotypes provided by five different breeding programs exhibited a wide range of plant mortality during both years of the trial. Noticeable differences in average mortality among all genotypes assessed during both years of the trial were found, with the total average mortality during year one of the trial being higher than mortality during year two. For example, the mortality of commonly grown susceptible cultivar 'Monterey' had a higher mortality in year one (78.8%) compared to year two (11.5%). Aforementioned lack of knowledge and identification of resistant R-genes and large-effect quantitative trait loci are found to be under large environmental influence (Castro and Lewers 2016; Pincot et al. 2020; Zorrilla-Fontanesi et al. 2011) which may explain differences in mortality between common cultivars of year one and year two of the trial.

Environmental conditions such as ambient temperature may have contributed to differences in disease progression and mortality. Although, Figures 3-4 and 3-7 show that the daily average temperature during both years of the trial were not noticeably different, with average daily temperature during the experimental period for both years being roughly 21°C. Verticillium wilt progression is favored by cool temperatures (temperature range of 24°C to 28°C) with overcast weather interspersed with warm, bright days (Ellis 2016). High temperatures above 27°C exacerbate disease symptoms due to limited water

movement because of vascular colonization, yet *V. dahliae* does not colonize plants at higher rates due to these increases in temperature (Ellis 2016). A sharp increase in temperature near the end of year one (Fig. 3-7) may have contributed to an increase in disease symptoms. Although the total length of observations during year one of the trial was 266 days and 258 days for year two of the trial, this difference did not contribute to a drastic reduction in mortality. Differences between levels of natural soil inoculum during both years of the trial are also hypothesized to play a role in plant mortality, yet the higher inoculum level during year two did not result in overall higher plant mortality. Thus, these differences between years one and two show the importance of examining host resistance over multiple years and under different environmental conditions and locations.

The University of California, Davis, in conjunction with the California Strawberry Commission, has publicly available information showing wide degrees of variability between strawberry resistance to *V. dahliae* (California Strawberry Commission 2022). On a scale of 1 to 4, with 1 being resistant and 4 being susceptible, it shows that no cultivar that is publicly available has complete resistance to *V. dahliae.* During year one of the trial, eight cultivars including Fronteras, UCD_Warrior, UCD_Victor, Monterey, Cabrillo, UCD_Valiant, UCD_Royal Royce, and UCD_Moxie were assessed, and our results correspond with publicly available information. During year two of the trial, nine cultivars including UCD_Victor, UCD_Warrior, Monterey, UCD_ Royal Royce, UCD_Valiant, Finn, Mojo, UCD_Moxie, and 17C242P023 were assessed and our results correspond with publicly available information.

Average percent mortality with standard error at or below 25% over the four replicated plots is used to classify genotypes as resistant (Shaw et al. 2010). Further, we can reference AUDPC values to confirm our assessment. During year one of the trial, only one breeding line (17EDN019) showed mortality below 25% and a rAUDPC value of 0.00, though it was not included in year two. During year two of the trial, eleven cultivars and elite breeding lines showed mortality below 25%. Of these eleven cultivars, six were included in year one (BG-9.3128, SB_12_102-056, Monterey, Moxie, 16C100P023, and 16C029P012) and all resulted in mortality above 25% and rAUDPC values above 0.00. This exemplifies the importance of repeating resistance screenings due to variation in mortality between years.

More work identifying host plant resistance to Verticillium wilt is needed as the industry shifts away from other disease management techniques like fumigation. Future investigations into genetic resistance to Verticillium wilt is needed to assess variability over the years, as well as assess performance in different locations. The Central Coast of California harbors extremely favorable environmental conditions for *V. dahliae*, thus findings over these two years should be used in comparison with previous and future investigations to possibly identify resistant genes as well as providing growers with relevant information regarding publicly available genotypes and their performance in growing systems threatened by *V. dahliae*.

Table 3-1. The 74 strawberry entries in the 2020-2021 (Year one) and 2021-2022 (Year two) growing seasons.

Year 1		*Year 2*	
Program	Cultivar/Line	Program	Cultivar/Line
PSI	BG-4.352	PSI	BG-4.352
PSI	BG-4.367	PSI	BG-4.367
PSI	BG-6.3024	PSI	BG-6.3024
PSI	BG-9.3128	PSI	BG-9.3128
PSI	BG-9.3147	PSI	BG-9.3147
PSI	BG-10.3169	PSI	BG-10.3169
PSI	BG-10.3181	PSI	BG-10.3181
PSI	PS-9271	PSI	PS-9271
PSI	PS-10.116	PSI	PS-10.116
PSI	PE-7.2059	PSI	PE-7.2059
PSI	PE-7.2054	PSI	PE-7.2054
PSI	PEP-12.6010	PSI	PEP-12.6010
PSI	PEP-12.607	PSI	SBS-12.102.056
PSI	SB_12_53-118		
PSI	SB_12_102-066		
UCD	Warrior	UCD	Warrior
UCD	Victor	UCD	Victor
UCD	Moxie	UCD	Moxie
UCD	Valiant	UCD	Valiant
UCD	Royal Royce	UCD	Royal Royce
UCD	Monterey	UCD	Monterey
UCD	16C555P053	UCD	16C555P053
UCD	16C108P060	UCD	16C108P060
UCD	16C029P012	UCD	16C029P012
UCD	Fronteras	UCD	Mojo
UCD	Cabrillo	UCD	Finn
UCD	17EDN015	UCD	17C138P062
UCD	17EDN019	UCD	17C121P097
UCD	17C123P051	UCD	17C242P023
UCD	16C100P023	UCD	17C138P021
DR	Big Sur	DR	Big Sur
DR	Mariposa	DR	Mariposa
DR	Maverick	DR	Maverick
DR	Pomona	DR	Petra

DR	Xareni	DR	Minerva
DR	Yunuen	DR	Purisima
DR	Ravina	DR	Medusa
DR	Rosalind	DR	Dayana
DR	Liliana		
DR	Fortaleza		
DR	Challenger		
DR	Ailene		
DR	Casmalia		
DR	Laredo		
DR	Veronica		
Lassen	Sweet Ann	Lassen	Sweet Ann
Lassen	Ruby June	Lassen	Sierra
Lassen	152X14	Lassen	Camila
Lassen	152X18	Lassen	002Y03
Lassen	078X01	Lassen	152X15
Lassen	074X04	Lassen	193W33
		Lassen	122X08
		Lassen	145T39
		Lassen	146T54
		Lassen	143T35
		Planasa	Salma
		Planasa	Sayulita
		Planasa	15-105R
		Planasa	18-006R
		Planasa	18-145R

Table 3-2. The 23 strawberry cultivars and elite breeding lines (genotypes) common to year one and year two of the trials in ranking order by percent plant mortality as of 26 July 2021 and 18 July 2022.

Cultivar/ Elite Breeding Line	Plant Mortality[x] (%)			
	2020-2021		2021-2022	
	Mean[y]	SE[z]	Mean[y]	SE[z]
Years 1 & 2				
UCD_Valiant	54.1	4.9	22.5	10.3
Big Sur	58.6	14.7	28.7	3.4
Mariposa	63.8	13.9	23.5	5.1
BG-9.3128	64.0	8.9	13.6	4.5
Sweet Ann	67.2	13.2	23.9	4.6
16C029P012	67.5	11.8	14.2	3.2
PE-7.2059	69.7	14.1	35.5	12.8
Monterey	78.8	9.2	11.5	6.7
BG-6.3024	80.9	2.4	84.1	9.4
PS-9271	81.2	8.9	24.2	3.7
PE-7.2054	82.4	5.9	34.1	7.8
16C555P053	82.5	6.3	47.9	15.5
Maverick	83.8	8.9	54.1	8.0
UCD_Royal Royce	85.9	5.6	63.9	6.8
BG-10.3169	87.8	7.1	52.3	18.3
16C108P060	88.8	6.4	43.2	6.9
UCD_Victor	90.8	4.1	66.7	9.9
BG-4.367	92.5	7.5	70.5	11.4
UCD_Warrior	93.4	2.5	69.6	10.6
PEP-12.6010	94.8	2.2	27.3	16.2
BG-9.3147	95.0	1.3	80.7	12.1
BG-10.3181	97.5	0.0	56.8	7.8
BG-4.352	100.0	0.0	34.1	7.8

[x] Percent mortality as of 26 July 2021 and 18 July 2022, 266 and 258 days after transplanting, respectively.

[y] Mean values calculated from four plot replicates.

[z] Standard error derived from four plot replicates.

"-" no data available for this year of the trial.

Table 3-3. The 51 strawberry cultivars and elite breeding lines (genotypes) included in year one of the trial in ranking order by percent plant mortality as of 26 July 2021.

Cultivar/ Elite Breeding Line	Plant Mortality[x] (%) 2020-2021			
	Mean[y]	SE[z]	Mean[y]	SE[z]
Year 1				
17EDN019	13.0	7.3	-	-
Pomona	37.1	15.4	-	-
Fronteras	47.3	13.2	-	-
152X18	47.5	8.5	-	-
Xareni	49.1	6.5	-	-
16C100P023	50.0	8.8	-	-
Valiant	54.2	4.9	-	-
Ailene	58.1	10.6	-	-
152X14	58.5	13.8	-	-
Big Sur	58.6	14.7	-	-
Moxie	58.8	15.9	-	-
Mariposa	63.8	13.9	-	-
BG-9.3128	64.0	8.9	-	-
17EDN015	65.9	9.7	-	-
Sweet Ann	67.2	13.2	-	-
16C029P012	67.5	11.8	-	-
PE-7.2059	69.8	14.1	-	-
Challenger	72.2	9.2	-	-
074X04	78.8	17.6	-	-
Monterey	78.8	9.2	-	-
Rosalind	79.9	6.9	-	-
BG-6.3024	80.1	2.4	-	-
PS-9271	81.2	8.9	-	-
Ravina	81.3	6.8	-	-
PE-7.2054	82.4	5.9	-	-
16C555P053	82.5	6.3	-	-
Maverick	83.8	8.9	-	-
Fortaleza	83.8	8.3	-	-
Yunuen	85.0	7.2	-	-
UCD_Royal Royce	86.0	5.7	-	-

Ruby June	86.3	8.4	-	-
Liliana	87.5	5.8	-	-
BG-10.3169	87.8	7.1	-	-
16C108P060	88.8	6.4	-	-
SB_12.53-118	88.8	8.4	-	-
078X01	90.0	15.3	-	-
Victor	90.8	4.1	-	-
17C123P051	91.1	4.2	-	-
Cabrillo	92.3	4.5	-	-
BG-4.367	92.5	7.5	-	-
Laredo	92.6	7.4	-	-
Warrior	93.4	2.5	-	-
Veronica	93.6	4.3	-	-
PEP-12.6010	94.8	2.2	-	-
BG-9.3147	95	1.3	-	-
PS-10.116	96.2	3.8	-	-
Casmalia	96.3	2.5	-	-
SB_12.102-056	96.4	3.8	-	-
BG-10.3181	97.5	0.0		
PEP-12.6007	98.8	1.3	-	-
BG-4.352	100.0	0.0	-	-

[x] Percent mortality as of 26 July 2021, 266 days after transplanting.
[y] Mean values calculated from four plot replicates.
[z] Standard error derived from four plot replicates.
"-" no data available for this year of the trial.

Table 3-4. The 51 strawberry cultivars and elite breeding lines (genotypes) included in year one of the trial in ranking order by percent plant mortality as of 18 July 2022.

Cultivar/ Elite Breeding Line	Plant Mortality[x] (%) 2021-2022			
	Mean[y]	SE[z]	Mean[y]	SE[z]
Year 2				
17C242P023	-	-	1.5	1.5
17C138P021	-	-	2.8	2.8
Mojo	-	-	2.9	1.7
UCD_Moxie	-	-	7.6	1.4
Sierra	-	-	8.9	1.2
002Y03	-	-	10.5	3.1
15-105R	-	-	11.3	4.7
Monterey	-	-	11.5	6.7
BG-9.3128	-	-	13.6	4.5
SBS-12.102.056	-	-	13.6	4.5
16C029P012	-	-	14.2	3.2
PS-10.1160	-	-	21.1	4.1
UCD_Valiant	-	-	22.5	10.3
Mariposa	-	-	23.5	5.1
Sweet Ann	-	-	23.9	4.6
PS-9271	-	-	24.2	3.7
143T35	-	-	26.8	7.2
PEP-12.6010	-	-	27.3	16.2
Big Sur	-	-	28.7	3.4
Medusa	-	-	29.3	5.3
Minerva	-	-	30.7	5.5
Petra	-	-	32.3	10.4
BG-4.352	-	-	34.1	7.8
PE-7.2054	-	-	34.1	7.8
Purisima	-	-	34.6	9.4
PE-7.2059	-	-	34.8	13.5
145T39	-	-	35.0	7.4
193W33	-	-	36.3	12.3
Finn	-	-	37.9	3.5
18-006R	-	-	39.3	12.1

152X15	-	-	40.3	8.7
146T54	-	-	41.7	8.2
16C108P060	-	-	43.2	6.9
17C138P062	-	-	43.2	11.5
122X08	-	-	43.3	10.6
17C121P097	-	-	43.6	11.5
Salma	-	-	43.9	13.1
Camila	-	-	44.7	11.1
Dayana	-	-	45.9	12.5
16C555P053	-	-	47.9	15.5
Sayulita	-	-	50.0	10.2
BG-10.3169	-	-	52.3	18.3
Maverick	-	-	52.7	7.9
BG-10.3181	-	-	56.8	7.8
UCD_Royal Royce	-	-	63.9	6.8
18-145R	-	-	65.8	11.5
UCD_Victor	-	-	66.7	9.9
UCD_Warrior	-	-	69.6	10.6
BG-4.367	-	-	70.5	11.4
BG-9.3147	-	-	80.7	12.1
BG-6.3024	-	-	84.1	9.4

[x] Percent mortality as of 18 July 2022, 258 days after transplanting.
[y] Mean values calculated from four plot replicates.
[z] Standard error derived from four plot replicates.
"-" no data available for this year of the trial.

Table 3-5. Strawberry cultivars and elite breeding lines in ranking order by area under the disease progress curve (AUDPC) as of 26 July 2021 and 18 July 2022.

Cultivar/ Elite Breeding Line	AUDPC[w]					
	2020-2021			2021-2022		
	Mean	SE	rAUDPC[v]	Mean	SE	rAUDPC[v]
Years 1 & 2						
UCD_Valiant	139.0	21.5	0.01	122.5	64.0	0.01
Big Sur	155.8	23.5	0.03	178.5	54.6	0.01
16C029P012	251.5	69.1	0.01	45.5	12.0	0.00
UCD_Victor	335.5	36.9	0.01	399.0	103.6	0.02
UCD_Warrior	356.5	81.3	0.01	493.5	100.7	0.02
16C555P053	365.3	48.4	0.01	350.0	144.9	0.02
BG-9.3128	415.0	133.1	0.02	38.5	20.9	0.00
16C108P060	417.8	92.0	0.02	311.5	89.3	0.01
Sweet Ann	422.0	90.1	0.02	185.5	66.3	0.01
Mariposa	425.8	183.4	0.03	150.5	25.2	0.01
PE-7.2059	431.0	189.4	0.02	192.5	86.8	0.01
PS-9271	476.8	85.1	0.02	119.0	35.5	0.01
UCD_Royal Royce	493.8	98.6	0.02	262.5	15.5	0.01
Monterey	556.8	285.4	0.02	70.0	45.9	0.00
BG-4.367	613.8	137.5	0.02	318.5	89.7	0.01
PEP-12.6010	660.0	102.3	0.03	164.5	106.4	0.01
PE-7.2054	666.5	85.5	0.03	140.0	60.5	0.01
Maverick	813.0	67.4	0.03	469.0	65.0	0.02
BG-6.3024	877.5	133.1	0.03	580.9	135.8	0.03
BG-10.3169	1017.5	194.5	0.04	322.0	125.6	0.01
BG-4.352	1035.5	45.2	0.04	164.4	51.5	0.01
BG-10.3181	1041.5	89.9	0.04	329.0	88.3	0.01
BG-9.3147	1194.5	81.9	0.05	601.8	121.3	0.03

[w] AUDPC = Area under the disease progress curve during the season ratings (18 observations).
[v] rAUDPC = Relative area under the disease progress curve.
"-" no data available for this year of the trial.

Table 3-6. Strawberry cultivars and elite breeding lines for year 1 of the trial in ranking order by area under the disease progress curve (AUDPC) as of 26 July 2021.

Cultivar/ Elite Breeding Line	AUDPC[w]					
	2020-2021			2021-2022		
	Mean	SE	rAUDPC[v]	Mean	SE	rAUDPC[v]
17EDN019	55.5	36.2	0.00	-	-	-
Pomona	108.8	45.2	0.01	-	-	-
152X18	114	45	0.00	-	-	-
16C100P023	125.5	15.2	0.00	-	-	-
Valiant	139	21.5	0.01	-	-	-
Xareni	142.8	34.6	0.00	-	-	-
Big Sur	155.8	23.5	0.03	-	-	-
Moxie	220.3	63.4	0.01	-	-	-
16C029P012	251.5	69.1	0.01	-	-	-
Ailene	254.5	47.5	0.01	-	-	-
152X14	259.5	74.2	0.01	-	-	-
Challenger	312.8	118.2	0.02	-	-	-
Fronteras	317.0	130.1	0.01	-	-	-
17EDN015	327.8	123.0	0.01	-	-	-
Victor	335.5	36.9	0.01	-	-	-
Warrior	356.5	81.3	0.01	-	-	-
16C555P053	365.3	48.4	0.01	-	-	-
Ravina	369.8	54.8	0.02	-	-	-
17C123P051	371.0	121.6	0.01	-	-	-
Rosalind	400.0	118.9	0.01	-	-	-
BG-9.3128	415.0	133.1	0.02	-	-	-
16C108P060	417.8	92.0	0.02	-	-	-
Sweet Ann	422.0	90.1	0.02	-	-	-
Mariposa	425.8	183.4	0.03	-	-	-
PE-7.2059	431.0	189.4	0.02	-	-	-
Cabrillo	473.5	78.1	0.02	-	-	-
PS-9271	476.8	85.1	0.02	-	-	-
UCD_Royal Royce	493.8	98.6	0.02	-	-	-
Yunuen	494.5	63.5	0.01	-	-	-
Monterey	556.8	285.4	0.02	-	-	-
074X04	583.5	130.7	0.02	-	-	-
BG-4.367	613.8	137.5	0.02	-	-	-

Casmalia	637.0	75.9	0.01	-	-	-
PEP-12.6010	660.0	102.3	0.03	-	-	-
PE-7.2054	666.5	85.5	0.03	-	-	-
SB_12.102-056	666.8	59.7	0.03	-	-	-
Ruby June	718.8	86.0	0.03	-	-	-
Liliana	752.0	103.1	0.02	-	-	-
Fortaleza	755.3	167.5	0.01	-	-	-
SB_12.53-118	779.8	123.2	0.03	-	-	-
Maverick	813.0	67.4	0.03	-	-	-
Veronica	824.3	98.9	0.03	-	-	-
Laredo	826.5	122.7	0.03	-	-	-
BG-6.3024	877.5	133.1	0.03	-	-	-
078X01	880.8	137.7	0.03	-	-	-
PS-10.116	944.3	142.3	0.04	-	-	-
PEP-12.6007	953.3	46.1	0.04	-	-	-
BG-10.3169	1017.5	194.5	0.04	-	-	-
BG-4.352	1035.5	45.2	0.04	-	-	-
BG-10.3181	1041.5	89.9	0.04			
BG-9.3147	1194.5	81.9	0.05	-	-	-

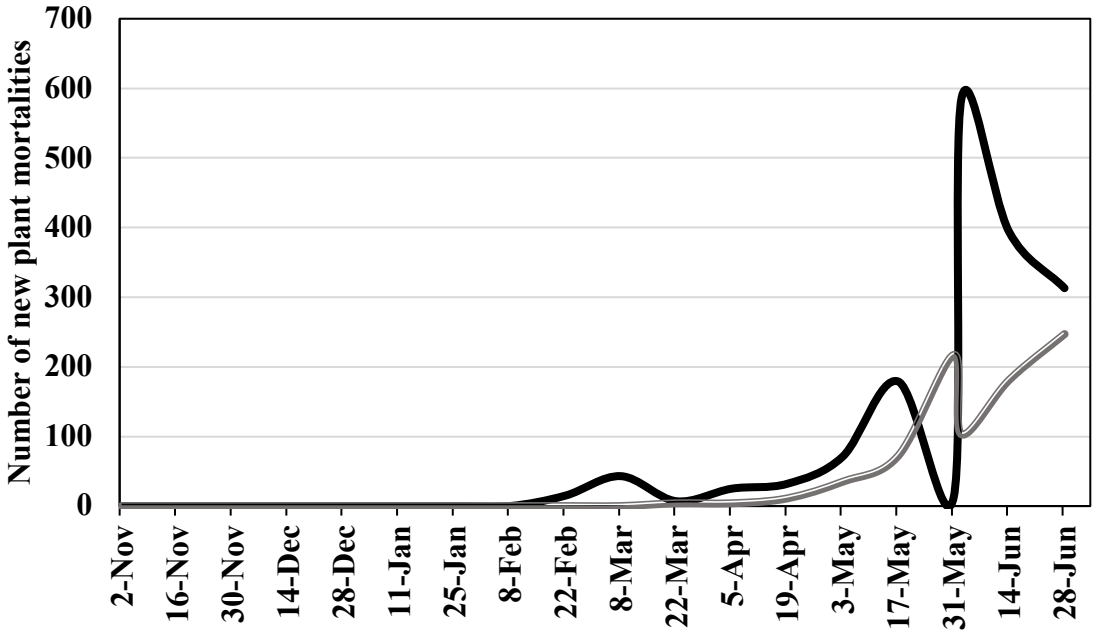
[w] AUDPC = Area under the disease progress curve during the season ratings (18 observations).
[v] rAUDPC = Relative area under the disease progress curve.
"-" no data available for this year of the trial.

Table 3-7. Strawberry cultivars and elite breeding lines for year 2 of the trial in ranking order by area under the disease progress curve (AUDPC) as of 18 July 2022.

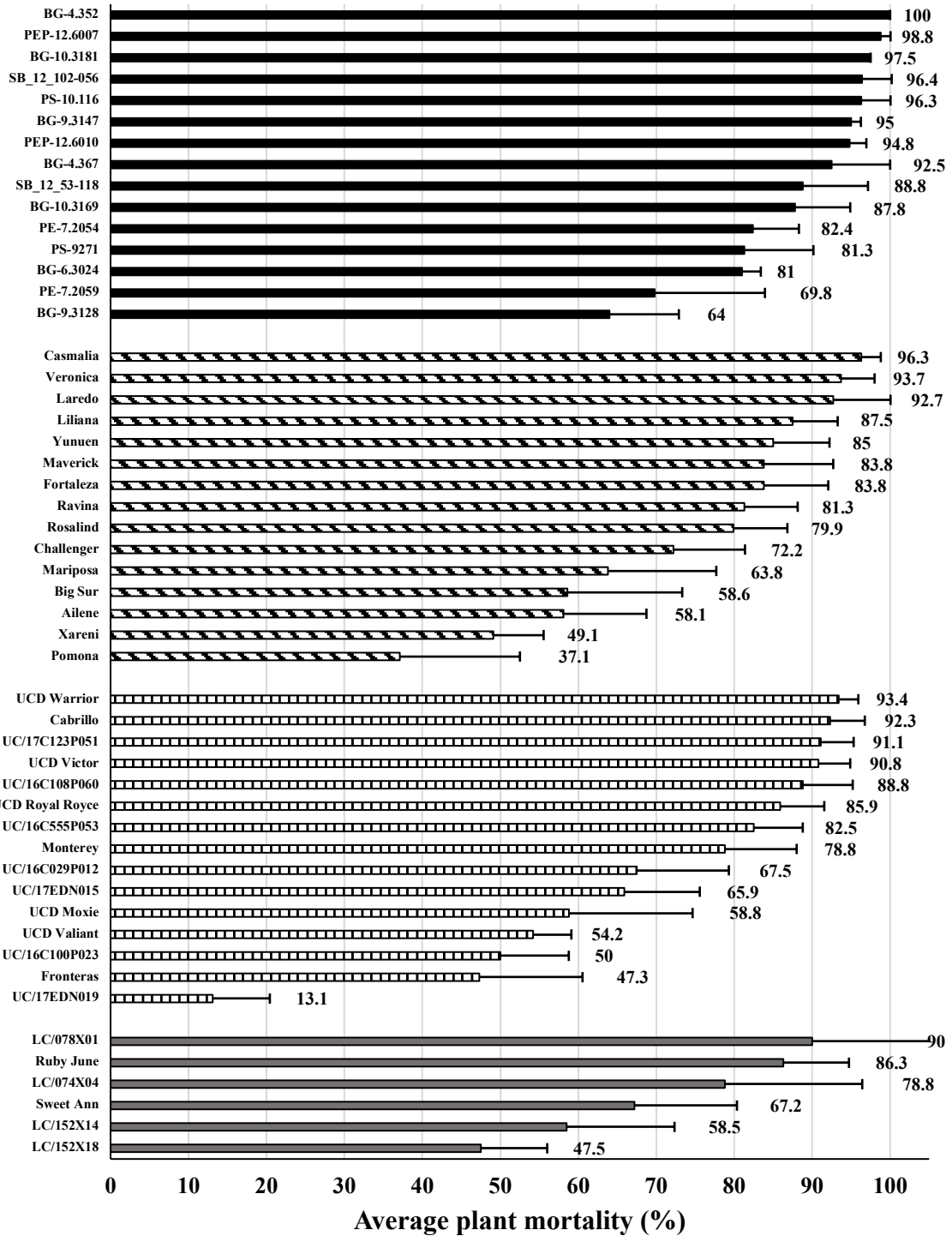
Cultivar/ Elite Breeding Line	AUDPC[w]					
	2020-2021			2021-2022		
	Mean	SE	rAUDPC[v]	Mean	SE	rAUDPC[v]
Mojo	-	-	-	14.0	9.9	0.00
17C138P021	-	-	-	14.0	14.0	0.00
17C242P023	-	-	-	17.5	17.5	0.00
002Y03	-	-	-	31.5	10.5	0.00
UCD_Moxie	-	-	-	38.5	12.0	0.00
BG-9.3128	-	-	-	38.5	20.9	0.00
16C029P012	-	-	-	45.5	12.0	0.00
15-105R	-	-	-	45.5	12.0	0.00
Monterey	-	-	-	70.0	49.5	0.00
Sierra	-	-	-	84.0	19.0	0.00
PS-10.1160	-	-	-	111.9	20.5	0.01
PS-9271	-	-	-	119.0	35.5	0.01
UCD_Valiant	-	-	-	122.5	64.0	0.01
PE-7.2054	-	-	-	140.0	60.5	0.01
SBS-12.102.056	-	-	-	150.4	85.9	0.01
Mariposa	-	-	-	150.5	25.2	0.01
BG-4.352	-	-	-	164.4	51.5	0.01
PEP-12.6010	-	-	-	164.5	106.4	0.01
Petra	-	-	-	178.5	75.5	0.01
Big Sur	-	-	-	178.5	54.6	0.01
Sweet Ann	-	-	-	185.5	66.3	0.01
PE-7.2059	-	-	-	192.5	86.8	0.01
143T35	-	-	-	203.0	64.0	0.01
Minerva	-	-	-	223.8	55.5	0.01
UCD_Royal Royce	-	-	-	262.5	15.5	0.01
Medusa	-	-	-	262.5	107.0	0.01
17C121P097	-	-	-	273.0	77.6	0.01
Purisima	-	-	-	276.4	74.3	0.01
146T54	-	-	-	276.5	60.0	0.01
145T39	-	-	-	301.0	78.9	0.01
122X08	-	-	-	304.5	82.9	0.01
16C108P060	-	-	-	311.5	89.3	0.01

BG-4.367	-	-	-	318.5	89.7	0.01
BG-10.3169	-	-	-	322.0	125.6	0.01
Sayulita	-	-	-	329.0	46.3	0.01
BG-10.3181	-	-	-	329.0	88.3	0.01
Finn	-	-	-	329.3	83.7	0.01
17C138P062	-	-	-	336.0	95.0	0.01
Salma	-	-	-	339.5	111.6	0.01
18-006R	-	-	-	346.5	131.8	0.02
16C555P053	-	-	-	350.0	144.9	0.02
152X15	-	-	-	353.5	74.4	0.02
193W33	-	-	-	364.0	115.6	0.02
Dayana	-	-	-	374.4	136.1	0.02
UCD_Victor	-	-	-	399.0	103.6	0.02
Camila	-	-	-	461.9	116.1	0.02
Maverick	-	-	-	469.0	65.0	0.02
UCD_Warrior	-	-	-	493.5	100.7	0.02
18-145R	-	-	-	574.0	168.5	0.02
BG-6.3024	-	-	-	580.9	135.8	0.03
BG-9.3147	-	-	-	601.8	121.3	0.03

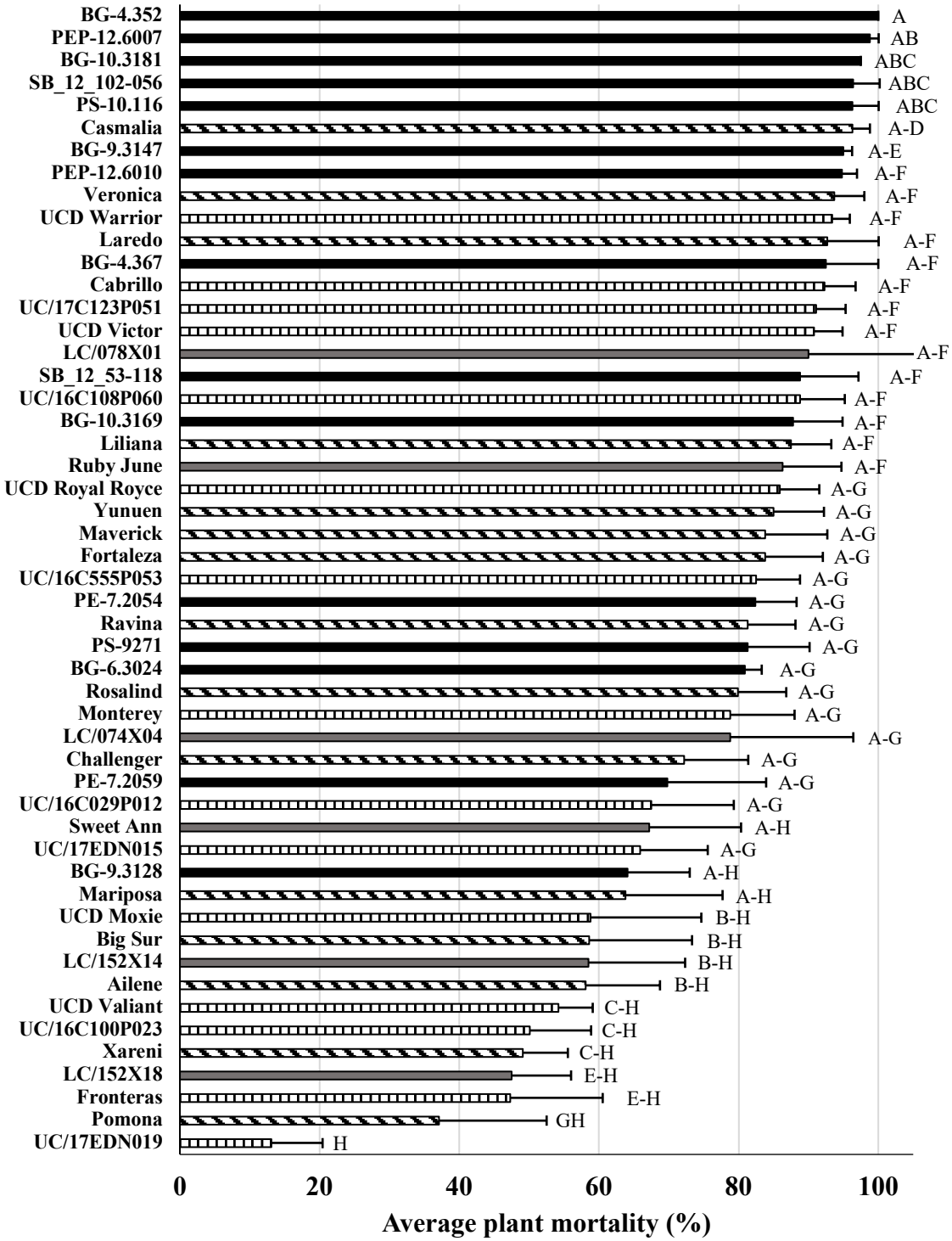
[w] AUDPC = Area under the disease progress curve during the season ratings (18 observations).
[v] rAUDPC = Relative area under the disease progress curve.
"-" no data available for this year of the trial.



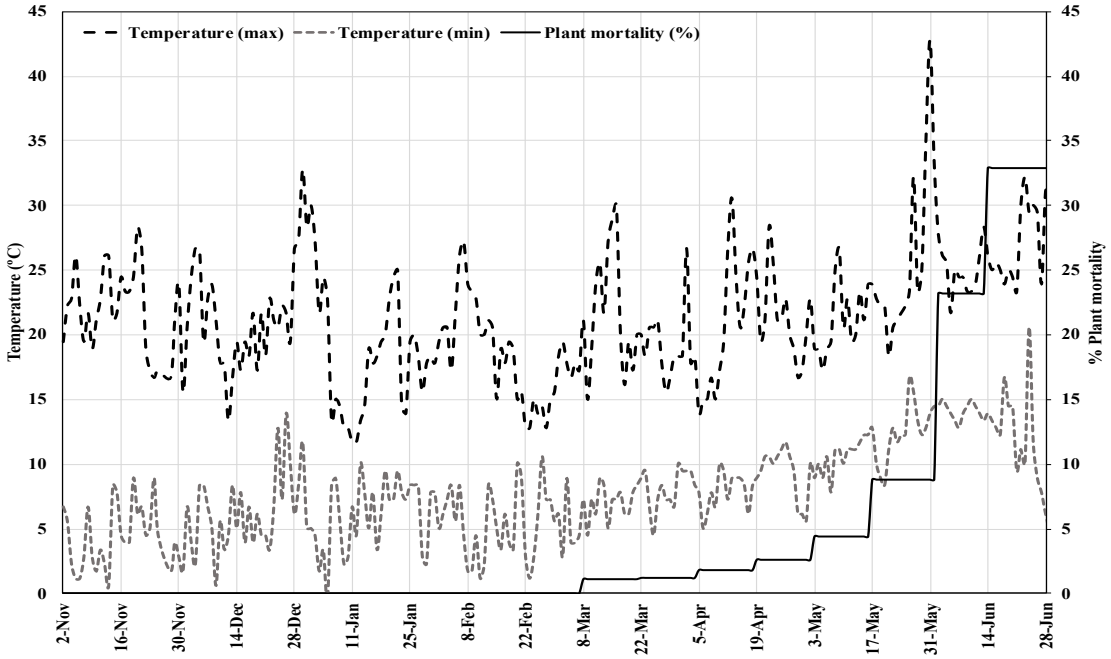
3-1. Number of new plant mortalities across all genotypes over time due to Verticillium wilt for year 1 (black) and year 2 (grey) of the trial.



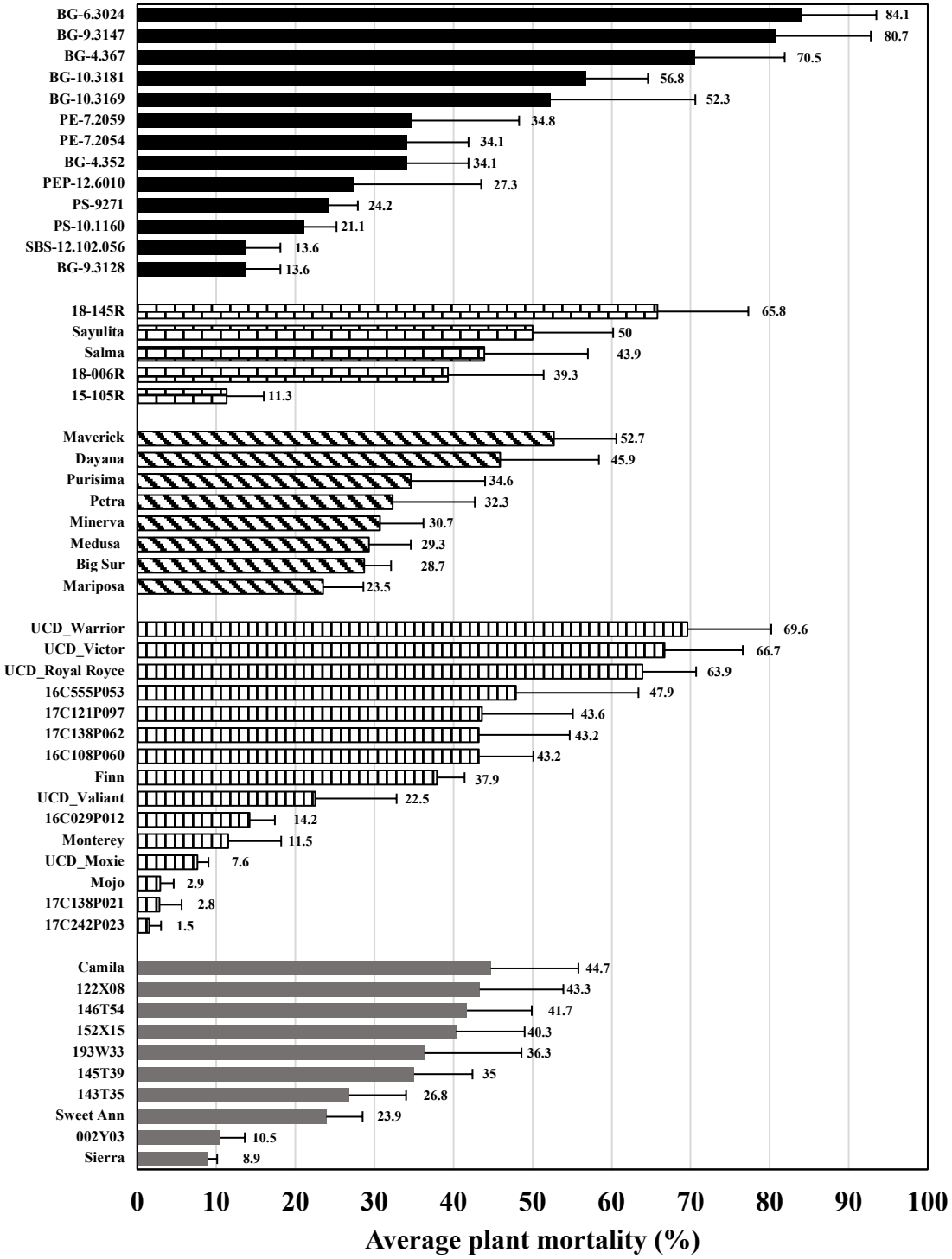
3-2. Average plant mortality due to Verticillium wilt as of 26 July 2021 (266 days after transplant) from highest mortality to lowest mortality (by breeding program). Average values were calculated by percent mortality of four replicate plots. Error bars represent standard error of the mean.



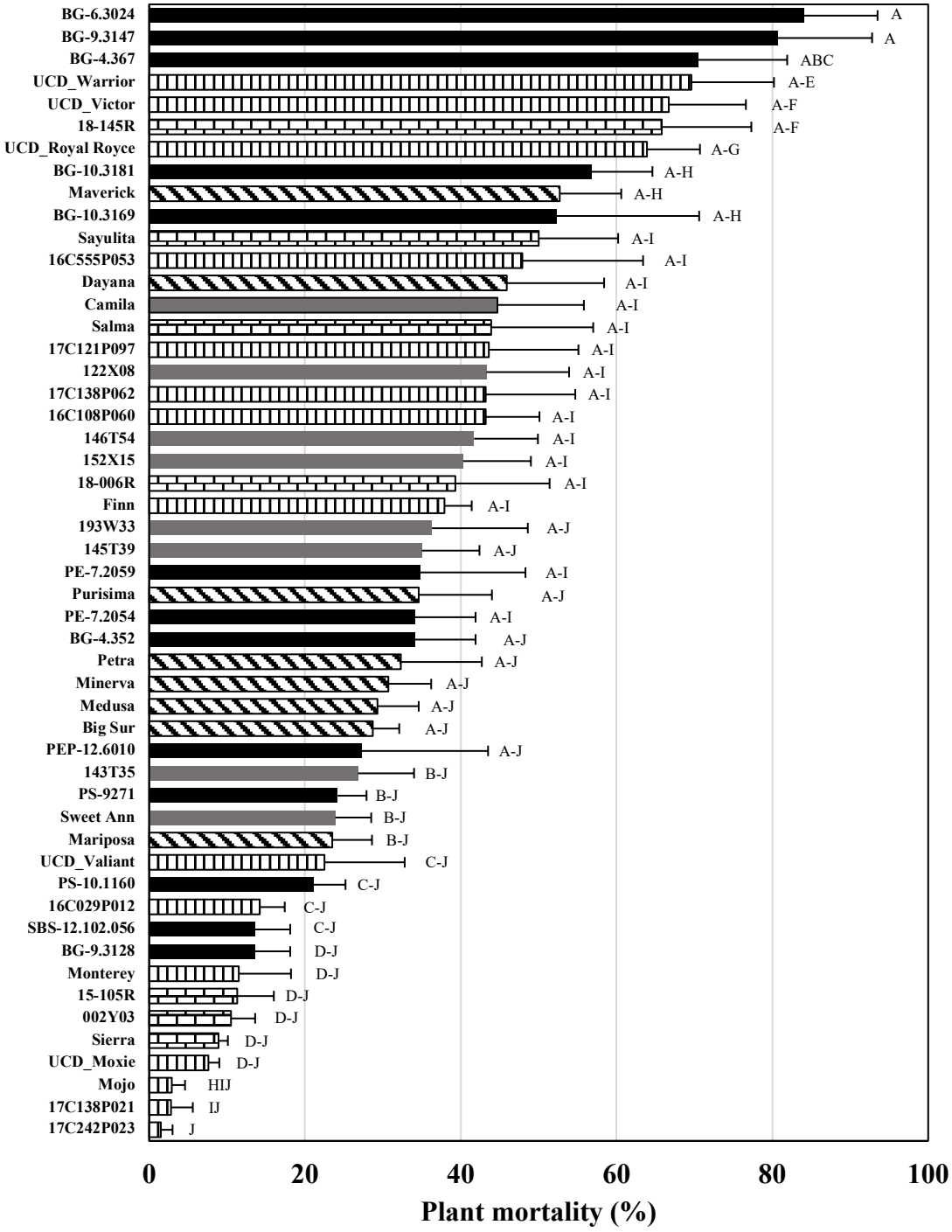
3-3. Average plant mortality due to Verticillium wilt as of 26 July 2021 (266 days after transplant). Average values were calculated by percent mortality of four replicate plots. Error bars represent standard error of the mean. Genotypes not connected by the same letter are significantly different ($F = 5.79$, $P < 0.001$).



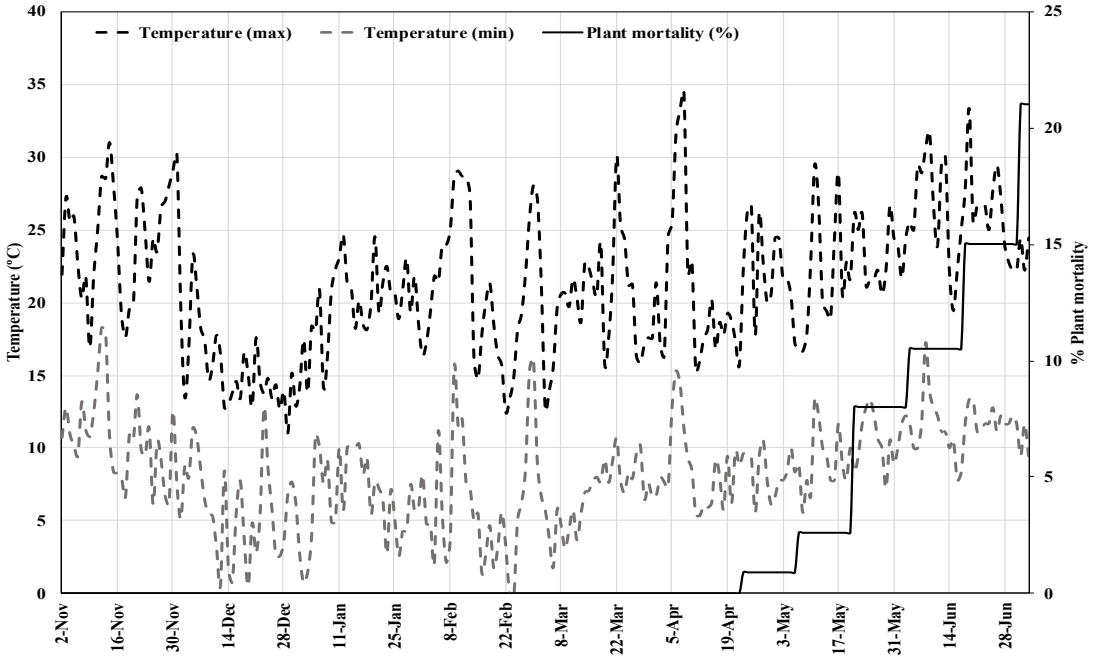
3-4. Maximum/minimum air temperature and overall plant mortality percentage during the 2020-2021 growing season. Average daily maximum temperature during the trial period was 21.2° C.



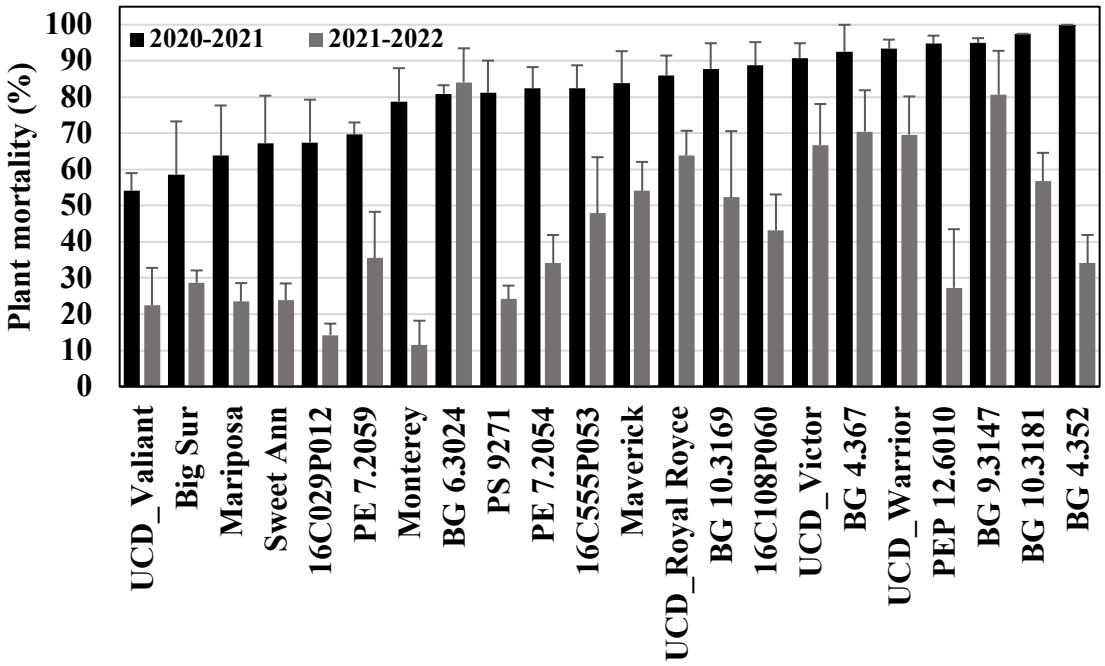
3-5. Average plant mortality due to Verticillium wilt as of 18 July 2022 (258 days after transplant) from highest mortality to lowest mortality (by breeding program). Average values were calculated by percent mortality of four replicate plots. Error bars represent standard error of the mean.



3-6. Average plant mortality due to Verticillium wilt as of 18 July 2022 (258 days after transplant) from highest mortality to lowest mortality. Average values were calculated by percent mortality of four replicate plots. Error bars represent standard error of the mean. Genotypes not connected by the same letter are significantly different ($F = 5.49$, $P < 0.001$).



3-7. Maximum/minimum air temperature and overall plant mortality percentage during the 2021-2022 growing season. Average daily maximum temperature during the trial period was 21.4°C.



3-8. Percent plant mortality due to Verticillium wilt for genotypes common to the 2020-21 and 2021-22 growing seasons as of 26 July 2021 and 18 July 2022. Error bars represent standard error of the mean.

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