UNIVERSITY OF CALIFORNIA
RIVERSIDE

Influence of Soil Moisture and Location of Infection on Development of Macrophomina Charcoal Rot of Strawberry

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Plant Pathology

by

Lindsey Rose Pedroncelli

March 2023

Dissertation Committee:
Dr. Alexander I. Putman, Chairperson
Dr. Patricia Manosalva
Dr. James E. Adaskaveg

The Dissertation of Lindsey Rose Pedroncelli is approved:

_____

_____

_____

Committee Chairperson

University of California, Riverside

ACKNOWLEDGEMENTS

When I began my Ph.D. journey, I knew that I would learn a lot about plant pathology, conduct interesting research, make and learn from mistakes, and meet many driven, intelligent people. What I didn't know, however, was the level at which I would experience all these things or how much I would learn about myself along the way. I have changed and grown a lot over the last several years and I am really proud of myself for not giving up. But I did not do this on my own. I would not have made it to this point without the village of supporters, advisors, friends, and family who were there for me every step of the way.

First, I would like to express my gratitude towards my advisor, Dr. Alexander Putman. Thank you for accepting me into your lab and embarking on this journey with me. I learned so much from you about conducting research and doing thorough science, but I also learned how to be a better communicator, mentor, leader, and manager. Thank you for providing me with the space to do that. Thank you for allowing me to pursue opportunities outside of my research, especially Science to Policy, and for encouraging me to attend and present at numerous conferences and meetings. Thank you for your support and understanding, most notably during the pandemic when there were many unknowns.

Many thanks to all former and current members of the Putman Lab for their assistance with lab, greenhouse, and field work, especially with planting and processing plant samples: Valentina Valencia Bernal, Hannah Ayala, Anita Behari, Sonali Singh, Alyssa Soloria, Nader Mostowfi, Andrew Gruhn, Brian Nguyen, Alyssa Monroy,

Kristopher Turner, and Evelyn Martinez Lopez. Without your help I would not have been able to accomplish all that I did. Thank you not only for your help, but for your company in the lab.

I am very grateful to have had Patricia Manosalva and James E. Adaskaveg on my guidance committee for the past 5 years. Thank you for your assistance, input, and expertise with planning experiments and comprehending data.

Thank you, Andre Biscaro, for your irrigation management advice and general support throughout the years. Irrigation management is an art and there is so much more that goes into it than meets the eye. Thank you for your input and expertise on this crucial part of my Ph.D. research. And thank you for helping me present this research at extension meetings. This experience was invaluable to me.

Thank you, Peter Henry, for sharing your *M. phaseolina* quantification protocol and other research ideas with me over the years. Being able to bounce ideas back and forth and develop projects with you was extremely helpful to me.

Thank you very much to everyone who helped make the field experiment possible. Thank you to AG Kawamura, Carlos Rangel, and Mark Lopez of Orange County Produce and the Orange County Farm Bureau for helping us prepare and bed-up the field with your equipment. Thank you to Romain Langlois at Hortau for installing and helping maintain the tensiometers used in the field. I am also extremely grateful for everyone at UCANR South Coast Research and Extension Center in Irvine, California: Darren Haver, Chris Martinez, George Nieblas, Eric Palmer, and Naomi Robles, thank you for helping to set up and maintain the experiment, and especially for responding to

my almost daily irrigation requests. This project was very demanding, but you were always there to help in any way possible.

I had the opportunity to organize and participate in many outreach and education events throughout graduate school. These events are some of the highlights of my graduate school experience. Thank you to Tammy Majcherek and Jason Suppes at South Coast REC for supporting my outreach endeavors and helping me organize field days and events. Thank you so much to Andrea Machuca of Femme and Forage for collaborating with me, supporting me, using my strawberries at your events, and inviting me to educate your audience about California agriculture and strawberries.

Thank you to Planasa, Crown Nursery LLC, and Lassen Canyon Nursery, Inc. for the plants used in my projects. And many thanks to the USDA NIFA Specialty Crop Research Initiative for funding this research.

I was very lucky to have several other mentors and advisors providing me valuable insight on my research projects over the years. Thank you to Steven Knapp, Glenn Cole, and Bruce Campopiano of the UC Davis Strawberry Breeding Program, Thomas Gordon of UC Davis, Steve Koike of TriCal Diagnostics, Frank Martin and Michael Matson of the USDA ARS, Alyssa Burkhardt, and Shashika Hewavitharana of the Cal Poly San Luis Obispo Strawberry Center.

Thank you very much to Kelly Ivors and Gerald Holmes, who mentored me while I was an undergraduate at Cal Poly San Luis Obispo. You were the first plant pathologists I ever met, and you helped me discover my passion for plant pathology. You encouraged me to go to graduate school and to pursue a Ph.D. It felt like a blessing to continue

working together on strawberries and *M. phaseolina*. I hope that we continue to stay connected.

Outside of my research, I was very involved in the Science to Policy Program at UCR. I am very grateful to be a part of the inaugural cohort of graduates from the UCR Science to Policy Graduate Certificate Program. Thank you to Susan Hackwood and Doug Brown for leading these efforts, welcoming me into the program, and fostering my passion for science policy and communication. Thank you for allowing me to pursue the aspects of science policy that strongly interested me, helping me become a strong leader, and showing me how to be a successful scientist in the policy realm. During my time in Science to Policy I gained knowledge and skills that I otherwise would have never been exposed to. I made friends and created memories that I will carry with me forever. This experience changed my life and was a major highlight of my time in graduate school.

There were many moments during graduate school when I couldn't remember why I was here. There were many moments when I wanted to give up. But there were even more moments when I was encouraged to keep going. Thank you to the people who were there for me in those moments. To my family, my friends, and my colleagues: you have kept me going through some of the darkest, most difficult moments over the last five and a half years. You have reminded me why I am doing this and why it is so important.

To my graduate school friends, my 'Cadang Cadang gang', from the moment I met you at recruitment in February of 2017, I knew we would be lifelong friends. I am

forever grateful for you all and the support we have provided each other through this process. You are family to me.

To my friends outside of graduate school, thank you for your understanding, for your unwavering support, and for reminding me that there is life outside of the lab.

To my family, especially my parents Laura and Rick, you have always provided me with all of the tools that I need to be successful, but you truly went above and beyond to support me through graduate school. I would not have made it through this without you.

A huge thank you to my husband, Chandler Gietzen, for sticking by my side through it all. I know the last five and a half years were not easy for me, and they were not easy for us, but we made it.

Lastly, thank you to my dog, Raisin, who I adopted four months into graduate school. Thank you for being the best dog a girl could ask for. No matter how long I was away from home or how short of a walk I could take her on, she was always the most excited to see me and spend time with me. She has been and continues to be my light through the darkest times.

DEDICATION

I dedicate this dissertation to Dr. Joseph Herbert Immel, Jr., Ph.D., one of my high school science teachers, who suddenly passed away in 2016. I can't remember the last time I saw you or the last time we spoke. I don't think we had ever talked about me going to graduate school, it was only a small thought it my mind when you passed in 2016. The time we spent together was short and I didn't understand the impact that having a Ph.D. scientist as a high school teacher would have on me as a young scientist. I do now.

I thought of you often during graduate school and wished I could reach out to you for advice. You unknowingly had a huge impact on me and my scientific career. I will forever be grateful for you. Thank you.

ABSTRACT OF THE DISSERTATION

Influence of Soil Moisture and Location of Infection on Development of Macrophomina Charcoal Rot of Strawberry

by

Lindsey Rose Pedroncelli

Doctor of Philosophy, Graduate Program in Plant Pathology
University of California, Riverside, March 2023
Dr. Alexander I. Putman, Chairperson

Macrophomina phaseolina is an economically important pathogen that causes charcoal rot of strawberries (*Fragaria* x *ananassa*). The disease cycle on strawberries is not well understood and there are currently very few ways to manage charcoal rot. We performed a field study to investigate the role of soil moisture in charcoal rot development and *M. phaseolina* colonization on strawberries. Bare-root transplants were inoculated or uninoculated and maintained at either a high, optimal, or low soil moisture level based on tensiometers placed in each treatment. Randomly selected plants from each treatment were sampled for pathogen colonization every 2 to 4 weeks and all plants were rated for disease severity every 2 weeks starting at symptom onset. In two seasons, low soil moisture significantly increased charcoal rot mortality among inoculated 'Monterey' and 'Fronteras' plants, but high soil moisture only significantly affected mortality among inoculated Fronteras plants in one year of the study. Among inoculated

Monterey plants, high soil moisture reduced colonization of crowns in two years of the study. Soil moisture did not influence root colonization in any year of the study. These results indicate that the role of soil moisture in colonization of *M. phaseolina* on strawberries may be cultivar dependent and the extent of pathogen colonization may not directly relate to plant mortality.

To investigate whether *M. phaseolina* directly colonizes the roots or crown of the strawberry plant, we performed a greenhouse study with Monterey strawberry plants and 3 inoculation treatments (control, root-, and crown-inoculated) sampled at 5 timepoints (3, 7, 14, 21, and 28 days post-inoculation). To quantify the number of *M. phaseolina* colonies/g root or crown tissue, tissue was dried, ground, bleached, rinsed, and poured into Petri dishes in a mixture with molten media. All plants were asymptomatic throughout the experiment, however *M. phaseolina* was detected in plant roots and crowns within 28 days after inoculation. This study begins to elucidate early events in the disease cycle of Macrophomina charcoal rot on strawberries.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

CHAPTER 1

CHAPTER 2

APPENDIX A

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

APPENDIX B

APPENDIX C

GENERAL INTRODUCTION

California Strawberry Production

California is a global leader in strawberry (*Fragaria* × *ananassa*) production, accounting for nearly 90% of United States domestic supply and from 2016 to 2019 averaging $415 million in exports annually (CDFA 2017a, 2018a, 2019a, 2020). From 2016 to 2019, an average of 15,419 hectares of strawberries were grown in the state with an average farm gate value of $2.3 billion annually (CDFA 2016, 2017b, 2018b, 2019b).

Strawberry production in California primarily occurs in three regions: Salinas-Watsonville, Santa Maria Valley, and Oxnard-Ventura. The primary production season, known as fall planting, begins with planting between mid-September and mid-November followed by fruit production from winter or spring through the summer. A second production season, known as summer planting, occurs in the Santa Maria Valley and Oxnard-Ventura regions, beginning with plantings between the end of May through the end of July to produce fruit in the fall (Bolda et al. 2015).

Strawberry plants are vegetatively propagated to preserve the genetics of the cultivar (Strand 2008). Daughter plants are produced from the runners of mother plants and are grown in both high and low elevation nurseries in Northern California. Freshly dug bare-root transplants from high elevation nurseries are transported to growers around the nation for fall planting. Low elevation plants called ‘frigo’ plants are kept in cold storage and sent out to growers for summer plantings.

For many years strawberry growers used a combination of methyl bromide and chloropicrin to fumigate the soil before planting (Koike et al. 2016). This was highly

effective at managing many soilborne diseases, pests, and weeds in their fields. However, in 1990 the Montreal Protocol recognized methyl bromide as an ozone depleting chemical and planned to phase it out of use by 2005, but strawberry growers in the U.S. were given a critical use exemption that allowed them to use methyl bromide until December 2016 (USDA ERS 2000; Holmes et al. 2020). Additionally, strawberry nurseries were granted a quarantine and pre-shipment exemption, allowing them to use methyl bromide to limit the spread of soilborne pathogens and pests (Holmes et al. 2020). As counties in California began to phase-out the use of methyl bromide, strawberry growers were left with very few other management options. Because of this, several new and re-emerging diseases began to appear in strawberry production regions (Koike et al. 2013).

Disease Discovery

In 2006, a few years after beginning to phaseout the use of methyl bromide, strawberry growers in Orange County, CA started noticing an increase in the number of plants collapsing and dying (Koike 2008). Plants were stunted and the older, outer leaves of the plant wilted and became necrotic while the inner, younger leaves of the plant remained green and alive. When the crown of the plant was cut open it appeared rotted with orange to brown discoloration. Two novel pathogens, *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae*, were found associated with strawberry plant collapse throughout California (Koike et al. 2013). At first, *M. phaseolina* was only reported in Ventura and Orange counties, but within five years was found in Santa Cruz, Santa Clara, Alameda, and Sacramento counties. *F. oxysporum* f. sp. *fragariae* was first

reported in Ventura County, but within four years was found in Monterey County. Fields that were no longer being treated with the combination of methyl bromide and chloropicrin were found to be most affected by these two novel pathogens.

Macrophomina charcoal rot was first reported on strawberries in Illinois in 1958 (Tweedy and Powell 1958). Reports of the disease on strawberries did not appear again until 2005, when it was reported in Florida, and then in 2008 in Orange County, California (Mertely et al. 2005; Koike 2008). Since then, it has been reported in many other countries including Spain, Tunisia, Pakistan, Chile, and Israel, making charcoal rot on strawberries a global concern (Avilés et al. 2008; Hajlaoui et al. 2015; Koike et al. 2013; Qamar et al. 2019; Sánchez et al. 2013; Zveibil and Freeman 2005).

Pathogen Characteristics

Macrophomina charcoal rot is caused by *Macrophomina phaseolina* (Tassi) Goid., a fungus in the Ascomycota: Botryosphaeriaceae (Islam et al. 2012). *M. phaseolina* produces brown to grey septate hyphae and black, melanized microsclerotia, which consist of 50 to 200 hyphal cells (Kaur et al. 2012). Microsclerotia are embedded in the mycelium and range in size from 50 to 150 µm depending on the host and growth media type (Kaur et al. 2012). *M. phaseolina* isolates from strawberry have been shown to produce irregularly shaped microsclerotia that are 55 to 190 µm long and 44 to 135 µm wide (Mertely et al. 2005; Koike 2008). On solid growth media, colonies have white to gray mycelium that becomes darker with age as microsclerotia form and become embedded in the medium.

M. phaseolina produces dark, ostiolate pycnidia with hyaline, cylindrical conidia (Koike 2008; Kaur et al. 2012). Pycnidia are typically 100 to 200 µm in diameter and contain 10 to 15 µm long rod-shaped conidia. Pycnidia and conidia are not a major part of the disease cycle and are not common in culture, but are most often seen embedded in host tissue (Kaur et al. 2012).

M. phaseolina microsclerotia are believed to serve as the primary source of inoculum and are the survival structure of the fungus (Short et al. 1980; Kaur et al. 2012). Microsclerotia persist in the soil and host debris through periods of extreme temperatures, low moisture levels, and limited nutrient availability (Kaur et al. 2012). Shokes et al. (1977) investigated the effects of soil moisture on *M. phaseolina* and showed that *M. phaseolina* can grow and survive in soils that are very dry, and suggested that this gives the fungus a competitive advantage over other microbes in the soil. The ability to survive under these adverse conditions is likely a primary factor in the historic prevalence and severity of Macrophomina charcoal rot in geographic regions commonly affected by drought. However, *M. phaseolina* consistently causes losses in the irrigated strawberry production systems of California.

M. phaseolina has a large host range of about 500 plant species and has historically been a major problem on corn, sorghum, and legume crops (Young 1949; Kaur et al. 2012). Despite this large host range, there is evidence of host specificity within the population of *M. phaseolina* isolates from strawberry plants in California and Florida (Koike et al. 2016; Peres et al. 2007; Burkhardt et al. 2019). Peres et al. (2007) indicated that the population of *M. phaseolina* in Florida that infects strawberry plants is

unable to infect other crops, and vice versa. Koike et al. (2016) characterized several California isolates of *M. phaseolina* from strawberry, cantaloupe, and watermelon using 65 simple sequence repeat (SSR) markers. Almost all isolates from strawberry grouped separately from cantaloupe and watermelon. Additionally, comparative genomics has identified candidate genes present in isolates from strawberry but absent in isolates from other hosts that may be associated with host specificity (Burkhardt et al. 2019). Because of this host specificity it is possible that the behavior of *M. phaseolina* on other hosts may not be applicable to strawberry.

Management

Following the phaseout of methyl bromide as a pre-plant fumigant, strawberry growers were left with very few effective management strategies for many soilborne diseases of strawberry. As with most soilborne plant diseases, it is recommended that strawberry growers avoid fields with a history of charcoal rot and plant pathogen-free transplants. However, *M. phaseolina* is widely distributed within most strawberry production areas in California (Koike et al. 2016), which are narrow portions of land along the coast in a few coastal valleys. Strawberry nurseries are exempt from the Montreal Protocol and are allowed to use methyl bromide, therefore strawberry transplants should be pathogen-free. However, recently there was an outbreak of Phytophthora root rot associated with infected transplants, demonstrating the difficulty in growing completely pathogen-free transplants (Holmes et al. 2020).

Several alternatives to methyl bromide have been tested, however they are less effective against *M. phaseolina* compared to other soilborne pathogens (Koike et al.

2013). Alternatives to chemical fumigation, such as soil solarization and anaerobic soil disinfestation, have shown efficacy against soilborne plant pathogens, pests, and weeds, however their use at a large scale is difficult and may not be economically viable for all growers (Hartz et al. 2019; Holmes et al. 2020).

The use of crop rotations is an effective way of managing soilborne diseases. Koike et al. (2016) showed in a greenhouse study that mustard, oat, rye, fava bean, and vetch may serve as good rotation crops because they will not increase *M. phaseolina* inoculum levels in the soil. Brassica crops have been shown to effectively mitigate severity of other soilborne diseases (Subbarao et al. 2007), but are not effective at controlling *M. phaseolina* (Muramoto et al. 2016). Though there are several options for rotation crops, these crops are not always economically viable for farmers to grow for several years in rotation with strawberry. Additionally, crop rotation is not as effective as soil fumigation (Subbarao et al. 2007).

Biological control is another option that has been explored to control soilborne pathogens. Several *Trichoderma* spp. have been shown to effectively control *in vitro* growth of *M. phaseolina* and may be effective at mitigating infection on greenhouse plants, however their use can be difficult at large scales (Khalifa et al. 2019). Establishing a population of these biologicals that can be sustained over time to effectively manage soilborne pathogens is difficult and requires a lot of care and attention (Mazzola and Freilich 2017).

Host Resistance

One of the most promising ways to mitigate damage by soilborne diseases is to use cultivars that are tolerant or resistant to the pathogen. Unfortunately, currently, there are no strawberry cultivars harboring complete resistance, but commercial cultivars exhibit a range of susceptible phenotypes.

Koike et al. (2013) screened six cultivars for resistance to *M. phaseolina* and showed that 'Albion' was the most susceptible, followed by moderately susceptible 'San Andreas' and 'Camarosa', then the less susceptible 'Ventana', 'Palomar', and 'Monterey'. According to resistance screening trials conducted across several years and locations by the University of California, Davis Strawberry Breeding Program, there are no varieties currently classified as completely resistant and there are only three classified as moderately resistant: 'UCD Warrior', 'Grenada', and 'UCD Mojo' (Knapp and Cole 2018a, 2018b, 2022b, 2022c, 2022a; Cole and Knapp n.d.). Nine varieties are classified as moderately susceptible, including 'Petaluma' and 'Fronteras', and 15 varieties are classified as susceptible, including 'Monterey'. The Strawberry Center at California Polytechnic State University, San Luis Obispo has been conducting resistance screening trials on about 90 strawberry cultivars annually since 2017 and has shown that the susceptibility to *M. phaseolina* varies among study years (Table i) (Winslow et al. 2017; Ivors et al. 2018; Mansouripour et al. 2019, 2020; Wang et al. 2021).

The Strawberry Center and UC Davis trials highlight the major differences in susceptibility between cultivars and show that other factors may play a role in cultivar susceptibility to *M. phaseolina.* For example, Wang et al. (2022, *unpublished*)

investigated the epidemiology of charcoal rot using data from the Strawberry Center trials. They found a positive correlation between either soil temperature or air temperature and final plant mortality. Though soil and air temperature appear to play a role in disease development of Macrophomina charcoal rot on strawberries, soil moisture was not included as a factor and its role should be investigated further.

Although strawberry cultivars exhibit a range of susceptibility, there is little information on genetic sources of resistance to charcoal rot in strawberry. Nelson et al. (2021) identified three quantitative trait loci (QTL) in *Fragaria x ananassa* that increase resistance to *M. phaseolina* but do not confer complete resistance: *FaRMp1*, *FaRMp2*, and *FaRMp3*. *FaRMp1* and *FaRMp2* were found in commercial strawberry germplasm while the source of *FaRMp3* was found in a known resistant accession of strawberry. *FaRMp3* confers stronger resistance than *FaRMp1* and *FaRMp2*, and further studies are being conducted to assess the effects of stacking all three loci (Nelson et al. 2021). In other hosts, QTL for charcoal rot resistance have been identified in common bean and several candidate genes related to resistance were found in soybean (Hernández-Delgado et al. 2009; Coser et al. 2017). However, the genetic basis for resistance to *M. phaseolina* remains unclear.

Soil Moisture

Historically, *M. phaseolina* has been problematic in rainfed agriculture. Early reports of the disease mention that hosts with a seedling or maturity stage during cool, wet parts of the year nearly avoid becoming infected by *M. phaseolina* and that late season irrigation can help to manage disease symptoms (Young 1949). In the first report

of charcoal rot on strawberries, Tweedy and Powell (1958) noted that high temperatures and dry soil were required for disease development, and that late season irrigation may help to decrease disease severity. Recent studies have shown that disease severity is increased in plants experiencing drought stress compared to plants given an adequate amount of water (Pande et al. 1990; Diourte et al. 1995; Mayek-Pérez et al. 2002). Diourte et al. (1995) showed that sorghum plants subjected to water stress post-flowering had increased disease severity compared to plants that were irrigated for the full season. A similar study was conducted by Pande et al. (Pande et al. 1990) where plants were subjected to drought stress at various stages throughout the season. In one treatment, though drought stress was initiated at an early growth stage, disease symptoms did not appear until the sorghum plants were nearly mature, however the length of drought stress was positively correlated with disease severity. On common bean, drought stress increased disease severity and reduced the incubation period compared to fully irrigated plants (Mayek-Pérez et al. 2002). Results from these studies indicate that soil moisture influences charcoal rot development.

Zveibil et al. (2012) evaluated the effects of soil moisture and heat stress on charcoal rot development on greenhouse-grown strawberry plants. Plants were inoculated with a water suspension of *M. phaseolina* microsclerotia and were kept at either 25°C or 30°C under 12-hour day/night light conditions and were given 200 ml of water either every 4 days (not drought-stressed) or every 10 days (drought-stressed). At the end of the study there was no difference in plant mortality between drought-stressed or non-drought-stressed plants grown at either 25°C or 30°C, indicating that water stress does

not significantly affect plant mortality due to *M. phaseolina*. However, to our knowledge the influence of soil moisture on Macrophomina charcoal rot of strawberry has not been investigated in a field setting.

In addition to disease severity, increased soil moisture has been shown to reduce colonization of *M. phaseolina* on host plants. A greenhouse study on soybean and sunflower investigated the effects of soil moisture on colonization at three growth stages: seedling, flowering, and maturity (Jordaan et al. 2019). Increased soil moisture significantly reduced colonization of both soybean and sunflower stems at plant maturity. A similar study on soybean showed that root colonization by *M. phaseolina* was greater in drought-stressed plants than in fully irrigated plants, and drought stress early in the season had a strong effect on root colonization (Kendig et al. 2000). Arias et al. (2013) showed that on pine, plants kept at a high soil moisture level became infected by *M. phaseolina* but remained asymptomatic. However, no information is available on the role of soil moisture in *M. phaseolina* colonization of strawberry.

Host-Pathogen Interactions

The monocyclic disease cycle and infection process of *M. phaseolina* have been extensively studied in soybean and common bean. On soybean, it has been shown that microsclerotia germinate and form multiple strands of hyphae which grow both transversely and longitudinally along the surface of the root (Ammon et al. 1975). Hyphae form pear-shaped appressoria both on the ridges and in the depressions of the soybean roots and then use turgor pressure to break through the cell wall of the plant (Ammon et al. 1975; Bressano et al. 2010). Following penetration, fungal hyphae grow

intercellularly within roots before using turgor pressure and cell wall degrading enzymes to penetrate and disintegrate the cells and grow intracellularly (Ammon et al. 1974; Mayek-Pérez et al. 2002; Islam et al. 2012). Hyphae can then grow into the xylem and microsclerotia can develop within the xylem vessels, which leads to symptom development (Ilyas and Sinclair 1974; Rajeswari et al. 2019; Chowdhury et al. 2014; Mayek-Pérez et al. 2002). For strawberry, both the roots and the crown of the plant may be exposed to *M. phaseolina* in the soil upon transplant, however it is unclear where *M. phaseolina* is first penetrating the plant and growing during the infection process.

The timing of symptom development differs among hosts (Ilyas and Sinclair 1974; Meyer et al. 1974; Kaur et al. 2012). On soybean and common bean, symptoms may appear on seedlings, but in some cases symptoms do not appear until plants are mature (Meyer et al. 1974; Gupta et al. 2012; Abawi et al. 1990). On fall planted strawberry in California, symptoms are typically seen in the spring on mature plants that are producing a heavy fruit load, but in Florida symptoms may appear soon after planting in October and again in the spring (Koike et al. 2013; Baggio et al. 2021). First, the older, outer leaves of the plant wilt and die, while the younger leaves remain green and alive, but within a few weeks the entire plant collapses and dies.

Interestingly, though *M. phaseolina* has historically been characterized as a necrotrophic pathogen, two studies provide evidence for a hemibiotrophic lifestyle (Chowdhury et al. 2017; Schroeder et al. 2019). Chowdhury et al. (2017) investigated signaling pathways and transcriptional responses to *M. phaseolina* infection on both a susceptible and a resistant sesame variety. They showed that *M. phaseolina* goes through

a distinct biotrophic phase, followed by a transition phase, and then a necrotrophic phase. The biotrophic phase lasted 24 to 38 hours in the susceptible and resistant varieties, respectively, and was characterized by intercellular growth of thick primary hyphae in the epidermal cells. During this phase there was also an increase in expression of the biotrophic marker gene *BAS3* (biotrophy associated secreted protein). As the switch from biotrophy to necrotrophy occurred, expression of the biotrophic marker gene decreased and expression of the necrotrophic marker gene *NIP* (necrosis inducing protein) increased. The necrotrophic phase was characterized by inter- and intra-cellular growth of thin secondary hyphae that caused host tissues to become necrotic. Microsclerotia then formed on the sesame roots and eventually blocked the xylem vessels, leading to wilting of the plant.

Schroeder et al. (2019) characterized *Arabidopsis thaliana* host defense responses to *M. phaseolina* infection via mRNA-seq analysis. They found that 28% of the genes that were upregulated in *A. thaliana* during infection by *M. phaseolina* were also upregulated during infection by the biotrophic oomycete *Hyaloperonospora arabidopsidis*. Genes associated with salicylic acid signaling, which is known to be involved in the plant's immune response to biotrophic pathogen infection, were also upregulated during infection by *M. phaseolina*. These two studies are the first to suggest that *M. phaseolina* is a hemibiotroph, however this has not been investigated in populations affecting strawberry.

To develop management strategies that are accessible and beneficial to strawberry growers, more information on the disease cycle of charcoal rot on strawberries is needed.

Having a better understanding of the host-pathogen interactions within this pathosystem will also help researchers breed tolerant and resistant strawberry cultivars. The main objectives of this dissertation research were to: i) elucidate the role of soil moisture in colonization of strawberry plants by *M. phaseolina* and investigate the use of irrigation schedules as a tool to manage soil moisture and symptoms of Macrophomina charcoal rot of strawberries, and ii) quantify the temporal dynamics of early events during the infection process of *M. phaseolina* on strawberry roots and crowns.

LITERATURE CITED

Abawi, G. S., Corrales, P., and Marcial, A. 1990. Root rots of beans in Latin America and Africa: Diagnosis, research methodologies, and management strategies. Cent. Int. Agric. Trop. 114.

Ammon, V., Wyllie, T. D., and Brown, M. F. 1974. An ultrastructural investigation of pathological alterations induced by *Macrophomina phaseolina* (Tassi) Goid in seedlings of soybean, *Glycine max* (L.) Merrill. Physiol. Plant Pathol. 4:1–4.

Ammon, V., Wyllie, T. D., and Brown, M. F. 1975. Investigation of the infection process of *Macrophomina phaseolina* on the surface of soybean roots using scanning electron microscopy. Mycopathologia. 55:77–81.

Arias, S. G., Pons, R. R., and Von Stowasser, E. S. 2013. Temporal analysis of charcoal root rot in forest nurseries under different pathogen inoculum densities and soil moisture content. Trop. Plant Pathol. 38:179–187.

Avilés, M., Castillo, S., Bascon, J., Zea-Bonilla, T., Martín-Sánchez, P. M., and Pérez-Jiménez, R. M. 2008. First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathol. 57:382–382.

Baggio, J. S., Ruschel, R. G., Noling, J. W., and Peres, N. A. 2021. Physical, cultural, and chemical alternatives for integrated management of charcoal rot of strawberry. Plant Dis. 105:295–304.

Bolda, M., Surendra K. Dara, Fallon, J., Sanchez, M., and Peterson, K. 2015. Strawberry Production Manual For Growers on the Central Coast. Available at: https://ucanr.edu/sites/santabarbaracounty-new/files/228579.pdf.

Bressano, M., Lorena Giachero, M., Luna, C. M., and Ducasse, D. A. 2010. An in vitro method for examining infection of soybean roots by *Macrophomina phaseolina*. Physiol. Mol. Plant Pathol. 74:201–204 Available at: http://dx.doi.org/10.1016/j.pmpp.2009.12.003.

Burkhardt, A. K., Childs, K. L., Wang, J., Ramon, M. L., and Martin, F. N. 2019. Assembly, annotation, and comparison of *Macrophomina phaseolina* isolates from strawberry and other hosts. BMC Genomics. 20:1–18.

CDFA. 2017a. California Agricultural Exports 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017AgExports.pdf.

CDFA. 2018a. California Agricultural Exports 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgExports.pdf.

CDFA. 2019a. California Agricultural Exports 2018-2019. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/AgExports2018-2019.pdf.

CDFA. 2020. California Agricultural Exports 2019-2020. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf.

CDFA. 2016. California Agricultural Statistics Review 2015-2016. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016Report.pdf.

CDFA. 2017b. California Agricultural Statistics Review 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016-17AgReport.pdf.

CDFA. 2018b. California Agricultural Statistics Review 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgReport.pdf.

CDFA. 2019b. California Agricultural Statistics Review 2018-2019. Available at: https://www.cdfa.ca.gov/statistics/PDFs/2018-2019AgReportnass.pdf.

Chowdhury, S., Basu, A., and Kundu, S. 2017. Biotrophy-necrotrophy switch in pathogen evoke differential response in resistant and susceptible sesame involving multiple signaling pathways at different phases. Sci. Rep. 7:1–17 Available at: http://dx.doi.org/10.1038/s41598-017-17248-7.

Chowdhury, S., Basu, A., Ray Chaudhuri, T., and Kundu, S. 2014. In-vitro characterization of the behaviour of *Macrophomina phaseolina* (Tassi) Goid at the rhizosphere and during early infection of roots of resistant and susceptible varieties of sesame. Eur. J. Plant Pathol. 138:361–375.

Cole, G., and Knapp, S. J. Disease Resistance and Yield. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/SitePages/Breeding.aspx#disease-resistance-and-yield.

Coser, S. M., Chowda Reddy, R. V., Zhang, J., Mueller, D. S., Mengistu, A., Wise, K. A., et al. 2017. Genetic architecture of charcoal rot (*Macrophomina phaseolina*) resistance in soybean revealed using a diverse panel. Front. Plant Sci. 8:1–12.

Diourte, M., Starr, J. L., Jeger, M. J., Stack, J. P., and Rosenow, D. T. 1995. Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water stress on disease development in sorghum. Plant Pathol. 44:196–202.

Gupta, G. K., Sharma, S. K., and Ramteke, R. 2012. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). J. Phytopathol. 160:167–180.

Hajlaoui, M. R., Mnari-Hattab, M., Sayeh, M., Zarrouk, I., Jemmali, A., and Koike, S. T. 2015. First report of *Macrophomina phaseolina* causing charcoal rot of strawberry in Tunisia. New Dis. Reports. 32:14.

Hartz, T. K., DeVay, J. E., and Elmore, C. L. 2019. Solarization is an effective soil disinfestation technique for strawberry production. HortScience. 28:104–106.

Hernández-Delgado, S., Reyes-Valdés, M. H., Rosales-Serna, R., and Mayek-Pérez, N. 2009. Molecular markers associated with resistance to *Macrophomina phaseolina* (Tassi) Goid. in common bean. J. Plant Pathol. 91:163–170.

Holmes, G. J., Mansouripour, S. M., and Hewavitharana, S. S. 2020. Strawberries at the crossroads: Management of soilborne diseases in California without methyl bromide. Phytopathology. 110:956–968.

Ilyas, M. B., and Sinclair, J. B. 1974. Effects of plant age upon development of necrosis and ocurrence of intraxylem sclerotia in soybean infected with *Macrophomina phaseolina*. Phytopathology. 64:156–157.

Islam, M. S., Haque, M. S., Islam, M. M., Emdad, E. M., Halim, A., Hossen, Q. M. M., et al. 2012. Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics. 13:1.

Ivors, K., Winslow, J., Mansouripour, M., Gomez, B., Blauer, K., and Holmes, G. 2018. Evaluating Host Resistance to Macrophomina Crown Rot (Macrophomina phaseolina) in Strawberry - 2018. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Stop 4 Macrophomina cv. - Winslow.pdf.

Jordaan, E., van der Waals, J. E., and McLaren, N. W. 2019. Effect of irrigation on charcoal rot severity, yield loss and colonization of soybean and sunflower. Crop Prot. 122:63–69 Available at: https://doi.org/10.1016/j.cropro.2019.04.026.

Kaur, S., Dhillon, G. S., Brar, S. K., Vallad, G. E., Chand, R., and Chauhan, V. B. 2012. Emerging phytopathogen *Macrophomina phaseolina*: Biology, economic importance and current diagnostic trends. Crit. Rev. Microbiol. 38:136–151.

Kendig, S. R., Rupe, J. C., and Scott, H. D. 2000. Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. Plant Dis. 84:895–900.

Khalifa, E.-S. Z., Amer, G. A.-A., Bakr, R. A., and Hamad, A. S. 2019. Bio-control efficacy of Trichoderma against strawberry charcoal rot disease. Egypt. J. Crop Prot. 14:1–10.

Knapp, S. J., and Cole, G. 2018a. Day-neutral cultivar advancement & research update. Available at: http://knapp.plantsciences.ucdavis.edu/wp-content/uploads/2018/05/UCD-DAY-NEUTRAL-Cultivar-Strawberry-Field-Day-Handout-3-29-2018.pdf.

Knapp, S. J., and Cole, G. 2018b. Short-day cultivar advancement & research update. Available at: http://knapp.plantsciences.ucdavis.edu/wp-content/uploads/2018/05/UCD-SHORT-DAY-Cultivar-Strawberry-Field-Day-Handout-3-29-2018.pdf.

Knapp, S. J., and Cole, G. 2022a. UC-Davis Strawberry Breeding Program Oxnard field day short-day cultivar advancement and research update, Oxnard, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD SHORT-DAY Cultivar Strawberry Field Day Handout Oxnard.pdf?csf=1&web=0&cid=a224dfbc-06d9-4d07-892a-b8dababdc2f5.

Knapp, S. J., and Cole, G. 2022b. UC Davis Strawbery Breeding Program day-neutral cultivar advancement, Prunedale, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD DAY-NEUTRAL Cultivar Strawberry Field Day Handout Prunedale.pdf?csf=1&web=0&cid=f0b6100e-70f6-40f5-9639-9aa215e894e0.

Knapp, S. J., and Cole, G. 2022c. UC Davis Strawbery Breeding Program day-neutral cultivar advancement, Santa Maria, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD DAY-NEUTRAL Cultivar Strawberry Field Day Handout Santa Maria.pdf?csf=1&web=0&cid=bd902b6a-3868-449b-9362-473a68343048.

Koike, S. T. 2008. Crown rot of strawberry caused by *Macrophomina phaseolina* in California. Plant Dis. 92:1253.

Koike, S. T., Arias, R. S., Hogan, C. S., Martin, F. N., and Gordon, T. R. 2016. Status of *Macrophomina phaseolina* on strawberry in California and preliminary characterization of the pathogen. Int. J. Fruit Sci. 16:148–159 Available at: http://dx.doi.org/10.1080/15538362.2016.1195313.

Koike, S. T., Gordon, T. R., Daugovish, O., Ajwa, H., Bolda, M., and Subbarao, K. 2013. Recent developments on strawberry plant collapse problems in California caused by Fusarium and Macrophomina. Int. J. Fruit Sci. 13:76–83.

Mansouripour, S. M., Blauer, K. A., and Holmes, G. J. 2019. Evaluating Host Resistance to Macrophomina Crown Rot in Strawberry - 2019. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/4_Macrophomina - Mansouripour.pdf.

Mansouripour, S. M., Blauer, K. A., and Holmes, G. J. 2020. Evaluating Host Resistance to Macrophomina Crown Rot in Strawberry - 2020. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/documents/06MANSOURIPOUR_Macrophomina_Field Day handout_2020.pdf.

Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J. A., and Simpson, J. 2002. Water relations, histopathology and growth of common bean (*Phaseolus vulgari*s L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol. Mol. Plant Pathol. 60:185–195.

Mazzola, M., and Freilich, S. 2017. Prospects for biological soilborne disease control: Application of indigenous versus synthetic microbiomes. Phytopathology. 107:256–263.

Mertely, J., Seijo, T., and Peres, N. 2005. First report of *Macrophomina phaseolina* causing a crown rot of strawberry in Florida. Plant Dis. 89:434 Available at: http://dx.doi.org/10.1094/PD-89-0434A.

Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1974. Factors affecting charcoal rot of soybean seedlings. Phytopathology. 64:845–849.

Muramoto, J., Shennan, C., Zavatta, M., Baird, G., Toyama, L., and Mazzola, M. 2016. Effect of anaerobic soil disinfestation and mustard seed meal for control of charcoal rot in California strawberries. Int. J. Fruit Sci. 16:59–70 Available at: http://dx.doi.org/10.1080/15538362.2016.1199993.

Nelson, J. R., Verma, S., Bassil, N. V., Finn, C. E., Hancock, J. F., Cole, G. S., et al. 2021. Discovery of three loci increasing resistance to charcoal rot caused by *Macrophomina phaseolina* in octoploid strawberry. G3. 11.

Pande, S., Mughogho, L. K., and Karunakar, R. I. 1990. Effect of moisture stress, plant population density and pathogen inoculation on charcoal stalk rot of sorghum. Ann. Appl. Biol. 116:221–232.

Peres, N. A., Baggio, J. S., and Mertely, J. C. 2007. Charcoal Rot of Strawberries Caused by Macrophomina phaseolina. Available at: https://edis.ifas.ufl.edu/publication/PP161.

Qamar, M. I., Ghanzafar, M. U., Abbas, M. F., Zainab, R., Andleeb, S., Shafiq, M., et al. 2019. First report of *Macrophomina phaseolina* causing crown and root rot on strawberry in Sargodha, Pakistan. Plant Dis. 103:1420.

Rajeswari, P., Angappan, K., Sivakumar, M., and Vijayalakshmi, D. 2019. Histopathology of *Macrophomina phaseolina* infested mulberry (*Morus* spp.) root under varied soil moisture regime. Int. J. Chem. Stud. 7:4044–4048.

Sánchez, S., Gambardella, M., Henríquez, J. L., and Díaz, I. 2013. First report of crown rot of strawberry caused by *Macrophomina phaseolina* in Chile. Plant Dis. 97:996.

Schroeder, M. M., Lai, Y., Shirai, M., Alsalek, N., Tsuchiya, T., Roberts, P., et al. 2019. A novel Arabidopsis pathosystem reveals cooperation of multiple hormonal response-pathways in host resistance against the global crop destroyer *Macrophomina phaseolina*. Sci. Rep. 9:1–14 Available at: http://dx.doi.org/10.1038/s41598-019-56401-2.

Shokes, F. M., Lyda, S. D., and Jordan, W. R. 1977. Effect of water potential on the growth and survival of *Macrophomina phaseolina*. Phytopathology. 77:239–241.

Short, G. E., Wyllie, T. D., and Bristow, P. R. 1980. Survival of *Macrophomina phaseolina* in soil and residues of soybean. Phytopathology. 70:13–17.

Strand, L. 2008. Integrated Pest Management for Strawberries Second Edition. University of California Division of Agriculture and Natural Resources.

Subbarao, K. V., Kabir, Z., Martin, F. N., and Koike, S. T. 2007. Management of soilborne diseases in strawberry using vegetable rotations. Plant Dis. 91:964–972.

Tweedy, B., and Powell, D. 1958. Charcoal rot on strawberry in Illinois. Plant Dis. Report. 42:107.

USDA ERS. 2000. Economic implications of the methyl bromide phaseout.

Wang, Y., Hewavitharana, S. S., and Holmes, G. J. 2021. Evaluating host resistance to Macrophomina crown rot in strawberry - 2021. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/J. Macrophomina Field Day 2021.pdf.

Winslow, J., Brantley, R., Holmes, G., and Ivors, K. 2017. Evaluating host resistance to Macrophomina crown rot (*Macrophomina phaseolina*) in Strawberry. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Field Day Handout Mp Host Resistance V6 updated.pdf.

Young, P. A. 1949. Charcoal rot of plants in East Texas. ed. TEXAS Agricultural Experiment Station. Available at: https://www.cabdirect.org/cabdirect/abstract/19501700783.

Zveibil, A., and Freeman, S. 2005. First report of crown and root rot in strawberry caused by *Macrophomina phaseolina* in Israel. Plant Dis. 89:1014.

Zveibil, A., Mor, N., Gnayem, N., and Freeman, S. 2012. Survival, host-pathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. Plant Dis. 96:265–272.

Table i. Average percent plant mortality due to Macrophomina charcoal rot at the end of each season of the cultivar resistance screening trials conducted at the Strawberry Center at California Polytechnic State University, San Luis Obispo[a].

	Average Percent Mortality in Each Trial Year				
Cultivar	2017	2018	2019	2020	2021
Monterey	69.1	26.3	21.9	27.1	0.0
Petaluma	25.1	19.0	18.1	12.0	NA
Fronteras	40.3	11.3	10.6	73.3	1.6

[a] Winslow et al. 2017; Ivors et al. 2018; Mansouripour et al. 2019, 2020; Wang et al. 2021

CHAPTER 1

The Role of Soil Moisture in Disease Development of Charcoal Rot of Strawberries caused by *Macrophomina phaseolina*

ABSTRACT

Charcoal rot, caused by the soilborne fungus *Macrophomina phaseolina*, is one of the most economically important diseases affecting strawberry (*Fragaria* × *ananassa*) production in California. There are currently very few ways to manage charcoal rot, however previous studies on non-strawberry hosts have shown that proper soil moisture management can limit pathogen colonization of the plants and decrease disease severity. We performed field studies for three seasons from 2018 to 2020 in Irvine, California, to investigate the role of soil moisture in disease development and management of charcoal rot of strawberries. Bare-root transplants of two cultivars, Monterey and either Petaluma (2018–2019) or Fronteras (2019–2020 and 2020–2021), were inoculated or not inoculated (control) and maintained at either a high (5 kPa), optimal (10 kPa), or low (30 kPa alternating with 60 kPa) soil moisture level based on tensiometers placed in each treatment. Randomly selected plants from each treatment were sampled for pathogen colonization every 2 to 4 weeks and all plants were visually rated for disease severity every 2 weeks starting at symptom onset. In the 2019–2020 and 2020–2021 seasons, low soil moisture significantly increased charcoal rot mortality among the inoculated Monterey and Fronteras plants compared to optimal soil moisture by at least 12% and 25%, respectively. Among inoculated Fronteras plants in the 2019–2020 season, mortality was significantly lower in the high soil moisture treatment compared to both optimal and low. Colonization of crowns was reduced by high soil moisture among inoculated Monterey plants in two years of the study, but soil moisture did not influence root colonization in any year of the study. These results indicate that the role of soil

moisture in colonization of strawberries by *M. phaseolina* may vary among cultivars, and the extent of pathogen colonization may not directly relate to disease severity. Our results show that maintaining optimal soil moisture (≤10 kPa) can help strawberry growers avoid increased plant mortality due to charcoal rot.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is an important crop in California. Production acreage averaged 15,419 hectares, and the farm gate value averaged $2.3 billion annually from 2016 to 2019 (CDFA 2016, 2017b, 2018b, 2019b). California accounts for nearly 90% of domestic strawberry production, and from 2016 to 2019 exports averaged $415 million annually, making California a global leader in strawberry production (CDFA 2017a, 2018a, 2019a, 2020).

In 2006, California strawberry growers started noticing an increase in the number of plants collapsing and dying. Plants were stunted and the older, outer leaves of the plant wilted and became necrotic while the inner, younger leaves of the plant remained green and alive. When the crown of the plant was cut open, dark orange to brown discolored decay was observed (Koike 2008). These are symptoms of charcoal rot, a new disease affecting California strawberry production following the phaseout of methyl bromide as a preplant fumigant (Koike et al. 2013). Since the first report in Orange County, California, charcoal rot has been reported in all major strawberry production regions throughout the state, as well as Florida and several other countries, making charcoal rot of strawberries a global concern (Avilés et al. 2008; Hajlaoui et al. 2015; Koike et al. 2013; Qamar et al. 2019; Sánchez et al. 2013; Zveibil and Freeman 2005; Mertely et al. 2005).

There are very few widely effective strategies to manage charcoal rot. Crop rotation, anaerobic soil disinfestation, and biological controls are promising approaches but often do not suppress disease below economic thresholds and can be difficult to implement on a large scale. Planting disease-resistant cultivars is a strategy to manage

many plant diseases; however, susceptibility of strawberry cultivars to charcoal rot varies greatly, and there are currently no highly resistant cultivars (Holmes et al. 2020). Strawberry growers throughout the state need new ways to manage this disease.

Charcoal rot, which may also be referred to as crown rot or root rot, is caused by *Macrophomina phaseolina* (Tassi) Goid., a soilborne fungus in the Ascomycota: Botryosphaeriaceae (Islam et al. 2012). *M. phaseolina* has a very wide host range of about 500 plant species including soybeans, sorghum, and corn (Gupta et al. 2012; Islam et al. 2012; Kaur et al. 2012). Despite this wide host range, there are genomic and genetic evidence suggesting host specificity in *M. phaseolina* populations infecting strawberries in California and Florida (Burkhardt et al. 2019; Koike et al. 2016; Peres et al. 2007).

M. phaseolina produces brown to gray septate hyphae and melanized microsclerotia that are considered the primary inoculum for disease for their ability to survive in the soil and plant debris from season to season (Kaur et al. 2012; Short et al. 1980). The microsclerotia can also survive hot, dry conditions for long periods of time, historically making charcoal rot a major problem in production systems that are not irrigated or that experience drought (Kaur et al. 2012). Several studies have shown that disease incidence and severity are increased in sorghum and common bean plants experiencing water stress compared to plants given an adequate amount of water (Diourte et al. 1995; Mayek-Pérez et al. 2002; Pande et al. 1990).

Zveibil et al. (2012) performed a study on strawberries and found that there was no difference in plant mortality between water-stressed and non-water-stressed plants grown at 25°C or 30°C, indicating that water stress does not significantly affect plant

mortality due to *M. phaseolina*. However, plant mortality was higher at 30°C than at 25°C indicating that soil temperature has an effect on disease development (Zveibil et al. 2012). Sànchez et al (2019) evaluated the effects of water stress on charcoal rot development in four strawberry cultivars. They found that disease severity was higher in water-stressed plants than fully irrigated plants, but disease severity was highly dependent on cultivar. Both studies created a low soil moisture environment that caused the plants to experience water stress, however these studies were performed under greenhouse conditions, thus this still needs to be tested under field conditions.

In addition to disease severity, soil moisture has been shown to influence the growth of *M. phaseolina* in host plants. Jordaan et al. (2019) showed that increased soil moisture reduced colonization of soybean and sunflower plants by *M. phaseolina*. Kendig et al. (2000) evaluated the effects of soil moisture on colonization of soybean roots and soil microsclerotia density. Overall, low soil moisture, especially early in the season, had a strong effect on root colonization: *M. phaseolina* microsclerotia density in the roots was greater in plants that were not irrigated than in plants that were irrigated for the full season (Kendig et al. 2000). These studies suggest that soil moisture may be used as a management tool to restrict pathogen colonization, which in turn could reduce disease severity. However, it is unclear how soil moisture affects *M. phaseolina* colonization and charcoal rot development of strawberries.

The objectives of this study were to: i) elucidate the role of soil moisture in *M. phaseolina* colonization on strawberry plants, and ii) investigate the use of irrigation schedules as a tool to manage soil moisture and symptoms of charcoal rot of strawberries.

MATERIALS AND METHODS

Pathogen isolate and storage. *M. phaseolina* isolate GL1310 was obtained from a strawberry plant exhibiting symptoms of charcoal rot in Orange County, CA in 2007, and was acquired from T.R. Gordon (University of California, Davis). The virulence of this isolate was independently confirmed to be similar to isolates from the main strawberry clade of the species (F. Martin, *personal communication*). Isolate GL1310 was maintained in long-term storage by first growing on autoclaved filter paper placed on top of potato dextrose agar (PDA; BD Difco, Franklin Lakes, NJ) in a 15 mm × 100 mm Petri dish. After transferring an actively growing colony, cultures were incubated at 19°C to 22° in the dark until the filter paper was covered with microsclerotia. The filter paper was then dried in a laminar flow hood for two to three days, and stored in a paper coin envelope at 4°C.

***M. phaseolina* inoculum preparation.** To prepare inoculum, isolate GL1310 was retrieved from storage by placing a small piece of colonized filter paper onto each of three PDA Petri dishes. Dishes were sealed with a double layer of Parafilm M (PM992, Bemis Company Inc., Sheboygan Falls, WI) and incubated for one week in the dark at 19°C to 22°C. A total of 328 sub-cultures were made and incubated under the same conditions in a clear plastic storage box (176FBPC18266, Choice Foodservice Equipment Company, Layton, UT) covered with aluminum foil for three to four weeks until fully colonized with microsclerotia.

Inoculum consisted of a slurry that was prepared in batches (T. Gordon, *personal communication*). In each batch, twelve cultures of *M. phaseolina* were combined with

400 ml of autoclaved deionized water in a blender (Model WF2211214, Waring Commercial Products, Torrington, CT). Twenty milliliters of autoclaved 0.35% water agar was used to rinse the blender to recover inoculum on the sides of the blender. To this blended inoculum mixture, 1,850 ml of autoclaved 0.35% water agar was added to a final volume of 2.5 liters. Each 2.5 liter batch of inoculum slurry was poured into an autoclaved carboy and stored at 5°C until it was used the following day to inoculate plants.

Field experiment – preparation. A field study was established at the University of California Agriculture and Natural Resources South Coast Research and Extension Center in Irvine, CA. Each experimental repeat consisted of a single-cycle crop that was planted in October and terminated in June, which is the standard fall-planting schedule for the Ventura County and Southern Coast (Orange and San Diego Co.) production districts. During the 2018–2019 season, the study site was located at the north corner of a 1-ha field and commercial strawberries were planted in the remainder of the field. Commercial strawberries were not planted during the 2019–2020 and 2020–2021 seasons, and the study site was moved to the opposite end of the field to be closer to the main irrigation line. Planting and termination dates can be found in Table A-1.

Field preparation, pre-plant fertilizer application, bed formation, laying of drip tape and plastic, and plant hole punching were performed by a local contractor following standard industry practices. The study area measured about 0.02 ha and consisted of beds with 1.6 m center-to-center spacing, each with four rows of plants with a 38.1-cm spacing between plants. A 22-7-10 slow-release fertilizer at a rate of 897 kg per ha was applied

prior to laying plastic. Two drip lines (Aqua-Traxx EA550667, The Toro Company, Bloomington, MN) with a flow rate of 254 liters per min per 30 m were placed in each bed with 15 cm spacing at a depth of 3.8 cm.

Plants were established with impact sprinkler (Model 14VH, A01619, Rain Bird Corporation, Azusa, CA) irrigation for the first 4 weeks. Specific dates for these events for each season are included in Table A-1. Irrigation water samples were collected and sent to an analytical laboratory (Fruit Growers Laboratory, Inc. Santa Paula, CA) in October 2018 prior to planting and in January 2021 to determine the suitability of the water (e.g., concentration of cations, anions, and minor elements; pH; electroconductivity; and sodium absorption rate) for irrigating strawberry.

Nutrient and pest management was performed following standard commercial practices for the Southern California coast. In the 2018–2019 season, the field study received the same applications as the commercial crop. A description of fertilizer application methods during the season is provided in Appendix A, and complete lists of all fertilizer and pesticide applications are presented in Table A-2 and Table A-3, respectively. Fruit harvest was performed following standard commercial practices.

Field experiment – treatment layout. The treatment structure was a 2 (cultivar) × 2 (inoculum) × 3 (soil moisture) factorial design. The treatments were arranged in a split-plot design, with soil moisture randomly assigned to main plots and the four cultivars × inoculum levels randomly assigned to subplots. Soil moisture main plots were separated by lateral furrows that were made by manually excavating the bed with a shovel, therefore their length varied from 11 m to 12 m within the field and among

seasons. Main plots were arranged in a randomized complete block design with four replicate blocks, each of which consisted of one linear bed. Each cultivar × inoculum subplot was approximately 3 m long (variation due to aforementioned main plot length variation) and contained 30 plants. See Fig. A-1 for a diagram of the treatment arrangement and the main plot and subplot dimensions. See Fig. A-2,Fig. A-3, and Fig. A-4 for the irrigation manifold design and drip irrigation layouts.

Field experiment – soil sampling. To quantify *M. phaseolina* in the soil prior to planting, soil samples were arbitrarily taken from the field at a depth of 15 to 25 cm using a hand trowel. In the 2018–2019 and 2019–2020 seasons, one sample per block was collected. In the 2020–2021 season, one sample was collected from 10 of 12 main plots. Quantification was performed using soil impaction plating with a modified Anderson sampler (Butterfield and DeVay 1977; Appendix A).

Additional pre-plant soil samples were taken in 2018 and 2019 for agronomic soil testing. Samples were taken arbitrarily throughout the experimental plot, avoiding areas where fertilizer was visible on the surface. These samples were sent to Fruit Growers Laboratory, Inc. in Santa Paula, CA to undergo the "comprehensive soil suitability analysis," which includes content of primary, secondary, and micro-nutrients.

Field experiment – cultivars and inoculation treatments. Three cultivars were used: 'Monterey', which is considered highly susceptible, and either 'Petaluma' or 'Fronteras', which are considered moderately susceptible to charcoal rot (Table A-4; Knapp and Cole 2018b, 2018a; Ivors et al. 2018; Winslow et al. 2017). Petaluma was used in the 2018–2019 season. Fronteras replaced Petaluma for the 2019–2020 and 2020–

2021 seasons because it is much more widely planted in commercial production. Both Petaluma and Fronteras are short-day cultivars that have a similar level of susceptibility to *M. phaseolina*, therefore Fronteras was an appropriate substitute for Petaluma (Knapp and Cole 2018b, 2018a; Ivors et al. 2018; Winslow et al. 2017).

Plants without secondary roots were discarded. Uninoculated control plants were planted first into each soil moisture treatment. To inoculate plants, roots and crown of a freshly dug bare-root transplant were placed into each cell of a 36-well traditional insert (Model CN-IKN, Greenhouse Megastore, Danville, IL) in a heavy duty tray (Model CN-FLHD, Greenhouse Megastore, Danville, IL). The inoculum slurry was then poured onto the roots and crown of each plant such that they were fully covered in the slurry. After five min, plants were removed from the cells and planted.

Field experiment – soil moisture treatments. Soil moisture was maintained at or below three matric potential thresholds: high, optimal, or low corresponding to excessive, ideal, and infrequent field irrigation schedules, respectively. Tensiometers (Model HXM-80, Hortau, San Luis Obispo, CA) monitored the matric potential (soil moisture) at 15.2 cm depth at one control plant per cultivar per soil moisture treatment in the 2018–2019 season, and two control plants per cultivar per soil moisture treatment in the 2019–2020 and 2020–2021 seasons. Representative plants of similar above ground size were chosen, and to avoid edge effects, tensiometers were located under plants in the center two plant rows.

The matric potential thresholds for the high and optimal treatments were defined as 5 and 10 kPa, respectively, for all three seasons. The threshold for the low treatment

was 30 kPa for the 2018–2019 season and alternating between 30 and 60 kPa after each irrigation event in the 2019–2020 and 2020–2021 seasons (Appendix A). An irrigation event was initiated when the tensiometers indicated that the soil moisture reached the predetermined threshold. Tensiometer readings were monitored daily and the average of the two (2018–2019 season) or four tensiometers (2019–2020 and 2020–2021 seasons) per soil moisture treatment was used to determine when to irrigate each treatment (Appendix A; Fig. A-5).

The software CropManage, an online irrigation and nutrient management tool (Cahn 2012), was used to calculate water amounts for each irrigation event. The CropManage calculation is based on total crop evapotranspiration ($ET_c = ET_o \times K_c \times$ days since last irrigation; where ET_c = crop evapotranspiration, ET_o = reference evapotranspiration, and K_c = crop coefficient), leaching requirement of the soil, and total precipitation. Average precipitation, air temperature, and ET_o data (Snyder and Pruitt 1992) were obtained from the California Irrigation Management Information System (CIMIS) Irvine station (number 75) located approximately 0.4 km from the study site. The strawberry-specific crop coefficient as described in Allen et al. (1998) was implemented in CropManage. To account for variation in water use over time due to increasing biomass, CropManage adjusts the crop coefficient according to the canopy cover. In our study, we estimated canopy cover using the phone application Canopeo. The adjustable crop coefficient for strawberry was evaluated and tested in field trials in the Oxnard production district (Cahn 2012), which is expected to be similar to the conditions at the study location in Irvine, CA.

In order to maintain similar leaching fractions among treatments and avoid differences in chlorine and nitrogen concentrations in the rootzone, which can negatively affect the overall health of the plant (Hartz et al. 2018; Strand 2008), irrigation was managed with the goal of delivering the same amount of water to each treatment by the end of the crop cycle. Fertilizer meters (Model 36FMPO.75, Netafim, Fresno, CA) were used to monitor the volume of water delivered to each treatment during each irrigation event throughout each season (Fig. A-6).

To ensure the correct amount of water was delivered to each treatment during each irrigation event, a single Hortau pressure transducer was placed in the drip tape in each of the three soil moisture treatments to remotely monitor water pressure during each irrigation event. To allow the irrigator to monitor the water pressure during an irrigation event, a handheld pressure gauge was manually connected to a testing fitting (101001149, Rivulis, San Diego, CA) placed in the drip tape at the end of most main plots in each soil moisture treatment. Water pressure was maintained at approximately 55 kPa.

Field experiment – isolation incidence data. To detect *M. phaseolina* colonization of strawberry, isolations from plants were performed every 2 weeks in the 2018–2019 season and every 4 weeks in the 2019–2020 and 2020–2021 seasons. The sampling plan was designed at the beginning of each season using a random number generator to determine the individual plants that were to be sampled at each time point. To avoid edge effects, the sampling plan only included the center two plant rows.

Entire plants were sampled and stored in plastic bags at 5°C for up to 4 days. In the 2018–2019 season, 2 plants per treatment were sampled every 4 weeks post-

inoculation until soil moisture treatments were initiated and then plants were sampled every 2 weeks until the end of the season (Appendix A). In the 2019–2020 and 2020–2021 seasons, 3 plants per treatment were sampled every 4 weeks.

Roots and crowns were thoroughly rinsed with deionized water. Twenty 3-cm lengths of primary roots and eight approximately 5-mm^3 pieces of the cortex and stele of the crown were placed in separate autoclaved specimen cups. All pieces were surface disinfested in 0.5% sodium hypochlorite for 2 min and then rinsed three times for 60 s each in autoclaved deionized water. Pieces were air-dried on sterile paper towels. Five root pieces were placed on each of four plates of Sorenson's NP-10 medium, and four crown pieces were placed on one plate each of amended potato dextrose agar (PDA+++) and Sorenson's NP-10. PDA+++ was prepared by first making PDA (Appendix A), except after cooling to 52°C, molten media was amended with 0.025 g per liter each of streptomycin sulfate (CAS: 3810-74-0, Thermo Fisher Scientific, Waltham, MA), chlortetracycline hydrochloride (CAS: 64-72-2, Sigma Aldrich, St. Louis, MO), and chloramphenicol (CAS: 56-75-7, Sigma Aldrich, St. Louis, MO).

Petri dishes were double-wrapped in Parafilm and stored in clear plastic storage boxes at 20°C to 26°C under ambient fluorescent light conditions. Plates were monitored for two weeks for growth of *M. phaseolina* and other fungi. Putative colonies of *M. phaseolina* were visually examined under a stereoscope, and subcultured for further identification. A tissue piece was considered positive for colonization if at least one *M. phaseolina* colony emanated from that piece. Percent isolation incidence was based on the number of pieces positive for *M. phaseolina* of the total number of pieces plated. A

subset of *M. phaseolina* isolates obtained was stored on toothpicks and filter paper at 19°C to 22°C.

Field experiment – charcoal rot data. Charcoal rot severity ratings began once symptoms were observed in at least one plant and continued every 2 weeks until the end of the season. Plants were individually rated on a 0 to 5 ordinal rating scale where: 0 = completely healthy; 1 = slight discoloration on the leaves; and a rating of 2, 3, 4, or 5 indicated that 0 to 25%, >25 to 50%, >50 to 75%, or >75 to 100% of the total number of leaves were completely necrotic, respectively (Fig. A-7). Plant mortality was calculated by dividing the total number of plants with a severity rating of 5 in each subplot by the total number of plants in the subplot on that date, which excludes plants that died before severity ratings began and those that had been destructively sampled.

Field experiment – diagnostic samples. Later in each season, dead plants were arbitrarily sampled, and crowns were plated onto PDA+++ and NP-10 as described above to confirm *M. phaseolina* as the causal agent.

Greenhouse experiment. An experiment was established in a greenhouse at UC Riverside. Elite Nursery Containers with a volume of 8.7 liters (CN-NCE, Greenhouse Megastore, Danville, IL) were filled with a soil mixture composed of 25% sand, 25% peat, 25% redwood mulch, and 25% perlite by volume with 2.48 kg per m^3 dolomitic lime and 3.97 kg per m^3 Osmocote Smart-Release Plant Food Plus (15-9-12, The Scotts Company LLC, Marysville, OH). Each pot was placed in a tray insert (CN-FLIN, Greenhouse Megastore, Danville, IL) to collect water flowing through the pots. Pressure

compensating spray stakes (Model 22500-002030, Netafim, Fresno, CA) with a flow rate of 12.11 liters per hour were placed in each pot.

The treatment structure was 2 (cultivar) × 2 (inoculum) × 3 (soil moisture). Both Monterey and Fronteras bare-root transplants were either inoculated with a *M. phaseolina* inoculum slurry as described above for the field experiment or left uninoculated (control). Transplants from the same box of plants used in the field study in each year were used in the greenhouse. Soil moisture treatments were high, moderate, and low soil moisture, and all treatments were arranged in a randomized complete block design with five replicates. Planting and termination dates can be found in Table A-1.

The frequency and length of irrigation events were adjusted as needed throughout the season as the plants grew and water demand changed. For most of the experiment, plants in the high soil moisture treatment were irrigated once per day for one min (a water depth rate of approximately 0.1 cm per min), while plants in the moderate soil moisture treatment were irrigated every three days for one min, and plants in the low soil moisture treatment were irrigated every seven days for one min.

Osmocote Smart-Release Plant Food Plus was added to the plants following the manufacturer's instructions, runners were trimmed as needed, ripe fruit were harvested weekly, and pesticide sprays were performed as needed. Disease severity ratings were taken for all plants every 5 to 25 days using the same disease severity rating scale described above. Dead plants were processed as previously described.

Data analysis. Organization and visualization of data were performed using the *tidyverse* (Wickham et al. 2019) packages *dplyr* 1.0.8, *tidyr* 1.2.0, *ggplot2* 3.3.5, *lubridate*

1.8.0, *readr* 2.1.2, *stringr* 1.4.0, *forcats* 0.5.1, *plotly* 4.10.0, *egg* 0.4.5, *cowplot* 1.1.1, *ggpubr* 0.4.0, and *agricolae* 1.3-5 in R 4.1.2 (R Core Team 2018). Data analysis was performed using SAS/STAT software in SAS System 9.4 for Windows. The influence of main effects for isolation incidence data and mortality data were analyzed with generalized linear mixed models using PROC GLIMMIX.

Isolation incidence data was analyzed using the binomial distribution with the cumulative logit link function. All observations were adjusted by adding 1 to eliminate zeros and to allow the model to converge. Sampling date was included as a fixed effect, separate (i.e., not interacting with) from the treatment factors. For root isolations, to allow the model to run for both the 2018–2019 and 2020–2021 seasons, replicate block was not included as a random effect. For root isolations in the 2019–2020 season, "random Soil moisture*Cultivar*Inoculum / subject = Block" was included as a random effect (Madden and Kriss 2016). For crown isolations, in order for the model to run and to minimize overdispersion, no random effects were included for any season. Any significant interaction terms for root or crown were examined using the *slice* statement in PROC GLIMMIX to examine the influence of the factor of primary interest within each level of the other factor(s). Contrast statements were used to compare the optimal soil moisture treatment to either the high or low soil moisture treatments.

Plant mortality was calculated by dividing the total number of plants with a severity rating of 5 in each subplot by the total number of plants in the subplot on that date, which excludes plants that died before severity ratings began and those that had been destructively sampled. The percent plant mortality was analyzed with the binomial

distribution with the logit link function. To allow the model to run, all values were adjusted by adding 1 to eliminate zeros. Rating date was included as a fixed effect in the interaction with the treatment factors, and "random intercept / subject=Block" was included as a random effect. Significant interactions were examined using the *slice* statement. Means were compared using Tukey's honestly significant test at $P \leq 0.05$. Analysis of disease severity (the full rating scale) is described in Appendix A.

Greenhouse disease severity data was analyzed using the multinomial distribution with the cumulative logit link function. Rating date was included as a fixed effect separate from the treatment factors and block was included via the random effect term "random intercept / subject=Block" (Stroup et al. 2018). Significant interactions were analyzed using the *slice* statement and contrast statements were used to compare the optimal soil moisture treatment to either the high or low soil moisture treatments.

RESULTS

Field experiment – preparation. The strawberry irrigation suitability analysis showed that the levels of chloride and pH of the irrigation water at South Coast Research and Extension Center were high for strawberries. In 2018 and 2021, there were 126 and 138 mg chloride per liter, respectively, and the pH was 7.9 in both years, but all other element levels were considered "good" for irrigating strawberry (Table A-5).

Field experiment – soil sampling. The comprehensive soil suitability analysis performed by Fruit Growers Laboratory, Inc. showed that the field site in 2018–2019 had a sandy loam (68.5% sand, 17.7% silt, and 13.8% clay) with a pH of 7.59 and 0.86%

organic matter. Sites in the 2019–2020 and 2020–2021 seasons, had a sandy clay loam (55% sand, 17.5% silt, and 27.5% clay) with a pH of 7.73 and 1.35% organic matter.

In soil samples obtained prior to inoculation for the 2018–2019 and 2019–2020 seasons, soil plating analyses showed that no *M. phaseolina* was recovered. In samples for the 2020–2021 season, 4 CFU/g soil were recovered from one sample, which is the detection limit of the assay, and no *M. phaseolina* was detected in the other 9 samples.

Field experiment – soil moisture treatments. To maintain similar leaching fractions among treatments as described above, the high soil moisture treatment was irrigated most frequently with smaller volumes of water administered during each irrigation event, while the low soil moisture treatment was irrigated least frequently with larger volumes of water administered during each irrigation event (Table A-6). In the 2018–2019 season, more water was delivered to the high soil moisture treatment than to optimal or low (Fig. A-6). In the 2019–2020 season, the total depth of water delivered to each soil moisture treatment was similar. In the 2020–2021 season less water was delivered to the low soil moisture treatment than the high and optimal soil moisture treatments (Fig. A-6). However, this difference was apparent only at the end of the season. Despite the long intervals between events in the low soil moisture treatment, after each event the amount of water delivered in the 2020–2021 season was similar among all three treatments.

Field experiment – isolation incidence data. During both the 2019–2020 and 2020–2021 seasons, *M. phaseolina* was first isolated from a randomly sampled plant 4 weeks after inoculation and planting (Fig. A-8). On the tissue piece level across all three

seasons, *M. phaseolina* was isolated from 8% of inoculated root pieces and less than 1% of control root pieces, and 9% of inoculated crown pieces and 3% of control crown pieces. *M. phaseolina* was isolated from 55% of inoculated plants and 7% of control plants across all three seasons (data not shown).

For roots, analysis of main effects revealed strong evidence for an influence of inoculum on isolation incidence in all three seasons and a cultivar × inoculum interaction in the 2019–2020 season ($P = 0.0243$) (Table 1-1). *M. phaseolina* was isolated more frequently from inoculated roots (3% and 12%) than from control roots (<1% in both seasons) on average in the 2018–2019 and 2020–2021 seasons, respectively (Table 1-2). Slicing the 2019–2020 season cultivar × inoculum interaction by inoculum revealed an effect of cultivar for inoculated plants ($P = 0.0005$) but not for control plants ($P = 0.8529$) (Table 1-1). For inoculated plants, *M. phaseolina* was isolated approximately twice as frequently on average from Monterey roots (11%) compared to Fronteras roots (6%) (Table 1-2).

For crowns, analysis of main effects showed evidence for a soil moisture × cultivar interaction in the 2019–2020 and 2020–2021 seasons (Table 1-3). Slicing by cultivar revealed an influence of soil moisture on isolation incidence in Monterey plants but not in Fronteras plants in both seasons. This slice was examined using contrast statements comparing the effect of high or low soil moisture treatments to the optimal treatment within each cultivar. For Monterey plants in the 2019–2020 and 2020–2021 seasons, on average *M. phaseolina* was isolated significantly more frequently from crowns in the optimal soil moisture treatment (10% in both seasons) compared to the high

soil moisture treatment (7% and 2%, respectively) whereas no significant difference was observed between the low and optimal soil moisture treatments (Table 1-4).

Additionally, a significant soil moisture × inoculum interaction ($P = 0.0010$) was observed in the 2020–2021 season for crown isolations (Table 1-3). Slicing the soil moisture × inoculum interaction by inoculum showed strong evidence for an influence of soil moisture on crown isolation incidence for inoculated plants ($P = 0.0029$) and weak evidence for an influence for control plants ($P = 0.0524$). Contrast statements revealed that for inoculated plants, on average, crown isolation incidence was significantly higher in the optimal soil moisture treatment (12%) compared with the high soil moisture treatment (3%) (Table 1-4). Isolation incidence was also significantly different between the optimal (12%) and low (11%) soil moisture treatments, but the magnitude of this difference was small. For control plants, contrast statements revealed that crown isolation incidence was significantly higher in the low soil moisture treatment (7%) compared to the optimal (1%), with no difference in crown isolation incidence between the optimal and high soil moisture treatments.

In the 2019–2020 season there was also a significant effect of inoculum on crown isolation incidence (Table 1-3). *M. phaseolina* was isolated from significantly more inoculated crown pieces (16%) than control crown pieces (3%) on average (Table 1-4).

Our results suggest that colonization of roots was primarily affected by inoculation, and no significant effect of soil moisture was observed. Colonization of crowns was primarily influenced by soil moisture and cultivar, with significantly lower

isolation incidence in the high soil moisture treatment compared to the optimal, an effect which was observed in Monterey but not Fronteras.

Field experiment – charcoal rot data. Symptoms of charcoal rot were first seen on 8 May 2019, 28 Apr. 2020, and 17 Apr. 2021 of each season. On the final rating date of each season, the average mortality incidence for non-inoculated and inoculated plants was 0% and 2%, 14% and 35%, and 30% and 46% in the 2018–2019, 2019–2020, and 2020–2021 seasons, respectively (data not shown). For the 2018–2019 season, the analysis model would not converge due to low overall mortality. To allow the model to run, the first rating date was removed from the 2019–2020 and 2020–2021 trials due to very low mortality. Analysis of main effects revealed a significant soil moisture × cultivar × inoculum interaction for the 2019–2020 ($P = 0.0374$) and 2020–2021 ($P = <.0001$) seasons (Table 1-5). This interaction was examined by slicing by each cultivar × inoculum combination. Because this interaction does not include rating date, this analysis was an examination of mortality averaged over all included rating dates in each season.

In the 2019–2020 and 2020–2021 seasons, there was a significant influence of soil moisture on plant mortality among inoculated Monterey and Fronteras plants (Table 1-5). Significantly more plants died in the low soil moisture treatment compared to the optimal and high soil moisture treatments by a factor of at least 1.6 (36% or 57% incidence in the low treatment as compared to 27% or 33% in the optimal and 26% or 35% in the high in both season, respectively) for Monterey and at least 1.8 (58% or 63% incidence in the low treatment as compared to 36% or 42% in the optimal and 31% or 44% in the high in both seasons, respectively) for Fronteras (Fig. 1-1). Significantly more

plants died in the optimal compared to the high treatment (36% and 31% incidence, respectively) only for Fronteras in the 2019–2020 season, but no significant difference was observed between optimal and high for Fronteras in 2020–2021 or for Monterey in either season (Fig. 1-1).

In the 2020–2021 season, there was also a significant influence of soil moisture on plant mortality for control treatments of both Monterey ($P < 0.0001$) and Fronteras ($P = 0.0346$) (Table 1-5). In the 2020–2021 season, means separation revealed that significantly fewer non-inoculated Monterey plants died in the optimal soil moisture treatment (14%) compared to the low (28%) and high (31%) soil moisture treatments across all rating dates (Fig. 1-1). Despite a significant ($P = 0.0346$) effect of soil moisture for non-inoculated Fronteras plants, no significant differences were observed in the mean separation (Fig. 1-1).

In addition to the soil moisture × cultivar × inoculum interaction described above, significant interactions were observed for inoculum × rating date in the 2019–2020 season and soil moisture × rating date in the 2020–2021 season (Table 1-5). Each of these interactions was sliced to examine the main effect for each rating date. On all dates except for the first, significantly more inoculated plants than control plants died in the 2019–2020 season (Table A-8). In the 2020–2021 season, significantly more plants in the low soil moisture treatment died compared to the optimal and high treatments on four dates, except for May 17 when low and high moisture were statistically similar (Table A-8).

Results for mortality data were similar to those for the charcoal rot severity ratings scale (Appendix A). In summary, mortality due to charcoal rot was mainly influenced by soil moisture; there were significantly more dead inoculated Monterey and Fronteras plants in the low soil moisture compared to the optimal soil moisture treatment.

Field experiment – diagnostic samples. *M. phaseolina* was isolated from 73%, 90%, and 90% of the dead plants that were specifically sampled for diagnosis in the 2018–2019, 2019–2020, and 2020–2021 seasons, respectively (data not shown).

Greenhouse experiment. Symptoms of charcoal rot appeared in March of the 2019–2020 and 2020–2021 experiments, but plants did not start dying until April of both seasons (data not shown). In both seasons, *M. phaseolina* was isolated from all 8 inoculated plants that were sampled for diagnosis (data not shown).

Analysis of main effects revealed significant cultivar × inoculum and soil moisture × cultivar interactions for the 2019–2020 season (Table A-11). Similar to the field experiment, rating date was not included in these interactions; therefore, the following analyses represent averages across all rating dates. Slicing the cultivar × inoculum interaction by cultivar revealed a significant effect of inoculum on Monterey, but not Fronteras plants. By the final rating date, 60% of inoculated Monterey plants had died compared to 13% of Monterey control plants (data not shown). Slicing the cultivar × soil moisture interaction by cultivar revealed a significant difference among soil moisture treatments for both cultivars (Table A-11). Contrast statements were used to compare either the low or high soil moisture treatment to the moderate treatment. For both Monterey and Fronteras, there was a significant difference in disease severity between

the high and moderate soil moisture treatments, where 7% and 27% of Monterey plants and 0% and 7% of Fronteras plants died, respectively, but there was no significant difference in disease severity between moderate and low (Fig. 1-2).

In the 2020–2021 season, analysis of main effects showed evidence for a significant three-way interaction (Table A-11). The influence of soil moisture within each level of cultivar × inoculum was examined by slicing, and contrast statements were used to compare either the low or high to the moderate soil moisture treatment. Disease severity was significantly lower in the high compared to the moderate soil moisture treatments for all four cultivar × inoculum combinations (Fig. 1-2). Similarly, disease severity was significantly lower in the moderate compared to the low soil moisture treatment for all combinations except for inoculated Fronteras plants. By the final rating date of the season, 100% of inoculated Monterey and Fronteras plants in the low and moderate soil moisture treatments had died (data not shown). Among all plants in the high soil moisture treatment, only 20% of inoculated Fronteras plant died (data not shown).

Overall, charcoal rot severity of greenhouse-grown strawberries was primarily affected by soil moisture, where disease severity was significantly reduced by high soil moisture compared to optimal soil moisture.

DISCUSSION

This study shows that low soil moisture exacerbates mortality of strawberries due to charcoal rot. To our knowledge, this is the first study documenting *M. phaseolina* colonization of strawberry plants prior to symptom development at different soil moisture

levels under field conditions. We found that soil moisture does not affect *M. phaseolina* colonization of the roots, but affects colonization of the crown, and this interaction varies by cultivar.

To our knowledge, this is also the first study to evaluate the effects of increased and reduced soil moisture on charcoal rot development on strawberries. The finding that low soil moisture significantly increases plant mortality was consistent over two seasons and two cultivars in field and greenhouse studies (Table 1-6). These results are contrary to what was reported by Zveibil et al. (2012) that did not find differences in *M. phaseolina*-related plant mortality in drought-stressed versus non-drought-stressed plants. However, the latter authors conducted their study in a controlled-temperature environment and reported very high disease incidence in their experiment, with 100% mortality of plants grown at a soil temperature of 30°C within 14 days of inoculation. Disease pressure may have been too high to detect an effect of soil moisture on charcoal rot. The different methods used by Zveibil et al. (2012), including use of a soilless growth medium and inoculating by drenching the medium with a microsclerotia-water suspension, may have accounted for the high disease pressure. Our mortality and charcoal rot severity results align with what has been shown for other hosts including sorghum, soybean, and sunflower, where charcoal rot symptoms were more severe under low soil moisture conditions (Diourte et al. 1995; Jordaan et al. 2019). In our field study, however, there was no statistically significant decrease in disease severity with high soil moisture, suggesting that increasing soil moisture may not be beneficial for disease

management. Our findings highlight the importance of proper irrigation management in fields where *M. phaseolina* is present.

Soil moisture in this study was maintained for each treatment during almost the entire period between transplant establishment and crop termination. Research on other crops examined the effect of soil moisture treatments that were initiated at specific times in the crop cycle. On sorghum, for example, inoculated plants that experienced water stress post-flowering had increased disease severity compared with plants that did not experience water stress (Diourte et al. 1995). On sunflower and soybean, reduced soil moisture had more of an effect on disease severity on mature plants than plants at the seedling or flowering stages (Jordaan et al. 2019). In our study, *M. phaseolina* was isolated from roots and crowns far in advance of symptom development, regardless of soil moisture. Future research on strawberries could include soil moisture treatments that are initiated or altered at different growth stages throughout the season such as before flower production peak, during flower production peak, or at the first signs of fruit ripening.

In our greenhouse studies, there was a statistically significant decrease in disease severity with high soil moisture, but no difference between low and moderate soil moisture. In contrast, in our field studies a decrease in mortality was observed with optimal compared to low soil moisture, with no difference between optimal and high soil moisture. In the field, tensiometers monitored soil moisture and CropManage was used to determine irrigation event lengths. In the greenhouse, attempts to use tensiometers to determine irrigation timing resulted in nonsensical readings and CropManage was not

used to determine the irrigation event length because it was developed for the field only. Instead, each soil moisture treatment in the greenhouse was irrigated on a schedule that was adjusted based on visual observations. It is possible that our assessments of the soil moisture levels did not match the actual conditions and therefore, the results are not comparable between the field and greenhouse. For example, the moderate soil moisture treatment in the greenhouse may have actually been insufficient, whereas the high soil moisture treatment could have been sufficient under greenhouse conditions. Still, we observed a trend of a significant decrease in disease severity associated with an increase in soil moisture between at least two soil moisture levels in both the field and greenhouse.

In our study, pathogen colonization differed between cultivars and soil moisture treatments. Throughout the season, *M. phaseolina* was isolated from root pieces on most sampling dates, but there was no evidence for an influence of soil moisture on isolation incidence. In contrast, *M. phaseolina* was isolated from significantly fewer Monterey crown pieces in the high compared to the optimal soil moisture treatment. Kendig et al. (2000) showed that colonization of soybean roots by *M. phaseolina* was greatest when plants were not irrigated and was least when plants were irrigated for the full season. The extent of colonization differed between the two cultivars used, but overall, when irrigation was terminated at flowering, there was an increase in soil microsclerotia density and colonization of soybean roots. When irrigation was initiated at flowering, however, soil microsclerotia densities decreased (Kendig et al. 2000). Similar results were found by Jordaan et al. (2019), where reduced soil moisture favored colonization of mature soybean and sunflower stems. Although pathogen colonization occurred at all

three soil moisture levels in our study, our results indicate that colonization of the crown may be reduced by high soil moisture.

We hypothesized that reducing soil moisture would lead to increased disease severity and increased pathogen colonization, but statistically, there was no association between these two parameters. For example, low soil moisture consistently increased mortality of Fronteras plants when compared to optimal, but no significant difference was observed between these two treatments for colonization of Fronteras roots or crowns (Table 1-6). In contrast, for inoculated Monterey plants, high soil moisture reduced crown colonization in two study years when compared to optimal, but a difference in mortality was only observed between the low and optimal soil moisture treatments (Table 1-6). These results indicate that the extent of pathogen colonization may not be a major factor in determining plant mortality.

In only one year of the study, the only factor that significantly affected root colonization besides inoculation was a cultivar × inoculum interaction. Soil moisture did not influence root colonization. Given findings of root colonization of soybean (Kendig et al. 2000), our negative result may question if our pathogen isolation methods were truly detecting pathogen propagules inside the plant. To better ensure that any *M. phaseolina* being detected is not from inoculum adhering to the root surface, more intensive washing methods should be used in future studies. In other studies investigating pathogen colonization of roots, roots were washed on a rotary shaker or by vortexing (Anderson et al. 1988; Scott et al. 2014). Including agitation in the washing step could help to remove soil, inoculum, and other debris from the roots. In some studies on non-

strawberry hosts, laboratory methods using seedlings were developed to assess root colonization (Bressano et al. 2010; Chowdhury et al. 2014; Hemmati et al. 2018). Further research should be done to develop more thorough methods to assess *M. phaseolina* colonization of strawberry roots and to potentially develop a laboratory assay to study these plant-pathogen interactions.

The level of charcoal rot and *M. phaseolina* isolation we observed on uninoculated control plots in the field studies was unexpected. The increase in non-inoculated plants where *M. phaseolina* was isolated from in each season (Fig. A-8) may be explained by the location of the study in each season. The entire field was fumigated in the fall of 2016 with chloropicrin (Pic-Clor 60), but the study in the first year was conducted in an area of the field that had been inoculated with the same isolate of *M. phaseolina* for an unrelated project in 2016–2017. However, *M. phaseolina* was not detected in pre-plant soil samples for this season. In the second year of the study, the study was in a different area of the field where commercial strawberry plants were grown the year prior. The third year of the study was located in the same area as the second year, and this was the only year that *M. phaseolina* was detected in pre-plant soil samples. Although the inoculum level required to cause disease on strawberry is unclear, it has been shown that infested strawberry debris can serve as an inoculum source in the following season (Baggio et al. 2019). In our study, it is possible that inoculum may have remained in the soil from the second to the third year, making it likely that uninoculated plants became infected. It is also possible that movement of irrigation water or splashing rain may have disseminated inoculum throughout the soil. In the future, other methods

such as qPCR should be used in addition to soil plating to detect *M. phaseolina* in soil samples (Burkhardt et al. 2018). Although more uninoculated plants became infected by *M. phaseolina* in the third year of the study, we still saw the same disease trends among the inoculated plants in each of the soil moisture treatments. In future studies, each trial should be conducted in a new area of the field and crop rotation should be used to decrease survival of inoculum from season to season.

Our observation of mortality in control plants in the field and greenhouse raises the possibility that transplants used were a source of *M. phaseolina* inoculum. The potting medium for our greenhouse studies was steamed at 100°C before use, and sprinklers were not used, which suggests the risk of environmental contamination was lower in the greenhouse compared to the field. Although the quarantine and pre-shipment exemption of the Montreal Protocol allows strawberry nurseries to use methyl bromide to fumigate their soils, a recent outbreak of Phytophthora root rot associated with infected transplants shows that pathogen movement from nurseries does occur (Holmes et al. 2020). However, it is also possible that abiotic stress due to excessively low soil moisture may have caused mortality of some control plants in the absence of *M. phaseolina*. For example, in the field, *M. phaseolina* was isolated from a majority of but not all dead control plants that were sampled, affirming that plants may have died from stress. Despite these observations, the presence of *M. phaseolina* on nursery transplants has not been reported to our knowledge.

This study highlights the importance of proper irrigation management for mitigating charcoal rot severity on strawberries. It provides an example for soil moisture

studies on soilborne pathogens and a framework for investigating the dynamics of soilborne pathogen colonization of plants in a field setting. Further research is warranted to identify factors that trigger *M. phaseolina* colonization, infection, and symptom development on strawberry plants in a field setting. The causal link between increased soil moisture and reduced crown colonization should also be assessed as this finding may be helpful in reducing inoculum levels.

LITERATURE CITED

Allen, R. G., Pereira, L. S., Raes, D., and Smith, M. 1998. *FAO Irrigation and Drainage Paper No. 56*.

Anderson, A. J., Habibzadegah-Tari, P., and Tepper, C. S. 1988. Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl. Environ. Microbiol. 54:375–380.

Avilés, M., Castillo, S., Bascon, J., Zea-Bonilla, T., Martín-Sánchez, P. M., and Pérez-Jiménez, R. M. 2008. First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathol. 57:382–382.

Baggio, J. S., Cordova, L. G., and Peres, N. A. 2019. Sources of inoculum and survival of *Macrophomina phaseolina* in Florida strawberry fields. Plant Dis. 103:2417–2424.

Bressano, M., Lorena Giachero, M., Luna, C. M., and Ducasse, D. A. 2010. An in vitro method for examining infection of soybean roots by *Macrophomina phaseolina*. Physiol. Mol. Plant Pathol. 74:201–204 Available at: http://dx.doi.org/10.1016/j.pmpp.2009.12.003.

Burkhardt, A. K., Childs, K. L., Wang, J., Ramon, M. L., and Martin, F. N. 2019. Assembly, annotation, and comparison of *Macrophomina phaseolina* isolates from strawberry and other hosts. BMC Genomics. 20:1–18.

Burkhardt, A., Ramon, M. L., Smith, B., Koike, S. T., and Martin, F. 2018. Development of molecular methods to detect *Macrophomina phaseolina* from strawberry plants and soil. Phytopathology. 108:1386–1394.

Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology. 67:1073–1078 Available at: https://www.apsnet.org/publications/phytopathology/backissues/Documents/1977Articles/Phyto67n08_1073.PDF.

Cahn, M. D. 2012. CropManage Overview: A web application for managing water and nitrogen fertilizer in lettuce. UCANR. Available at: http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=8501.

CDFA. 2017a. California Agricultural Exports 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017AgExports.pdf.

CDFA. 2018a. California Agricultural Exports 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgExports.pdf.

CDFA. 2019a. California Agricultural Exports 2018-2019. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/AgExports2018-2019.pdf.

CDFA. 2020. California Agricultural Exports 2019-2020. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf.

CDFA. 2016. California Agricultural Statistics Review 2015-2016. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016Report.pdf.

CDFA. 2017b. California Agricultural Statistics Review 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016-17AgReport.pdf.

CDFA. 2018b. California Agricultural Statistics Review 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgReport.pdf.

CDFA. 2019b. California Agricultural Statistics Review 2018-2019. Available at: https://www.cdfa.ca.gov/statistics/PDFs/2018-2019AgReportnass.pdf.

Chowdhury, S., Basu, A., Ray Chaudhuri, T., and Kundu, S. 2014. In-vitro characterization of the behaviour of *Macrophomina phaseolina* (Tassi) Goid at the rhizosphere and during early infection of roots of resistant and susceptible varieties of sesame. Eur. J. Plant Pathol. 138:361–375.

Diourte, M., Starr, J. L., Jeger, M. J., Stack, J. P., and Rosenow, D. T. 1995. Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water stress on disease development in sorghum. Plant Pathol. 44:196–202.

Gupta, G. K., Sharma, S. K., and Ramteke, R. 2012. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). J. Phytopathol. 160:167–180.

Hajlaoui, M. R., Mnari-Hattab, M., Sayeh, M., Zarrouk, I., Jemmali, A., and Koike, S. T. 2015. First report of *Macrophomina phaseolina* causing charcoal rot of strawberry in Tunisia. New Dis. Reports. 32:14.

Hartz, T. K., Cahn, M. D., Bolda, M. P., and Biscaro, A. S. 2018. Efficient nitrogen fertility and irrigation management in California strawberry production. Available at: http://ciwr.ucanr.edu/files/283985.pdf.

Hemmati, P., Zafari, D., Mahmoodi, S. B., Hashemi, M., Gholamhoseini, M., Dolatabadian, A., et al. 2018. Histopathology of charcoal rot disease (*Macrophomina phaseolina*) in resistant and susceptible cultivars of soybean. Rhizosphere. 7:27–34 Available at: https://doi.org/10.1016/j.rhisph.2018.06.009.

Holmes, G. J., Mansouripour, S. M., and Hewavitharana, S. S. 2020. Strawberries at the crossroads: Management of soilborne diseases in California without methyl bromide. Phytopathology. 110:956–968.

Islam, M. S., Haque, M. S., Islam, M. M., Emdad, E. M., Halim, A., Hossen, Q. M. M., et al. 2012. Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics. 13:1.

Ivors, K., Winslow, J., Mansouripour, M., Gomez, B., Blauer, K., and Holmes, G. 2018. Evaluating Host Resistance to Macrophomina Crown Rot (Macrophomina phaseolina) in Strawberry - 2018. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Stop 4 Macrophomina cv. - Winslow.pdf.

Jordaan, E., van der Waals, J. E., and McLaren, N. W. 2019. Effect of irrigation on charcoal rot severity, yield loss and colonization of soybean and sunflower. Crop Prot. 122:63–69 Available at: https://doi.org/10.1016/j.cropro.2019.04.026.

Kabir, Z., Bhat, R. G., and Subbarao, K. V. 2004. Comparison of media for recovery of *Verticillium dahliae* from soil. Plant Dis. 88:49–55.

Kaur, S., Dhillon, G. S., Brar, S. K., Vallad, G. E., Chand, R., and Chauhan, V. B. 2012. Emerging phytopathogen *Macrophomina phaseolina*: Biology, economic importance and current diagnostic trends. Crit. Rev. Microbiol. 38:136–151.

Kendig, S. R., Rupe, J. C., and Scott, H. D. 2000. Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. Plant Dis. 84:895–900.

Knapp, S. J., and Cole, G. 2018a. Day-neutral cultivar advancement & research update. Available at: http://knapp.plantsciences.ucdavis.edu/wp-content/uploads/2018/05/UCD-DAY-NEUTRAL-Cultivar-Strawberry-Field-Day-Handout-3-29-2018.pdf.

Knapp, S. J., and Cole, G. 2018b. Short-day cultivar advancement & research update. Available at: http://knapp.plantsciences.ucdavis.edu/wp-content/uploads/2018/05/UCD-SHORT-DAY-Cultivar-Strawberry-Field-Day-Handout-3-29-2018.pdf.

Knapp, S. J., and Cole, G. 2022a. UC-Davis Strawberry Breeding Program Oxnard field day short-day cultivar advancement and research update, Oxnard, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD SHORT-DAY Cultivar Strawberry Field Day Handout Oxnard.pdf?csf=1&web=0&cid=a224dfbc-06d9-4d07-892a-b8dababdc2f5.

Knapp, S. J., and Cole, G. 2022b. UC Davis Strawbery Breeding Program day-neutral cultivar advancement, Prunedale, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD DAY-NEUTRAL Cultivar Strawberry Field Day Handout Prunedale.pdf?csf=1&web=0&cid=f0b6100e-70f6-40f5-9639-9aa215e894e0.

Knapp, S. J., and Cole, G. 2022c. UC Davis Strawbery Breeding Program day-neutral cultivar advancement, Santa Maria, CA. Available at: https://calstrawberry1.sharepoint.com/sites/IndustryPortal-Landing/Breeding/2022 UCD DAY-NEUTRAL Cultivar Strawberry Field Day Handout Santa Maria.pdf?csf=1&web=0&cid=bd902b6a-3868-449b-9362-473a68343048.

Koike, S. T. 2008. Crown rot of strawberry caused by *Macrophomina phaseolina* in California. Plant Dis. 92:1253.

Koike, S. T., Arias, R. S., Hogan, C. S., Martin, F. N., and Gordon, T. R. 2016. Status of *Macrophomina phaseolina* on strawberry in California and preliminary characterization of the pathogen. Int. J. Fruit Sci. 16:148–159 Available at: http://dx.doi.org/10.1080/15538362.2016.1195313.

Koike, S. T., Gordon, T. R., Daugovish, O., Ajwa, H., Bolda, M., and Subbarao, K. 2013. Recent developments on strawberry plant collapse problems in California caused by Fusarium and Macrophomina. Int. J. Fruit Sci. 13:76–83.

Létourneau, G., Caron, J., Anderson, L., and Cormier, J. 2015. Matric potential-based irrigation management of field-grown strawberry: Effects on yield and water use efficiency. Agric. Water Manag. 161:102–113 Available at: http://dx.doi.org/10.1016/j.agwat.2015.07.005.

Madden, L., and Kriss, A. 2016. Generalized linear mixed models for data analysis in plant pathology. In *APS Annual Meeting*, Tampa, Florida. Available at: https://www.apsnet.org/meetings/annual/meetingarchives/2016Annual/Documents/2016 APS Program Book PDF.pdf.

Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J. A., and Simpson, J. 2002. Water relations, histopathology and growth of common bean (*Phaseolus vulgari*s L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol. Mol. Plant Pathol. 60:185–195.

Mertely, J., Seijo, T., and Peres, N. 2005. First report of *Macrophomina phaseolina* causing a crown rot of strawberry in Florida. Plant Dis. 89:434 Available at: http://dx.doi.org/10.1094/PD-89-0434A.

Pande, S., Mughogho, L. K., and Karunakar, R. I. 1990. Effect of moisture stress, plant population density and pathogen inoculation on charcoal stalk rot of sorghum. Ann. Appl. Biol. 116:221–232.

Peres, N. A., Baggio, J. S., and Mertely, J. C. 2007. Charcoal Rot of Strawberries Caused by Macrophomina phaseolina. Available at: https://edis.ifas.ufl.edu/publication/PP161.

Qamar, M. I., Ghanzafar, M. U., Abbas, M. F., Zainab, R., Andleeb, S., Shafiq, M., et al. 2019. First report of *Macrophomina phaseolina* causing crown and root rot on strawberry in Sargodha, Pakistan. Plant Dis. 103:1420.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.r-project.org/.

Sánchez, S., Gambardella, M., Henríquez, J. L., and Díaz, I. 2013. First report of crown rot of strawberry caused by *Macrophomina phaseolina* in Chile. Plant Dis. 97:996.

Sánchez, S., Grez, J., Contreras, E., Gil, P. M., and Gambardella, M. 2019. Physiological response and susceptibility of strawberry cultivars to the charcoal rot caused by *Macrophomina phaseolina* under drought stress conditions. J. Berry Res. 9:165–177.

Schabenberger, O. 2005. Introducing the glimmix procedure for generalized linear mixed models. In *SUGI 30 Proceedings*, Philadelphia, PA, p. 1–20. Available at: http://nesug.org/proceedings/nesug05/an/an4.pdf.

Scott, J. C., McRoberts, D. N., and Gordon, T. R. 2014. Colonization of lettuce cultivars and rotation crops by *Fusarium oxysporum* f. sp. *lactucae*, the cause of Fusarium wilt of lettuce. Plant Pathol. 63:548–553.

Short, G. E., Wyllie, T. D., and Bristow, P. R. 1980. Survival of *Macrophomina phaseolina* in soil and residues of soybean. Phytopathology. 70:13–17.

Snyder, R. L., and Pruitt, W. O. 1992. Evapotranspiration data management in California. In Irrigation and Drainage: Saving a Threatened resource—In Search of Solutions. ASCE. 128.

Strand, L. 2008. Integrated Pest Management for Strawberries Second Edition. University of California Division of Agriculture and Natural Resources.

Stroup, W. W., Barry, G., and Yeater, K. M. 2018. Chapter 16: Analysis of Non-Gaussian Data. In Applied Statistics in Agricultural, Biological, and Environmental Sciences. p. 449–509.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. 2019. Welcome to the Tidyverse. J. Open Source Softw. 4:1686.

Winslow, J., Brantley, R., Holmes, G., and Ivors, K. 2017. Evaluating host resistance to Macrophomina crown rot (*Macrophomina phaseolina*) in Strawberry. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Field Day Handout Mp Host Resistance V6 updated.pdf.

Zveibil, A., and Freeman, S. 2005. First report of crown and root rot in strawberry caused by *Macrophomina phaseolina* in Israel. Plant Dis. 89:1014.

Zveibil, A., Mor, N., Gnayem, N., and Freeman, S. 2012. Survival, host-pathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. Plant Dis. 96:265–272.

Table 1-1. The effect of cultivar, inoculum, and soil moisture on isolation incidence of *Macrophomina phaseolina* from strawberry roots for all randomly sampled plants in all three seasons.

	2018–2019				2019–2020				2020-2021			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	218	0.06	0.9422	2	67.88	0.19	0.8244	2	304	0.56	0.5705
Cultivar	1	218	0.53	0.4677	1	67.89	4.01	0.0491	1	304	1.86	0.1736
Soil Moisture × Cultivar	2	218	1.12	0.3274	2	67.88	0.77	0.4658	2	304	1.05	0.3496
Inoculum	1	218	8.01	0.0051	1	67.9	77.59	<.0001	1	304	186.80	<.0001
Soil Moisture × Inoculum	2	218	0.03	0.9746	2	67.88	0.24	0.7852	2	304	0.35	0.7023
Cultivar x Inoculum	1	218	0.58	0.4468	1	67.89	5.31	0.0243	1	304	0.23	0.6297
Control	–	–	–	–	1	67.89	0.03	0.8529	–	–	–	–
Inoculated	–	–	–	–	1	67.89	13.24	0.0005	–	–	–	–
Soil Moisture × Cultivar × Inoculum	2	218	0.84	0.4332	2	67.88	1.23	0.2993	2	304	0.66	0.5188
Sampling Date	10	218	7.27	<.0001	7	269	6.76	<.0001	8	304	13.56	<.0001

Table 1-2. Significant main and interaction effects on isolation incidence of *Macrophomina phaseolina* from strawberry roots.

Season	Effect[a]	Average isolation incidence (%)[b]		*P* value
		Control	Inoculated	
2018–2019	Inoculum	<1	3	0.0051
	Cultivar × Inoculum	-	-	0.4468
		Monterey	Fronteras	
2019–2020	Cultivar × Inoculum			0.0243
	Control	<1	<1	0.8529
	Inoculated	11	6	0.0005
		Control	Inoculated	
2020–2021	Inoculum	<1	12	<.0001
	Cultivar × Inoculum	-	-	0.6297

[a] Only the significant main and interactive effects from the overall analysis are shown here.

[b] Average isolation incidence was calculated from the number of root pieces positive for *M. phaseolina* divided by the total number of pieces sampled for each plant across all sampling dates.

Table 1-3. The effect of cultivar, inoculum, and soil moisture on isolation incidence of *Macrophomina phaseolina* from strawberry crowns for all randomly sampled plants in all three seasons.

	2018–2019				2019–2020				2020–2021			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	207	0.12	0.8900	2	263	0.50	0.6068	2	301	1.46	0.2346
Cultivar	1	207	0.49	0.4840	1	263	0.04	0.8484	1	301	1.55	0.2140
Soil Moisture × Cultivar	2	207	0.23	0.7930	2	263	4.15	0.0168	2	301	3.14	0.0448
Monterey	–	–	–	–	2	263	3.50	0.0317	2	301	4.22	0.0156
Petaluma/Fronteras	–	–	–	–	2	263	0.96	0.3849	2	301	0.30	0.7434
Inoculum	1	207	0.70	0.4031	1	263	40.96	<.0001	1	301	3.99	0.0467
Soil Moisture × Inoculum	2	207	0.08	0.9220	2	263	2.24	0.1081	2	301	7.07	0.0010
Control	–	–	–	–	2	263	0.97	0.3803	2	301	2.98	0.0524
Inoculated	–	–	–	–	2	263	2.08	0.1267	2	301	5.96	0.0029
Cultivar × Inoculum	1	207	0.49	0.4868	1	263	0.16	0.6920	1	301	2.08	0.1505
Soil Moisture × Cultivar × Inoculum	2	207	0.18	0.8346	2	263	0.66	0.5186	2	301	0.15	0.8582
Sampling Date	9	207	0.18	0.9961	7	263	15.69	<.0001	8	301	11.04	<.0001

Table 1-4. Effects of inoculum and cultivar on isolation incidence of *Macrophomina phaseolina* from strawberry crowns.

Effect[a]					Contrast[b]	
Factors	*P* value	Average isolation incidence (%)[c]			Optimal vs. Low	Optimal vs. High
2019–2020		Control	Inoculated			
Inoculum	<0.0001	3	16		-	-
		Low	Optimal	High		
Soil Moisture × Cultivar	0.0168					
Monterey	0.0317	14	10	7	0.8061	0.0258
Fronteras	0.3849	7	8	12	–	–
2020–2021						
Soil Moisture × Cultivar	0.0448					
Monterey	0.0156	9	10	2	0.9498	0.0124
Fronteras	0.7434	9	3	5	–	–
Soil Moisture × Inoculum	0.0010					
Control	0.0524	7	1	4	0.0153	0.1573
Inoculated	0.0029	11	12	3	0.0435	0.0008

[a] Only the significant main and interactive effects from the overall analysis are shown here.
[b] Contrast statements were used to evaluate the effect of low or high soil moisture treatment on crown isolation incidence when compared to optimal soil moisture.
[c] Average isolation incidence was calculated from the number of crown pieces positive for *M. phaseolina* divided by the total number of pieces sampled for each plant across all sampling dates.

Table 1-5. The influence of soil moisture, cultivar, and inoculum on strawberry mortality due to charcoal rot for the 2019-2020 and 2020-2021 seasons in a field study in Irvine, CA.

	2019–2020[a]				2020–2021[a]			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	144	34.89	<.0001	2	180	57.70	<.0001
Cultivar	1	144	7.36	0.0075	1	180	24.95	<.0001
Soil Moisture × Cultivar	2	144	2.20	0.1146	2	180	1.05	0.3523
Inoculum	1	144	162.08	<.0001	1	180	198.80	<.0001
Soil Moisture × Inoculum	2	144	8.43	0.0003	2	180	18.33	<.0001
Cultivar × Inoculum	1	144	32.04	<.0001	1	180	28.28	<.0001
Soil Moisture × Cultivar × Inoculum	2	144	3.36	0.0374	2	180	12.48	<.0001
Monterey Control	2	144	2.72	0.0694	2	180	18.81	<.0001
Monterey Inoculated	2	144	9.63	0.0001	2	180	47.85	<.0001
Fronteras Control	2	144	1.58	0.2099	2	180	3.43	0.0346
Fronteras Inoculated	2	144	47.00	<.0001	2	180	28.55	<.0001
Rating Date	3	144	90.01	<.0001	4	180	115.00	<.0001
Soil Moisture × Rating Date	6	144	0.67	0.6717	8	180	3.55	0.0008
Cultivar × Rating Date	3	144	1.23	0.3020	4	1	2.12	0.4701
Soil Moisture × Cultivar × Rating Date	6	144	0.47	0.8270	8	180	0.24	0.9838
Inoculum × Rating Date	3	144	4.67	0.0038	4	180	0.59	0.6722
Soil Moisture × Inoculum × Rating Date	6	144	0.34	0.9138	8	180	0.20	0.9910
Cultivar × Inoculum × Rating Date	3	144	1.00	0.3926	4	1	0.18	0.9244
Soil Moisture × Cultivar × Inoculum × Rating Date	6	144	0.30	0.9345	8	180	0.35	0.9435

[a] The first rating date was removed from each season in order for the analysis model to run.

Table 1-6. Summary of statistical effects on *M. phaseolina* isolation incidence from field-grown strawberry crowns, charcoal rot severity of greenhouse-grown strawberry plants, and charcoal rot mortality of field-grown strawberry plants.

			Relative difference vs. optimal[a]			
			Low		High	
Effect	Slice level	Variable[b]	2019	2020	2019	2020
Soil Moisture × Inoculum	Control	Crown colonization	n/a	Higher	n/a	NS
Soil Moisture × Inoculum	Inoculated	Crown colonization	n/a	~Lower	n/a	Lower
Soil Moisture × Cultivar	Fronteras	Crown colonization	n/a	n/a	n/a	n/a
Soil Moisture × Cultivar	Monterey	Crown colonization	NS	NS	Lower	Lower
Soil Moisture × Cultivar	Fronteras	Greenhouse severity	NS	n/a	Lower	n/a
Soil Moisture × Cultivar × Inoculum	Fronteras control	Greenhouse severity	n/a	Higher	n/a	Lower
Soil Moisture × Cultivar × Inoculum	Fronteras inoculated	Greenhouse severity	n/a	NS	n/a	Lower
Soil Moisture × Cultivar	Monterey	Greenhouse severity	NS	n/a	Lower	n/a
Soil Moisture × Cultivar × Inoculum	Monterey control	Greenhouse severity	n/a	Higher	n/a	Lower
Soil Moisture × Cultivar × Inoculum	Monterey inoculated	Greenhouse severity	n/a	Higher	n/a	Lower
Soil Moisture × Cultivar × Inoculum	Fronteras control	Field mortality	n/a	NS	n/a	NS
Soil Moisture × Cultivar × Inoculum	Fronteras inoculated	Field mortality	Higher	Higher	Lower	NS
Soil Moisture × Cultivar × Inoculum	Monterey control	Field mortality	NS	Higher	NS	Higher
Soil Moisture × Cultivar × Inoculum	Monterey inoculated	Field mortality	Higher	Higher	NS	NS

[a] Contrast statements were used to compare either the low or high soil moisture treatment to the optimal. n/a = contrasts not performed because the main effect slice was not significant at $P \leq 0.05$. NS = contrast not significant at $P \leq 0.05$.

[b] *M. phaseolina* colonization of the crown was monitored via sampling of randomly chosen plants from each treatment at 4-week intervals. 8 crown pieces were cut from each plant, surface sterilized, rinsed, and dried. For greenhouse severity and field mortality, each plant in the greenhouse and field was rated on a scale from 0 to 5, where 0 is a healthy plant and 5 = >75 to 100% of the total number of leaves were completely necrotic. Severity refers to all ratings and mortality refers to a rating of 5.

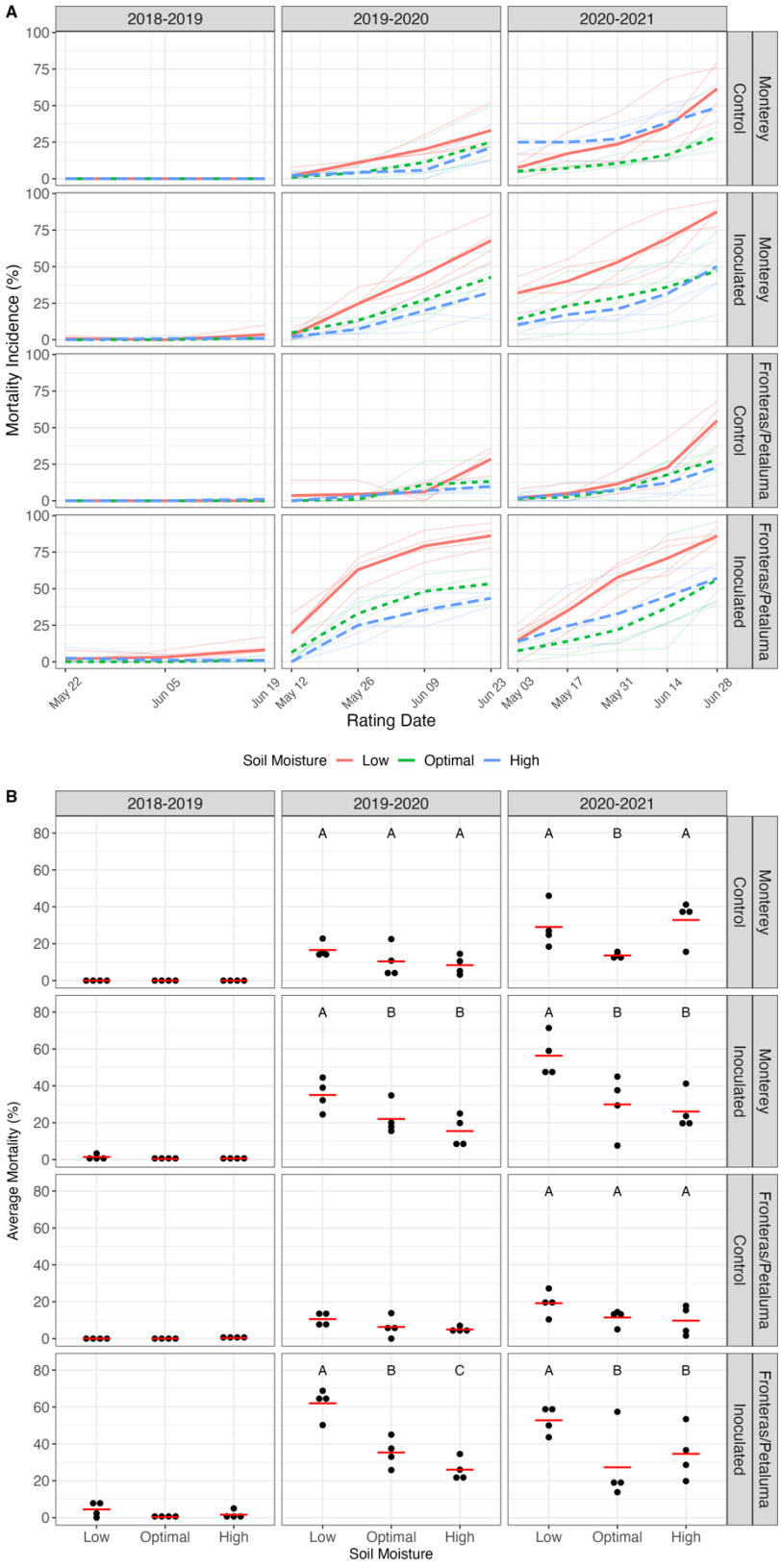
A
2018-2019
2019-2020
2020-2021
Monterey
Control
Inoculated
Fronteras/Petaluma
Mortality Incidence (%)
Rating Date
May 22
Jun 05
Jun 19
May 12
May 26
Jun 09
Jun 23
May 03
May 17
May 31
Jun 14
Jun 28
Soil Moisture
Low
Optimal
High
B
Average Mortality (%)
Low
Optimal
High
Soil Moisture

Fig. 1-1. Influence of soil moisture, cultivar, and inoculum on strawberry mortality due to charcoal rot in field studies in Irvine, CA. A) Percent incidence of plant mortality on each rating date, excluding the first rating date in each season. Charcoal rot severity was assessed for each plant on an ordinal 0 to 5 scale, where 5 = >75 to 100% of the total number of leaves were completely necrotic, every two weeks starting at the first appearance of disease symptoms until the end of the season. Plant mortality was calculated by dividing the total number of plants with a severity rating of 5 in each subplot by the total number of plants in the subplot on that date. B) Average percent incidence of plant mortality per subplot over all rating dates, excluding the first rating date in each season. Each data point represents the average percent incidence for all plants in a single replicate block and each red line represents the mean percent incidence of plant mortality across all subplots. Soil moisture treatment means were compared using Tukey's honestly significant test at $P \leq 0.05$. Letters indicate statistically significant differences between each soil moisture treatment. The absence of letters indicates that there was not a significant soil moisture × cultivar × inoculum interaction (2018–2019) or there was not a significant influence of soil moisture on mortality for the cultivar × inoculum combination (2019–2020).

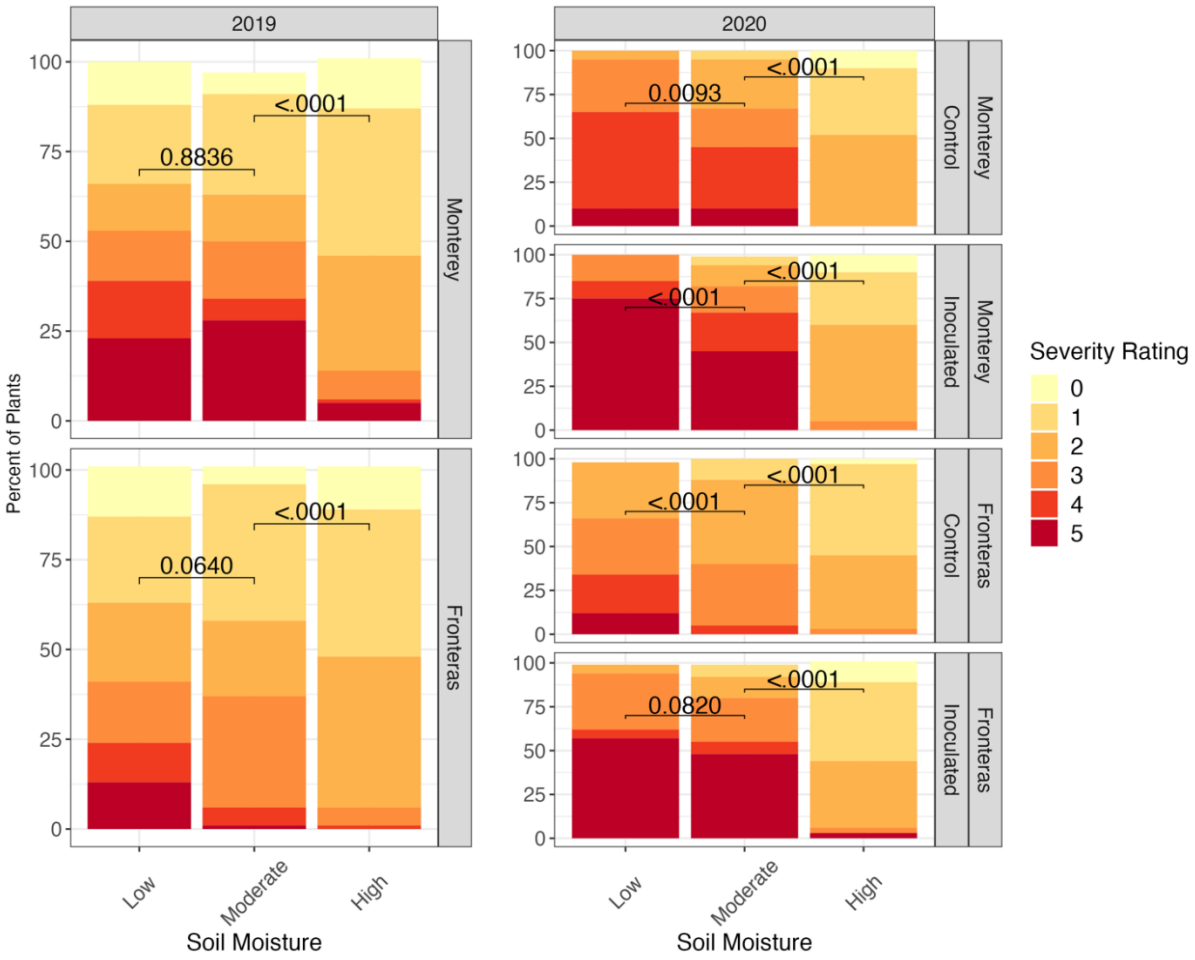

Fig. 1-2. Influence of soil moisture, cultivar, and inoculum on severity of charcoal rot of strawberry in greenhouse studies. Stacked raw data shows the percent of greenhouse plants across all rating dates with each disease severity rating for each significant treatment interaction during the 2019–2020 and 2020–2021 seasons. Brackets and adjacent *P*-values indicate results of contrast statements comparing disease severity among the soil moisture treatments. All plants were rated on a scale from 0 to 5, where 0 is a healthy plant and 5 is a dead plant.

CHAPTER 2

Colonization of Strawberry by *Macrophomina phaseolina* after Root or Crown Inoculations

ABSTRACT

Macrophomina phaseolina is an important pathogen causing charcoal rot on strawberries. It is unclear when and how *M. phaseolina* colonizes the roots or crowns of strawberry plants. A greenhouse study was conducted with 'Monterey' strawberry plants and three inoculum treatments (control, root-, and crown-inoculated) sampled at 5 timepoints (3, 7, 14, 21, and 28 days post-inoculation). A plating assay, which involved drying, grinding, washing, and sieving the tissue, then mixing with molten media, was used to quantify *M. phaseolina* in the roots and crown of each plant. Although all plants and plant parts were asymptomatic throughout the experiment, *M. phaseolina* was detected in roots and crowns of plants within 28 days of inoculation. This study provides a novel way to quantify asymptomatic infection of *M. phaseolina* on strawberries and begins to elucidate early events in the disease cycle of charcoal rot on strawberries.

INTRODUCTION

California is a global leader in strawberry (*Fragaria* × *ananassa*) production, accounting for nearly 90% of U.S. domestic production, with exports from 2016 to 2019 averaging $415 million annually (CDFA 2017a, 2018a, 2019a, 2020). Charcoal rot is a deadly disease affecting strawberries grown in California and Florida in the U.S., and several other countries including Israel, Chile, and Spain (Avilés et al. 2008; Koike 2008; Mertely et al. 2005; Sánchez et al. 2013; Zveibil and Freeman 2005). In California, charcoal rot was first reported on strawberries in 2006 in Orange County (Koike 2008). Since then, charcoal rot has been found in all major strawberry growing regions

throughout the state, threatening California's approximately 15,400 hectares of strawberry production (CDFA 2016, 2017b, 2018b, 2019b; Koike et al. 2013).

Charcoal rot is caused by the soilborne fungus *Macrophomina phaseolina* (Tassi) Goid. The pathogen has a large host range of about 500 plant species including soybeans, sesame, and common bean (Gupta et al. 2012; Islam et al. 2012; Kaur et al. 2012). On non-strawberry hosts the disease is often referred to as root rot, seedling blight, or charcoal rot because of the charcoal-like appearance of disease symptoms on the stem (Kaur et al. 2012). On strawberries, the disease is referred to as charcoal rot but is sometimes called root rot or crown rot (Tweedy and Powell 1958; Zveibil and Freeman 2005). Symptoms on strawberries include stunting, leaf wilting and necrosis, and plant collapse. Strawberry roots eventually become completely rotted and necrotic, and the crown exhibits internal orange to brown discoloration or rot (Zveibil and Freeman 2005; Koike 2008; Baino et al. 2011).

Development of charcoal rot symptoms can occur at different stages in the crop cycle, and there is evidence that *M. phaseolina* can exist as latent infections prior to symptom expression. On greenhouse-grown soybean and common bean seedlings, disease symptoms were visible within 7 to 38 days after inoculation, however in a field study, disease symptoms did not appear until plant maturity (Ilyas and Sinclair 1974; Meyer et al. 1974; Mayek-Pérez et al. 1997, 2002; Hemmati et al. 2018). Bruton et al. (1986) described that cantaloupe root infection was extensive 49 days after planting, but symptoms did not begin to develop until 85 to 90 days after planting. Events in the infection process leading to symptom development are generally the same among hosts.

Microsclerotia first germinate on the roots and form hyphae that infect and colonize the plant roots and crowns. Symptoms are typically initiated after extensive hyphal colonization of host tissue or formation of new microsclerotia in xylem vessels (Ilyas and Sinclair 1974; Islam et al. 2012; Chowdhury et al. 2014; Hemmati et al. 2018; Rajeswari et al. 2019).

On strawberries in Florida, symptoms may develop soon after planting in October and again in the spring due to high temperatures during both periods (Baggio et al. 2021). In fall plantings in California, symptoms of charcoal rot usually appear in the spring when plants are mature and have a heavy fruit load (Koike 2008; Koike et al. 2013). In a field study investigating the colonization of strawberry by *M. phaseolina* over the course of the season, we isolated the pathogen from roots and crowns of inoculated plants up to 6 months before symptoms first appeared and one month after inoculation and transplant (Pedroncelli et al. *unpublished*). However, it is unclear when strawberry plants initially become colonized by *M. phaseolina*.

Several studies on non-strawberry hosts have investigated the roots as the initial infection site for *M. phaseolina* (Ammon et al. 1974, 1975; Short et al. 1978; Hemmati et al. 2018). Though lesions and microsclerotia were observed on soybean stems, Pearson et al. (1984) sampled roots to assess *M. phaseolina* colonization throughout the season. Additionally, Bressano et al. (2010) developed an *in vitro* method to study the colonization of soybean roots and Chowdhury et al. (2014) specifically investigated the nature of *M. phaseolina* in the rhizosphere, as well as the infection and colonization of sesame roots. An exception is a study by Ilyas and Sinclair (1974) that reported disease

development from inoculating soybean stems through artificial wounds. On strawberries, symptoms are often seen in the crown, therefore inoculation is typically performed by placing cornmeal-sand-*M. phaseolina* inoculum on the crown and the soil surface adjacent to the crown after transplant (Winslow et al. 2017; Ivors et al. 2018; Mansouripour et al. 2019, 2020; Wang et al. 2021). However, to our knowledge, there are no studies that document the crown or stem of the plant as the infection court for natural infection, and it has not experimentally been shown whether *M. phaseolina* initially infects the roots or crown of the strawberry plant.

Taken together, the infection process and the location of *M. phaseolina* in strawberry prior to the appearance of charcoal rot symptoms remains unknown. The objective of this study was to quantify the temporal dynamics of early events in the infection process of *M. phaseolina* on strawberry roots and crown.

MATERIALS AND METHODS

Fungal storage and inoculum preparation. *M. phaseolina* isolate GL1310 was obtained from a strawberry plant exhibiting symptoms of charcoal rot sampled in Orange County, CA, in 2007, and was acquired from T.R. Gordon (University of California, Davis). The isolate was grown on potato dextrose agar (PDA) (FB0875713, Fisher Scientific, Hampton, NH). The fungus was stored as infested toothpicks in screw cap plastic tubes at 19°C to 22°C in the dark. This was done by placing autoclaved toothpicks onto PDA that was inoculated with a plug from an actively growing colony of *M. phaseolina*. Once the toothpicks were covered by the fungus, the toothpicks were removed and allowed to dry in a laminar flow hood for 2 to 3 days, then placed into

sterile tubes for storage. To grow the isolate from storage, microsclerotia were scraped from the toothpick onto PDA and single colonies were sub-cultured to new PDA plates.

M. phaseolina inoculum was prepared in a cornmeal sand mixture. Enriched yellow cornmeal (Great Value–Walmart, Bentonville, AR), aragonite aquarium sand (Model No. 2153529, CaribSea, Fort Pierce, FL), and deionized water were combined at a volume ratio of 1.1:0.4:0.4 in a 1-liter polycarbonate jar (Model No. 21161000, ThermoScientific Nalgene, Waltham, MA). The mixture was autoclaved for 1 h and the following day, the mixture was removed from each jar and mixed by hand. The jars were re-filled and autoclaved again for one hour. The mixture was shaken again and inoculated with five 4-mm PDA plugs of actively growing *M. phaseolina*. The negative control consisted of the cornmeal sand mixture only. The inoculated and control containers were incubated at 28 to 30°C and were shaken by hand every 2 to 4 days to encourage even growth throughout the jar. After 11 to 14 days, when the mixture was fully covered by microsclerotia, the inoculum was poured into a clear plastic storage box (Model No. 176FBPC18266, Choice Foodservice Equipment Company, Layton, UT). The lid was loosely placed on the box so the inoculum mixture could dry at 20°C to 26°C for 7 to 10 days under laboratory lighting conditions. The inoculum mixture was moved to a plastic crisper box (Model No. 295C, Pioneer Plastics, Dixon, KY) and stored in the dark at 20°C to 26°C for up to 43 days until use.

Greenhouse experiment. Experiments were established in a greenhouse at UC Riverside. Prior to planting, transplants of cultivar ‘Monterey’ were segregated into five groups by root density and crown size to ensure plants to be sampled at each timepoint

were similar in size from the beginning of the experiment (Fig. B-1). Plants in groups 1 and 2 had a crown of 0.6 to 1.3 cm in width without (Group 1) or with (Group 2) secondary roots. Crowns for plants in groups 3, 4, and 5 were 0.6 to 1.3 cm, 2 cm, and 2 to 2.5 cm wide, respectively, and had a mass of primary and secondary roots that was similar to each other. Plants in groups 1 and 2 were only used if larger plants were not available.

The treatment design was a 3 (inoculum) × 5 (timepoint) factorial with 5 single-plant replicate pots per inoculum × timepoint combination. Inoculum treatments included a root inoculation, crown inoculation, or control (uninoculated cornmeal sand mixture). A coffee filter (Model No. 20115, Bunn Commercial, Springfield, IL) was placed at the base of each 2.65 liter injection molded nursery container (Model CN-NCIM, Greenhouse Megastore, Danville, IL) to prevent loss of planting medium through drainage holes. Each container was filled with coarse sand and placed in a 20 cm square plant saucer (Model No. B093L1TGW2, UltraOutlet) to collect water flow-through. The same volume of mixture (19 to 25 g for the inoculated and 14 to 16 g for the uninoculated mixture) was added to each pot. Control plants were planted first and the uninoculated mixture was sprinkled onto the surface around the crown of the plant and folded into the upper 3 cm sand. The crown-inoculated plants were planted, watered, and the inoculum mixture was placed on the crown and on the sand around the crown. For the root-inoculated plants, sand was removed from each pot and the inoculum mixture was added at a depth of 10 to 12 cm below the surface. The plant was then placed in the pot so that some roots were in contact with the inoculum mixture, and then removed sand was

placed back into the pot. The root-inoculated plants were then watered. All plants were misted by hand using a hose fitted with a spray nozzle on the mist setting.

The timepoint for sampling was included as a treatment when creating the randomized design to predetermine which plants would be sampled at each of the five timepoints: 3, 7, 14, 21, and 28 days post inoculation (dpi). Six extra plants per inoculum treatment were included in the design to be used if plants that were predetermined to be sampled failed to grow. The experiment was conducted four times. In trials 1 and 2, freshly dug strawberry plants from a high elevation nursery were used. In trials 3 and 4, 'frigo' plants from a low elevation nursery were used. In trials 1, 2, and 3, plants were placed in a completely random design with the 6 extra plants per inoculum treatment directly adjacent to the experimental plants. In trial 4, plants were placed in a randomized complete block design with the extra plants included in each block.

A single 3.79 liter per hour drip emitter (Model XB-10PC, Rainbird, Azusa, CA) was installed in each container and set to water two to three times per day for one min each time. In trials 1 and 2, the drip emitter was placed directly next to the crown of the plant. For trials 3 and 4 the drip emitter was placed near the edge of the pot to avoid potentially moving the inoculum from the crown down to the roots.

Plant sampling. Plants were sampled at each of the five timepoints by gently removing the plant from the coarse sand and placing each in a plastic bag. The roots and crown of each plant were washed for approximately 30 s in a 1- to 2-liter beaker with deionized water to remove the sand, and then the leaves and petioles were gently removed. The roots were cut directly at the crown and roots and crown were placed in

separate autoclaved specimen cups, and were surface disinfested by submerging in a 0.5% sodium hypochlorite solution for two min, then rinsing three times with sterile deionized water. The roots and crown were then placed on autoclaved paper towels and allowed to dry in a biosafety cabinet for 4 to 5 h, and then further dried on a laboratory bench for three days at 20°C to 26°C.

Plating assay. To quantify the extent of *M. phaseolina* colonization, whole tissue samples were ground and poured into Petri dishes as a solution with molten media (Henry et al., *personal communication*). For this, after the second drying step, the roots and crowns were ground up separately in two 40-second pulses at 25,000 rpm in grinding chambers (Model MTT 40.10, No. 0020015380, IKA, Staufen, Germany) using a tube mill control (Model No. 0004180001, IKA). The ground tissue was then passed through a stainless-steel sieve with 425 µm square mesh pores (U.S. ASTM E11 standard #40, cat. no. 003SAW.425, Endecotts, Newtown, PA, USA) and a maximum of 0.15 g of sieved tissue was transferred into an autoclaved specimen cup. Seventy mg of tissue was used for DNA extraction and placed in a microcentrifuge tube (Appendix D). Fifty ml of 1% sodium hypochlorite solution was added to the cup with 0.15 g of tissue and mixed on a stir plate for 10 min. The bleached tissue was then passed through a stainless-steel sieve with 45 µm square mesh pores (U.S. ASTM E11 standard #325, cat. no. 003SAW.045, Endecotts) and rinsed using deionized water for several minutes until free of bleach odor. Autoclaved deionized water was then used as a final rinse and the tissue was transferred back into the cup. The tissue was then mixed with 90 ml of amended PDA and the solution was aliquoted by pouring into seven 15 mm × 100 mm Petri dishes. The

amended PDA was prepared by adding 1 ml Tergitol NP-10 (CAS: 127087-87-0, Sigma Aldrich, St. Louis, MO) per liter of PDA. After autoclaving for 30 min, 0.05 g of rifampicin (CAS: 13292-46-1, Research Products International Corp., Mount Prospect, IL) was added per liter media and mixed on a stir plate until use.

Petri dishes were placed into clear plastic boxes and incubated at 28 to 30°C in the dark. After six to ten days, the number of *M. phaseolina* colonies on each plate was counted. On the amended PDA, *M. phaseolina* colonies were circular with white to gray margins and a high density of microsclerotia throughout the colony (Fig. B-2). After 7 days of incubation, most colonies were 2.5 cm in diameter. Sometimes fluffy aerial mycelia grew from the center of the colony, making the colony slightly umbonate.

Inoculum washing test. A test was performed to assess the efficacy of the washing steps used to remove soil and inoculum from the plant surfaces. The experimental design was the same as previously described, except three replicate 'frigo' Monterey plants were used. In this test, plants were sampled immediately following inoculation using the methods described above. The plants were processed as previously described, except the beaker of water used for the initial wash step after sampling was changed between washing the root-inoculated and the crown-inoculated plants.

Data analysis. Organization and visualization of data was performed using the *tidyverse* (Wickham et al. 2019) packages *readr* 2.1.2, *tidyr* 1.2.0, *dplyr* 1.0.8, *ggplot2* 3.3.5, *lubridate* 1.8.0, *egg* 0.4.5, and *ggpubr* 0.4.0 in R 4.1.2 (R Core Team 2018). The number of colonies per gram of tissue was calculated for each Petri dish, and each individual dish was considered a sub-sample for visualization and analysis. Data analysis

was performed using SAS/STAT software in SAS System 9.4 for Windows. The influence of main effects was analyzed with generalized linear mixed models using PROC GLIMMIX with the negative binomial distribution and the logit link function. To allow the model to converge, all observations were adjusted by adding 1 to eliminate zeros, and a random effect statement was not included. Timepoint was analyzed as a fixed effect (i.e., interacting with the treatment factors). The two tissue types were analyzed separately, and any significant interactions were analyzed using the *slice* statement in PROC GLIMMIX to examine the influence of the primary factor of interest within each level of the other factor. Means within significant main effects were compared using Tukey's honestly significant test at $P \leq 0.05$.

RESULTS

Plating assay. *M. phaseolina* was isolated from root tissue of root-inoculated plants and crown tissue of crown-inoculated plants at the first timepoint, 3 dpi, in all four trials (Fig. 2-1, Fig. 2-2). Microsclerotia within *M. phaseolina* colonies conformed to the previously reported description: irregular in shape, measuring 67 to 170 µm × 44 to 133 µm, and starting out light gray then turning black as the colony grew (Koike 2008).

The weight of crown tissue processed in the plating assay was 0.15 g, 0.10 to 0.14 g, or 0.05 to 0.09 g for 65%, 20%, or 11% of the samples, respectively. The weight for 97% of the root samples was 0.15 g. Detection limits were 46.7, 70, or 140 colonies/g tissue for 0.15, 0.09, and 0.05 g of tissue, respectively.

To summarize colony data at the final sampling time (i.e., 28 dpi) for the four trials, the median number of colonies/g of crown tissue was zero for control plants and

ranged from 54 to 233/g tissue for crown-inoculated plants and from 0 to 162/g tissue for root-inoculated plants (Fig. 2-2). For roots, the median number of colonies was 0 for control plants and ranged from 0 to 187/g tissue for crown-inoculated plants and from 47 to 3360/g tissue for root-inoculated plants (Fig. 2-1). For crowns of crown-inoculated plants and roots of root inoculated plants, the median number of colonies detected at the final sampling was numerically higher than at the first timepoint in every trial (Fig. 2-1, Fig. 2-2).

For root and crown tissues, analysis of main effects revealed a significant treatment × timepoint interaction for each trial (Table 2-1). Given the observation of more colonies at the final timepoint compared to the first, we chose to slice this interaction by treatment to examine the time course of colonization. These slices revealed a significant effect of timepoint on the number of *M. phaseolina* colonies detected in both tissue types in both inoculation treatments across all four trials (Table 2-1). Among control plants, this slice revealed a significant effect of timepoint on the number of colonies detected in root tissue in trials 1, 3, and 4, and in crown tissue in trials 3 and 4 (Table 2-1).

Among root-inoculated plants, there were significantly more colonies on average detected in the root tissues at 28 dpi than 3 dpi in each trial (Fig. 2-1). The magnitude of this difference was a factor of 12, 160, 62, and 4,168 in trials 1, 2, 3, and 4, respectively. A similar significant difference was observed between 28 dpi and 7, 14, or 21 dpi in trials 2 and 3, but in trials 1 and 4 the average number of colonies detected at 21 dpi and 28 dpi was statistically similar (Fig. 2-1). In the crown tissues of root-inoculated plants, the

overall number of colonies detected was near zero for most timepoints. In these plants, the number of colonies detected at 28 dpi was significantly higher than 3 dpi by a factor of 3, 19, and 782 in trials 1, 2, and 4, respectively, whereas no differences were observed in trial 3 (Fig. 2-2). In summary, among the root tissue of root-inoculated plants, there were significantly more colonies detected at the final timepoint compared to the first in all four trials, but in crown tissue few colonies were detected, and trends were not consistent.

For crown-inoculated plants, significantly more colonies were detected in crown tissue at 28 dpi than at 3 dpi in all four trials (Fig. 2-2). In trials 1, 2, 3, and 4 the average number of colonies detected at 28 dpi was higher than what was detected at 3 dpi by factors of 4, 20, 3, and 5, respectively (Fig. 2-2). Among the root tissue of crown-inoculated plants, although the number of colonies detected significantly differed between some timepoints, there was not a consistent trend across all trials (Fig. 2-1). The average number of colonies detected in roots decreased from 3 dpi to 28 dpi in trial 1, but increased in trial 2 (Fig. 2-1). The average number of colonies detected in roots within trials 1 and 2 was numerically similar to each other, and when compared to the average number of colonies in trials 3 and 4 was numerically higher by a factor of at least 17. This difference between the two pairs of trials was associated with the change in the drip emitter placement. To summarize, among crown-inoculated plants the number of colonies detected in crowns at the final timepoint was significantly higher compared to the first timepoint for all four trials, whereas no consistent trend was observed in the number of colonies detected in root tissue.

M. phaseolina was detected in a total of 10 out of 100 control plants among all four trials. *M. phaseolina* was detected in one plant in trial 1, in two plants in trial 3, and in seven plants in trial 4 (Fig. 2-1, Fig. 2-2). Although the mean separation identified some significant differences, due to the low frequency of detection, there was no consistent trend among timepoints for either tissue (Fig. 2-1, 2-2).

Although not compared statistically, the number of colonies detected in control plants was numerically lower than that of inoculated plants (Fig. 2-1, 2-2). In trials 1, 2, and 3, no colonies were recovered from crowns or roots of control plants (data not shown). In trial 4, the average number of colonies detected in crowns and roots of control plants was lower than that of root-inoculated plants (by a factor of 56 and 1,871, respectively) or crown-inoculated plants (by a factor of 56 and 10, respectively). Overall, there were no meaningful statistical differences across timepoints for control plants and the average number of colonies detected in control plants was numerically much lower compared to inoculated plants.

Inoculum washing test. Colonies of *M. phaseolina* were not detected in crowns or roots of control plants or of roots of inoculated plants, except for one crown-inoculated plant where an average of 13 colonies/g tissue was detected. *M. phaseolina* was detected in the crown of all three crown-inoculated plants, with an average of 20 to 167 colonies/g tissue. *M. phaseolina* was detected in crowns of two root-inoculated plants, however the majority of colonies were detected in one plant with an average of 300 colonies/g tissue, whereas the other plants had an average of 0 and 20 colonies/g tissue (Fig. B-3).

Different numerical trends were observed between tissues in the washing test when compared to the 3- and 28-dpi timepoints of the inoculated trials. For root tissue, the total number of colonies detected in the washing test was less than or equal to what was detected in all four trials for both crown- and root-inoculated plants except for 3 dpi of trials 2 and 4 and 28 dpi of trial 3 (Fig. B-4). In contrast, numerous colonies were detected in crown tissue from the washing test. For crown tissue of crown-inoculated plants, the total number of colonies was numerically comparable between the washing test and 3 dpi of trials 1 and 4 but was lower in the washing test than at 28 dpi in all four trials (Fig. B-4). For root-inoculated plants, more colonies were detected in crown tissue in the washing test than 3 dpi in all four trials and at 28 dpi in trials 1, 2, and 3. For control plants, no colonies were detected in roots and crowns in the washing test. Overall, the total number of colonies detected in crowns of root-inoculated plants was higher in the washing test than during almost every timepoint in each trial. The total number of colonies detected in crowns of crown-inoculated plants and in the roots of both inoculated treatments in the washing test was lower than or comparable to the trials.

DISCUSSION

This is the first study to our knowledge to show that *M. phaseolina* can directly penetrate roots and crowns of strawberry plants within 28 days of inoculation. Direct infection by *M. phaseolina* of strawberry crowns via inoculation with infested toothpicks has been demonstrated previously (Koike 2008; Mertely et al. 2005), but the ability of *M. phaseolina* hyphae to directly penetrate the crown without wounding has not been

documented. This finding is important for understanding where *M. phaseolina* initially colonizes the strawberry plant.

Although *M. phaseolina* was consistently detected in root and crown tissues, all plants remained asymptomatic during the experiment. This aligns with what we showed in our previous field study, where *M. phaseolina* was detected in inoculated strawberry roots and crowns up to 6 months before symptoms developed (Pedroncelli et al. *unpublished*). This finding also is consistent with recent laboratory studies that provided insight into hemibiotrophic behavior of *M. phaseolina* during infection of non-strawberry hosts grown in Petri plates. For example, on sesame, Chowdhury et al. (2017) documented a distinct switch from biotrophy, where *M. phaseolina* asymptomatically produced thick intercellular hyphae, to necrotrophy, where the pathogen produced thinner intracellular hyphae and caused necrosis of host tissues. Schroeder et al. (2019) described changes in gene expression during infection of *Arabidopsis thaliana*, where 28% of the genes that were upregulated during infection by *M. phaseolina* were also upregulated during infection by the biotrophic Oomycota species *Hyaloperonospora arabidopsidis*. Future research to characterize these dynamics during *M. phaseolina* infection of strawberry would be valuable.

Our finding that strawberry can be colonized soon after exposure to *M. phaseolina*, which then undergoes a long latent phase, could have implications for managing charcoal rot. In fall plantings in Florida, it is common to see symptoms soon after planting and again in the spring, but in fall plantings in California it is not common to see symptoms until the spring (Koike et al. 2013; Baggio et al. 2021). Our previous

field study showed that low soil moisture exacerbates charcoal rot severity, and that high soil moisture leads to decreased crown colonization (Pedroncelli et al. *unpublished*). Therefore, strawberry growers should be mindful of environmental stresses, such as high temperature and low soil moisture, that could expedite colonization and symptom development.

For the crown tissues of root-inoculated plants, the number of colonies detected at 28 dpi was significantly higher than that detected at 3 dpi in trials 1, 2, and 4, although the magnitude of the change was not consistent across all four trials. This result potentially indicates that *M. phaseolina* grew from the roots to the crown within 28 days of inoculation. However, it is unclear if this colonization would lead to disease development. Future studies should extend the experiment beyond 28 dpi to determine the relative effect of the infection locations on charcoal rot development. Additionally, in our study we did not determine the depth of colonization into the crown. Future studies should dissect the crown into epidermal layers, the vasculature, and the inner pith to determine location of the pathogen before and during symptom development.

A large number of colonies were detected in the root tissue of crown-inoculated plants in trials 1 and 2, which was not expected. In these latter trials, water from the irrigation emitter was dripping on or directly next to the crown where inoculum was applied (Fig. B-5). It is possible that this flow of water moved crown-applied inoculum down to the roots. Therefore, in trials 3 and 4, emitters were moved to the edge of the pots. Subsequently, fewer colonies were detected in roots of crown-inoculated plants than in trials 1 and 2, which supports our hypothesis. For crowns, however, the number of

colonies detected in crown-inoculated plants remained fairly consistent for all four trials, suggesting that the location of the drip emitter had little to no effect on crown colonization.

Although *M. phaseolina* was detected in homogenized plant tissues at 3 dpi, it is difficult to distinguish whether new fungal growth or the inoculum itself was detected. This is because the total numbers of colonies detected in homogenized crowns or roots was sometimes similar or lower than the number of colonies detected in the crown or roots of the respective inoculum wash test. Other studies that utilize a similar method to quantify *M. phaseolina* do not indicate agitation or additional washing of roots to remove soil, debris, or inoculum (Kendig et al. 2000; Short et al. 1980; Pearson et al. 1984), although plants in these studies were infected by natural inoculum, soil-amended inoculum, or infested grain mixed with soybean seed. However, the post-hoc inoculum washing test that we performed showed that the inoculum was not fully removed from the plant. In a study investigating colonization of lettuce roots by *Fusarium oxysporum* f. sp. *lactucae*, feeder roots were washed in a solution of 1% sodium hexametaphosphate, a deflocculant, on a rotary shaker to remove soil particles (Scott et al. 2014). Employing a shaker or vortex to wash plant tissue and using a washing solution rather than water may help to remove any microsclerotia that have not penetrated the plant from the outer surface. Despite uncertainties regarding the first timepoint, the number of colonies detected in the roots of root-inoculated and crowns of crown-inoculated plants significantly increased by the final timepoint in all four trials. This trend indicates that the pathogen was colonizing these plant tissues and correlates with what has been shown

with *Arabidopsis thaliana*, where *M. phaseolina* can form microsclerotia inside of the plant within 48 h of inoculation (Schroeder et al. 2019).

Although *M. phaseolina* was isolated from roots and crowns of plants in the inoculum washing test, the total number of colonies detected in the roots in each treatment was lower in the washing test than at most timepoints in each experiment. Among crown tissue in the washing test, however, the average number of colonies detected was higher in one plant compared to the other two in both the root- and crown-inoculated treatments. A possible explanation for increased detection of *M. phaseolina* in the crown in the washing test is the washing methods that were used. Plants were washed by submerging in a beaker of water. The same beaker of water was used to wash all three plants in each inoculation treatment, and the last plant washed had the most colonies detected. This suggests that microsclerotia were washed off of roots or crowns, became suspended in the water, and then were disseminated among tissues of the following plant being washed. Trichomes and sites of petiole attachment are areas of strawberry crowns where dirt, debris, and microsclerotia can become deposited. Although the roots and crowns were thoroughly rinsed with water and then several times with bleach solution and sterile water, it is possible that microsclerotia became trapped in these locations. In future studies, plants should be rinsed in running water to ensure debris and microsclerotia are not re-deposited on the plant.

M. phaseolina was detected in several control plants in all four trials, but it is difficult to determine how they became infested with *M. phaseolina*. Control plants were always processed before inoculated plants for the plating assay. In addition, different

grinding chambers were used for control and inoculated plants, grinding chambers were sprayed with 0.5% sodium hypochlorite and thoroughly scrubbed with soap and water between plants from different sampling times, all materials were surface disinfested with 95% ethanol between samples, and all materials were wiped off or blown off with an electric duster between samples. It is unlikely that cross-contamination occurred during sample processing.

It is also possible that transplants were a source of *M. phaseolina.* Under the quarantine and pre-shipment exemption of the Montreal Protocol, strawberry nurseries can use methyl bromide for soil fumigation. However, a recent outbreak of Phytophthora root rot associated with infected strawberry transplants demonstrates that this kind of pathogen movement is possible (Holmes et al. 2020). In future studies, additional steps should be taken to ensure that transplants are not contaminated. Prior to the experiment, a subsample of transplants should be processed to assess levels of possible background pathogen contamination. Additionally, it has been shown that the infection rate of mother strawberry plants by *Verticillium dahliae* is higher than that of daughter plants (runners) (Gordon et al. 2002). It is possible that runner plants are also less likely to be contaminated with *M. phaseolina*. Researchers could obtain runner plants from field-grown transplants in a pathogen-free trellis system so they do not come into contact with any soil or potting medium until they are transplanted. Alternatively, runners could be propagated from tissue-cultured plants grown from meristem tissue, which is considered pathogen-free, but this may be too expensive and impractical for a large-scale experiment.

Our findings indicate that *M. phaseolina* can asymptomatically penetrate the roots and the crown of the plant within 28 days of inoculation, however it is still unclear where *M. phaseolina* is growing immediately prior to and during symptom development. The number of colonies detected in inoculated tissue significantly increased from the first timepoint to the last, but the magnitude of the difference was larger for inoculated roots than inoculated crowns. This baseline understanding of where *M. phaseolina* is able to initially colonize the strawberry plant can be used to design future research to elucidate the disease cycle of *M. phaseolina* on strawberry.

LITERATURE CITED

Ammon, V., Wyllie, T. D., and Brown, M. F. 1974. An ultrastructural investigation of pathological alterations induced by *Macrophomina phaseolina* (Tassi) Goid in seedlings of soybean, *Glycine max* (L.) Merrill. Physiol. Plant Pathol. 4:1–4.

Ammon, V., Wyllie, T. D., and Brown, M. F. 1975. Investigation of the infection process of *Macrophomina phaseolina* on the surface of soybean roots using scanning electron microscopy. Mycopathologia. 55:77–81.

Arias, S. G., Pons, R. R., and Von Stowasser, E. S. 2013. Temporal analysis of charcoal root rot in forest nurseries under different pathogen inoculum densities and soil moisture content. Trop. Plant Pathol. 38:179–187.

Avilés, M., Castillo, S., Bascon, J., Zea-Bonilla, T., Martín-Sánchez, P. M., and Pérez-Jiménez, R. M. 2008. First report of *Macrophomina phaseolina* causing crown and root rot of strawberry in Spain. Plant Pathol. 57:382–382.

Baggio, J. S., Ruschel, R. G., Noling, J. W., and Peres, N. A. 2021. Physical, cultural, and chemical alternatives for integrated management of charcoal rot of strawberry. Plant Dis. 105:295–304.

Baino, O. M., Salazar, S. M., Ramallo, A. C., and Kirschbaum, D. S. 2011. First report of *Macrophomina phaseolina* causing strawberry crown and root rot in Northwestern Argentina. Plant Dis. 95:1477.

Bressano, M., Lorena Giachero, M., Luna, C. M., and Ducasse, D. A. 2010. An in vitro method for examining infection of soybean roots by *Macrophomina phaseolina*. Physiol. Mol. Plant Pathol. 74:201–204 Available at: http://dx.doi.org/10.1016/j.pmpp.2009.12.003.

Bruton, B. D., Jeger, M. J., and Reuveni, R. 1986. *Macrophomina phaseolina* infection and vine decline in cantaloupe in relation to planting date, soil environment, and plant maturation. Plant Dis. 71:259–263.

CDFA. 2017a. California Agricultural Exports 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017AgExports.pdf.

CDFA. 2018a. California Agricultural Exports 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgExports.pdf.

CDFA. 2019a. California Agricultural Exports 2018-2019. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/AgExports2018-2019.pdf.

CDFA. 2020. California Agricultural Exports 2019-2020. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2020_Exports_Publication.pdf.

CDFA. 2016. California Agricultural Statistics Review 2015-2016. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016Report.pdf.

CDFA. 2017b. California Agricultural Statistics Review 2016-2017. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2016-17AgReport.pdf.

CDFA. 2018b. California Agricultural Statistics Review 2017-2018. Available at: https://www.cdfa.ca.gov/Statistics/PDFs/2017-18AgReport.pdf.

CDFA. 2019b. California Agricultural Statistics Review 2018-2019. Available at: https://www.cdfa.ca.gov/statistics/PDFs/2018-2019AgReportnass.pdf.

Chowdhury, S., Basu, A., and Kundu, S. 2017. Biotrophy-necrotrophy switch in pathogen evoke differential response in resistant and susceptible sesame involving multiple signaling pathways at different phases. Sci. Rep. 7:1–17 Available at: http://dx.doi.org/10.1038/s41598-017-17248-7.

Chowdhury, S., Basu, A., Ray Chaudhuri, T., and Kundu, S. 2014. In-vitro characterization of the behaviour of *Macrophomina phaseolina* (Tassi) Goid at the rhizosphere and during early infection of roots of resistant and susceptible varieties of sesame. Eur. J. Plant Pathol. 138:361–375.

Gordon, T. R., Kirkpatrick, S. C., Shaw, D. V., and Larson, K. D. 2002. Differential infection of mother and runner plant generations by *Verticillium dahliae* in a high elevation strawberry nursery. HortScience. 37:927–931.

Gupta, G. K., Sharma, S. K., and Ramteke, R. 2012. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). J. Phytopathol. 160:167–180.

Hemmati, P., Zafari, D., Mahmoodi, S. B., Hashemi, M., Gholamhoseini, M., Dolatabadian, A., et al. 2018. Histopathology of charcoal rot disease (*Macrophomina phaseolina*) in resistant and susceptible cultivars of soybean. Rhizosphere. 7:27–34 Available at: https://doi.org/10.1016/j.rhisph.2018.06.009.

Holmes, G. J., Mansouripour, S. M., and Hewavitharana, S. S. 2020. Strawberries at the crossroads: Management of soilborne diseases in California without methyl bromide. Phytopathology. 110:956–968.

Ilyas, M. B., and Sinclair, J. B. 1974. Effects of plant age upon development of necrosis and ocurrence of intraxylem sclerotia in soybean infected with *Macrophomina phaseolina*. Phytopathology. 64:156–157.

Islam, M. S., Haque, M. S., Islam, M. M., Emdad, E. M., Halim, A., Hossen, Q. M. M., et al. 2012. Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. BMC Genomics. 13:1.

Ivors, K., Winslow, J., Mansouripour, M., Gomez, B., Blauer, K., and Holmes, G. 2018. Evaluating Host Resistance to Macrophomina Crown Rot (Macrophomina phaseolina) in Strawberry - 2018. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Stop 4 Macrophomina cv. - Winslow.pdf.

Kaur, S., Dhillon, G. S., Brar, S. K., Vallad, G. E., Chand, R., and Chauhan, V. B. 2012. Emerging phytopathogen *Macrophomina phaseolina*: Biology, economic importance and current diagnostic trends. Crit. Rev. Microbiol. 38:136–151.

Kendig, S. R., Rupe, J. C., and Scott, H. D. 2000. Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. Plant Dis. 84:895–900.

Koike, S. T. 2008. Crown rot of strawberry caused by *Macrophomina phaseolina* in California. Plant Dis. 92:1253.

Koike, S. T., Gordon, T. R., Daugovish, O., Ajwa, H., Bolda, M., and Subbarao, K. 2013. Recent developments on strawberry plant collapse problems in California caused by Fusarium and Macrophomina. Int. J. Fruit Sci. 13:76–83.

Mansouripour, S. M., Blauer, K. A., and Holmes, G. J. 2019. Evaluating Host Resistance to Macrophomina Crown Rot in Strawberry - 2019. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/4_Macrophomina - Mansouripour.pdf.

Mansouripour, S. M., Blauer, K. A., and Holmes, G. J. 2020. Evaluating Host Resistance to Macrophomina Crown Rot in Strawberry - 2020. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/documents/06MANSOURIPOUR_Macrophomina_Field Day handout_2020.pdf.

Mayek-Pérez, N., Acosta-Gallegos, J. A., López-Castañeda, C., Lopez-Salinas, E., Cumpeán-Gutiérrez, J., and Acosta-Diaz, E. 1997. Resistance to *Macrophomina phaseolina* in common bean under field conditions references. Reports Bean Improv. Coop. Natl. Dry Bean Counc. Res. Conf. :99–100.

Mayek-Pérez, N., García-Espinosa, R., López-Castañeda, C., Acosta-Gallegos, J. A., and Simpson, J. 2002. Water relations, histopathology and growth of common bean (*Phaseolus vulgari*s L.) during pathogenesis of *Macrophomina phaseolina* under drought stress. Physiol. Mol. Plant Pathol. 60:185–195.

Mertely, J., Seijo, T., and Peres, N. 2005. First report of *Macrophomina phaseolina* causing a crown rot of strawberry in Florida. Plant Dis. 89:434 Available at: http://dx.doi.org/10.1094/PD-89-0434A.

Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1974. Factors affecting charcoal rot of soybean seedlings. Phytopathology. 64:845–849.

Pearson, C. A. S., Schwenk, F. W., Crowe, F. J., and Kelley, K. 1984. Colonization of soybean roots by *Macrophomina phaseolina*. Plant Dis. 68:1086–1088.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.r-project.org/.

Rajeswari, P., Angappan, K., Sivakumar, M., and Vijayalakshmi, D. 2019. Histopathology of *Macrophomina phaseolina* infested mulberry (*Morus* spp.) root under varied soil moisture regime. Int. J. Chem. Stud. 7:4044–4048.

Sánchez, S., Gambardella, M., Henríquez, J. L., and Díaz, I. 2013. First report of crown rot of strawberry caused by *Macrophomina phaseolina* in Chile. Plant Dis. 97:996.

Schroeder, M. M., Lai, Y., Shirai, M., Alsalek, N., Tsuchiya, T., Roberts, P., et al. 2019. A novel Arabidopsis pathosystem reveals cooperation of multiple hormonal response-pathways in host resistance against the global crop destroyer *Macrophomina phaseolina*. Sci. Rep. 9:1–14 Available at: http://dx.doi.org/10.1038/s41598-019-56401-2.

Scott, J. C., McRoberts, D. N., and Gordon, T. R. 2014. Colonization of lettuce cultivars and rotation crops by *Fusarium oxysporum* f. sp. *lactucae*, the cause of Fusarium wilt of lettuce. Plant Pathol. 63:548–553.

Short, G. E., Wyllie, T. D., and Ammon, V. D. 1978. Quantitative enumeration of *Macrophomina phaseolina* in soybean tissues. Phytopathology. 68:736.

Short, G. E., Wyllie, T. D., and Bristow, P. R. 1980. Survival of *Macrophomina phaseolina* in soil and residues of soybean. Phytopathology. 70:13–17.

Tweedy, B., and Powell, D. 1958. Charcoal rot on strawberry in Illinois. Plant Dis. Report. 42:107.

Wang, Y., Hewavitharana, S. S., and Holmes, G. J. 2021. Evaluating host resistance to Macrophomina crown rot in strawberry - 2021. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/J. Macrophomina Field Day 2021.pdf.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. 2019. Welcome to the Tidyverse. J. Open Source Softw. 4:1686.

Winslow, J., Brantley, R., Holmes, G., and Ivors, K. 2017. Evaluating host resistance to Macrophomina crown rot (*Macrophomina phaseolina*) in Strawberry. Available at: https://content-calpoly-edu.s3.amazonaws.com/strawberry/1/images/Field Day Handout Mp Host Resistance V6 updated.pdf.

Young, P. A. 1949. Charcoal rot of plants in East Texas. ed. TEXAS Agricultural Experiment Station. Available at: https://www.cabdirect.org/cabdirect/abstract/19501700783.

Zveibil, A., and Freeman, S. 2005. First report of crown and root rot in strawberry caused by *Macrophomina phaseolina* in Israel. Plant Dis. 89:1014.

Table 2-1. The effect of treatment and timepoint on the number of *M. phaseolina* colonies detected in the roots and crown of strawberry plants in all 4 experimental trials.

		Trial 1				Trial 2			
Tissue	Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Roots	Treatment	2	510	253.85	<.0001	2	510	340.88	<.0001
	Timepoint	4	510	14.49	<.0001	4	510	112.75	<.0001
	Treatment × Timepoint	8	510	26.43	<.0001	8	510	31.34	<.0001
	Control[a]	4	510	2.48	0.0432	4	510	0.00	1.0000
	Crown Inoculation[a]	4	510	27.83	<.0001	4	510	84.77	<.0001
	Root Inoculation[a]	4	510	37.90	<.0001	4	510	123.85	<.0001
Crown	Treatment	2	510	871.81	<.0001	2	510	533.04	<.0001
	Timepoint	4	510	11.87	<.0001	4	510	35.94	<.0001
	Treatment × Timepoint	8	510	13.20	<.0001	8	510	13.76	<.0001
	Control	4	510	0.00	1.0000	4	510	0.00	1.0000
	Crown Inoculation	4	510	40.78	<.0001	4	510	49.88	<.0001
	Root Inoculation	4	510	7.27	<.0001	4	510	49.88	<.0001
		Trial 3				Trial 4			
		Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Roots	Treatment	2	510	345.29	<.0001	2	510	731.22	<.0001
	Timepoint	4	510	16.32	<.0001	4	510	126.64	<.0001
	Treatment × Timepoint	8	510	25.93	<.0001	8	510	51.09	<.0001
	Control	4	510	2.48	0.0435	4	510	5.56	0.0002
	Crown Inoculation	4	510	20.07	<.0001	4	510	24.82	<.0001
	Root Inoculation	4	510	51.96	<.0001	4	510	220.37	<.0001
Crown	Treatment	2	510	473.73	<.0001	2	510	275.46	<.0001
	Timepoint	4	510	1.85	0.1173	4	510	58.25	<.0001
	Treatment × Timepoint	8	510	4.49	<.0001	8	510	21.70	<.0001
	Control	4	510	2.76	0.0270	4	510	8.83	<.0001
	Crown Inoculation	4	510	6.13	<.0001	4	510	5.54	0.0002
	Root Inoculation	4	510	2.76	0.0270	4	510	86.67	<.0001

[a] Timepoint means within each treatment were compared using Tukey's honestly significant test at $P \leq 0.05$.

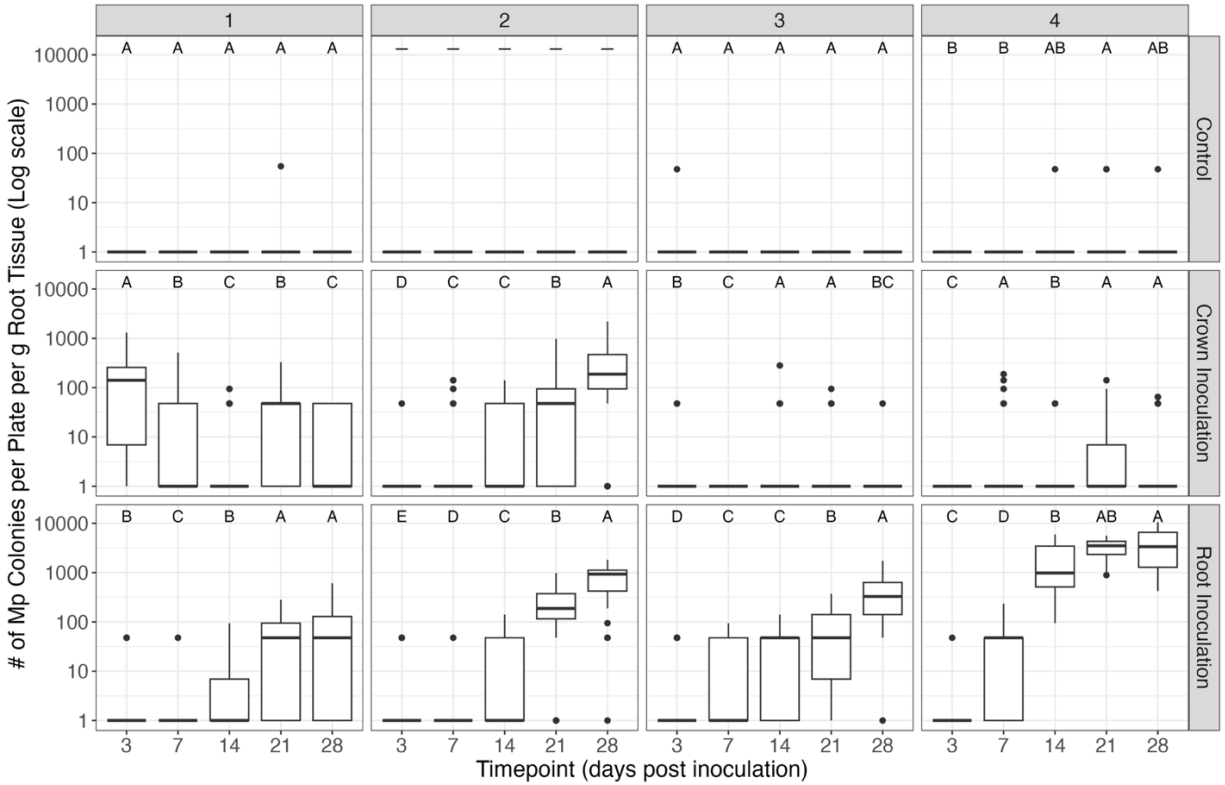

Fig. 2-1. Colonization of strawberry cultivar 'Monterey' roots as determined by inoculation location across four experimental greenhouse trials (1, 2, 3, and 4). Boxplots summarize raw data from five replicate plants and seven Petri dishes per plant for a total of 35 data points. The lower and upper limits of the box represent the 25th and 75th percentile of data (the first and third quartiles), respectively, with the horizontal bar within the box representing the median of the data. The vertical lines above and below the box extend to the largest and smallest data values, respectively, no more or less than 1.5 times the interquartile range (the distance between the first and third quartiles). Dots represent outliers in the data. Within each treatment, timepoint means were compared using Tukey's honestly significant test at $P \leq 0.05$. Letters indicate statistically significant differences among timepoints. A dash (–) indicates that the treatment × timepoint interaction was not significant and therefore the timepoint means were not compared. To account for zeros on the log scale, the data were adjusted by adding 1 to each value.

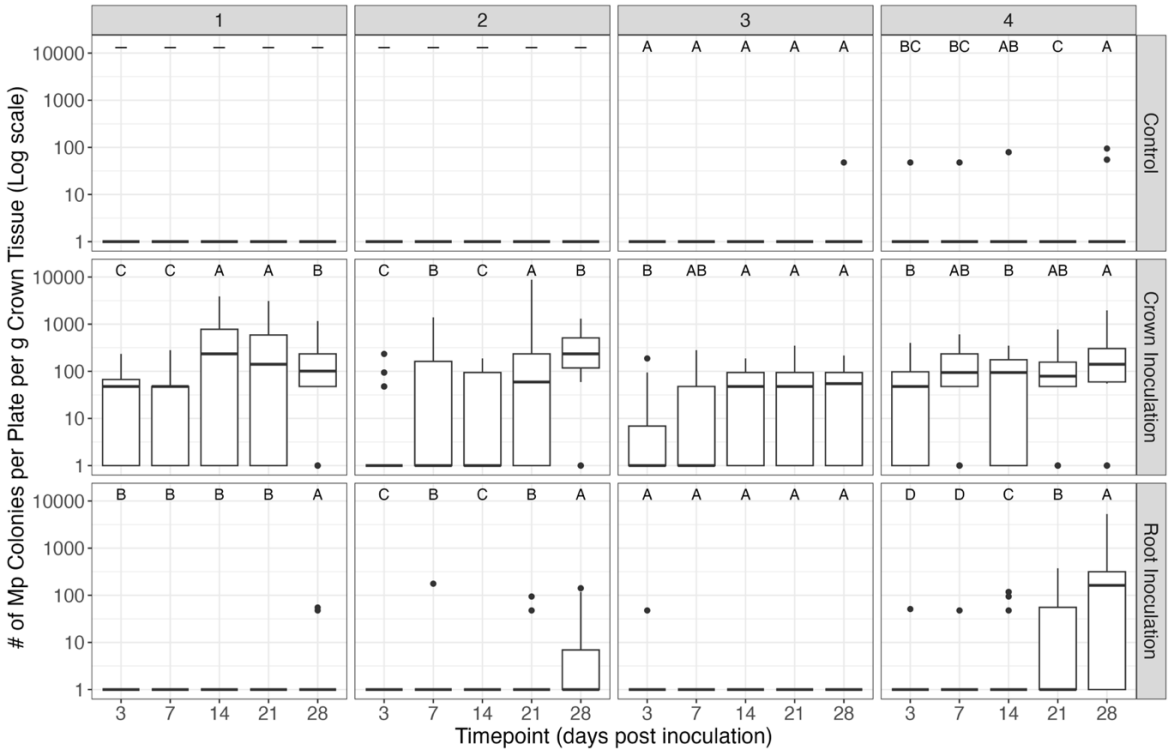

Fig. 2-2. Colonization of strawberry cultivar 'Monterey' crowns as determined by inoculation location across four experimental greenhouse trials (1, 2, 3, and 4). Boxplots summarize raw data from five replicate plants and seven Petri dishes per plant for a total of 35 data points. The lower and upper limits of the box represent the 25th and 75th percentile of data (the first and third quartiles), respectively, with the horizontal bar within the box representing the median of the data. The vertical lines above and below the box extend to the largest and smallest data values, respectively, no more or less than 1.5 times the interquartile range (the distance between the first and third quartiles). Dots represent outliers in the data. Within each treatment, timepoint means were compared using Tukey's honestly significant test at $P \leq 0.05$. Letters indicate statistically significant differences among timepoints. A dash (–) indicates that the treatment × timepoint interaction was not significant and therefore the timepoint means were not compared. To account for zeros on the log scale, the data were adjusted by adding 1 to each value.

APPENDIX A

Supplemental Methods, Results, and Discussion from Chapter 1

METHODS

Media preparation. PDA was prepared by mixing 39 g of PDA powder (213400, BD Difco, Franklin Lakes, NJ) with 1 liter of deionized water and autoclaving for 25 min on a liquid cycle with slow exhaust. Autoclaved PDA was cooled to 52°C and then poured into Petri dishes and stored at 4°C until use.

Sorenson's NP-10 is a two-part medium: Part 1 consists of 500 ml deionized water, 5.0 g polygalacturonic acid (P-3889 titration from oranges) (CAS: 25990-10-7, Sigma Aldrich, St. Louis, MO), and 1.2 g sodium hydroxide (CAS: 1310-73-2, Sigma Aldrich, St. Louis, MO); and Part 2 consists of 500 ml deionized water, 15.0 g granulated agar, 1.0 g potassium nitrate (CAS: 7757-79-1, Thermo Fisher Scientific, Waltham, MA), 1.0 g potassium phosphate monobasic (CAS: 7778-77-0, Thermo Fisher Scientific, Waltham, MA), 0.5 g potassium chloride (CAS: 7447-40-7, Thermo Fisher Scientific, Waltham, MA), 0.5 g magnesium sulfate heptahydrate (CAS: 10034-99-8, Thermo Fisher Scientific, Waltham, MA), and 0.5 ml Tergitol NP-10 (CAS: 127087-87-0, Sigma Aldrich, St. Louis, MO). After autoclaving for 25 min and cooling to 52°C, part 1 is added to part 2 and 0.05 g each of chloramphenicol (CAS: 56-75-7, Sigma Aldrich, St. Louis, MO), streptomycin sulfate (CAS: 3810-74-0, Thermo Fisher Scientific, Waltham, MA), and chlortetracycline hydrochloride (CAS: 64-72-2, Sigma Aldrich, St. Louis, MO) are directly added to the mixture. The medium is then poured into Petri dishes and stored at 4°C until use.

Field experiment – soil sampling. The samples were air-dried in paper bags in a greenhouse at UC Riverside for at least one week and then ground using a soil grinder

(H-4199, Humboldt, Elgin, IL). *M. phaseolina* colony forming units (CFU) were quantified (Butterfield and DeVay 1977). Aliquots of 0.05 g pulverized soil were plated onto each of five Petri dishes (a total of 0.25 g of soil per sample) containing Sorenson's NP-10 semi-selective medium (Kabir et al. 2004) placed below Stage 1 of a Two-Stage Viable Anderson Cascade Impactor (TE-10-860, Tisch Environmental, Cleves, Ohio). A sixth plate was placed below Stage 6 to cumulatively capture any particles that flowed through from the five Stage 1 plates. Following incubation in an incubator (Precision 818, Thermo Scientific Precision, Waltham, MA) at 30°C in the dark for 15 days, colonies of *M. phaseolina* were counted on each plate to calculate the number of CFU per gram of soil.

Field experiment – soil moisture treatments. Most well-managed strawberry fields maintain soil matric potential at or above 10 kPa most of the time, and Strand (2008) recommends irrigating strawberries when tensiometers at a depth of 15.2 cm read 10 to 15 kPa (Létourneau et al. 2015). Therefore 5 kPa means the soil is closer to saturation and most likely excessively wet, while 10 kPa is optimally wet. From 30 to 60 kPa the soil is excessively dry for strawberry production, however in our anecdotal experience this range is not uncommon to see in commercial fields. Alternating between 30 and 60 kPa allows the plants to become stressed without dying.

Irrigation was applied in the morning hours before peak evapotranspiration demand. In some cases, an irrigation event was initiated before reaching the threshold because waiting until the next day could allow soil moisture to continue to decline and exceed the threshold of the next-driest treatment. The decision to initiate an irrigation

event before the threshold had been reached was based on the trend of tensiometer readings and the weather forecast for that day via the phone application Weather Underground.

On business days, irrigation events were manually initiated and terminated by research staff. On weekends in the 2018–2019 season, irrigation events were manually initiated and terminated. During the 2019–2020 and 2020–2021 seasons, on weekends and holidays, a battery-operated irrigation controller (Model NODE400, Hunter Industries, San Marcos, CA) was programmed ahead of time to automatically initiate and terminate the events.

Field experiment - fertigation. Nitrogen was fertigated in 5- to 34-day intervals depending on the soil moisture treatment and irrigation frequency. In the 2019–2020 season, ammonium nitrate (AN20, 20-0-0, Nutrien, Calgary, Alberta, Canada) was applied at 11.21 kg nitrogen/ha/week starting in late January and continuing through the end of the season. Nitrate strip tests (MilliporeSigma, Burlington, MA) were used to monitor nitrate levels in the soil of each soil moisture treatment.

In the 2020–2021 season, N Phuric (15-0-0, Loveland Products Inc., Greeley, CO) was applied weekly following plant tissue analysis. Leaf samples were taken on 21 Feb. 2021 to assess nutrient levels when plants were showing signs of nutrient deficiency such as yellowing of older leaves. Eighty to 100 petioles and 40 to 60 leaf blades were sampled from both cultivars in each soil moisture treatment and were air dried in open paper bags at 20°C to 26°C overnight. Petiole and leaf blade samples were sent to Fruit Growers Laboratory, Inc. in Santa Paula, CA for the “strawberry plant tissue analysis”.

This revealed that Monterey and Fronteras plants in the three soil moisture treatments had ample levels of most macro- and micronutrients but were lacking zinc and copper. Since the irrigation water pH was 7.9, and strawberries prefer slightly acidic soils, N Phuric was applied at 8.97 kg of nitrogen/ha/week from mid-February and to the end of the season to acidify the soil. Specific application dates can be found in Table A-2.

Field experiment – isolation incidence data. The crowns and petioles of the plants sampled on 21 Nov. 2018 were processed. For plants sampled on 19 Dec. 2018 and 26 Jan. 2019, 4 root pieces and 8 crown pieces were processed. On 13 Feb. 2019 we stopped processing the petioles. The roots were cut into three sections: bottom, middle, and top. Four to five secondary roots from each section were surface disinfested, rinsed, and dried, then five roots were placed onto two plates each of NP-10. In March 2019 we started processing 20 roots instead of 10, and they were surface disinfested in 0.5% sodium hypochlorite bleach solution for 30 s.

In April 2019 we started processing larger primary roots that were directly attached to the crown instead of smaller roots. We cut the roots so that about 2.5 cm was left attached to the crown and then took at least one piece from each large root that had been cut from the crown. Roots were surface disinfested for 45 s in 0.5% sodium hypochlorite solution and then five root pieces were plated onto each of four plates of NP-10. We later discovered that surface disinfesting the roots for the same length of time as the crown (2 min) was sufficient and made it easy to process both roots and crown at the same time.

The roots and crowns of plants were thoroughly rinsed with deionized water. Twenty 3 cm lengths of primary roots, mainly from the top 6 to 8 cm of the roots directly attached to the crown, and eight 5 mm^3 pieces of the cortex and stele of the crown were cut from the plant. When a plant had several crowns, each was cut open to observe symptoms and pieces of more than one crown were sampled. If possible, both symptomatic and asymptomatic pieces were sampled from a symptomatic crown. Crown and root pieces were placed in separate autoclaved specimen cups and processed as described in Chapter 1.

Field experiment – mite damage data. In the 2020–2021 season, an outbreak of spider mites occurred in the study area. Mite damage appeared as a copper to red discoloration of leaves, and if the damage was severe, drying and death of leaves. Ratings were based on photos taken of each subplot on 17 Apr. 2021 and 31 May 2021 and consisted of a 1 to 4 ordinal scale where 1 = no mite damage, 2 = 0–50% of leaves exhibiting mite damage, 3 = 50% or more of the leaves exhibiting mite damage, and 4 = the plant has died.

Field experiment – diagnostic samples. To determine if mite damage was associated with mortality in the 2020–2021 season, 18 or 22 collapsed and necrotic plants were arbitrarily sampled from areas with high or low mite pressure, respectively. Plants were sampled to represent both inoculum treatments and cultivars from the low and high soil moisture treatments on 28 Jun. 2021. Plant crowns were plated onto PDA+++ and NP-10 for diagnosis as described above.

Data analysis. The disease severity rating scale is an ordinal scale that is partially qualitative because rating 1 does not represent a quantitative range. Therefore, disease severity data was analyzed using the multinomial distribution with the cumulative logit link function (Schabenberger 2005; Stroup et al. 2018). Sampling date was included as a fixed effect, separate (i.e., not interacting with) from the treatment factors. For field severity ratings "random intercept / subject = Block (Irrigation)" was included as the random effect term. Significant interactions were examined using the *slice* statement. Contrast statements were used to compare the optimal soil moisture treatment to either the high or low soil moisture treatments.

Spider mite severity was analyzed using the multinomial distribution with the cumulative logit link function without a random effect term (Schabenberger 2005; Stroup et al. 2018). Significant interactions were analyzed using the *slice* statement. Contrast statements were used to compare the optimal soil moisture treatment to either the high or low soil moisture treatments.

RESULTS

Field experiment – soil moisture treatments. There was more rainfall during the first two seasons of the study than the final year of the study (Table A-7; Fig. A-6). At planting and during the 7 days afterwards, the average air temperature was highest during the 2019–2020 season and lowest during the 2020–2021 season (Table A-7). When disease symptoms began to develop and disease severity ratings were first collected, the air temperature from 14 days before to 14 days after was also highest during the 2019–2020 season and lowest during the 2020–2021 season (Table A-7).

Field experiment – charcoal rot data. During each season, symptoms of charcoal rot were first seen on 8 May 2019, 28 Apr. 2020, and 17 Apr. 2021, and disease incidence increased as each season progressed and was numerically higher in inoculated than control plants (Fig. A-9).

Analysis of the main effects revealed a significant three-way interaction for each season (Table A-9). The influence of soil moisture within each level of cultivar × inoculum was examined by slicing. Within the short-day cultivars Petaluma and Fronteras, there was strong evidence for an effect of soil moisture on disease severity for inoculated plants but not for control plants in all three seasons. Contrast statements showed consistently over all three seasons that within the short-day cultivars, charcoal rot severity was significantly higher in the low soil moisture treatment when compared to the optimal soil moisture treatment (Table A-9; Fig. A-9). In contrast, no statistical difference was observed between the optimal and high soil moisture treatments.

Additionally, in the 2020–2021 season, there was evidence for an influence of soil moisture on disease severity for both Monterey control and Monterey inoculated plants (Table A-9). Contrast statements showed that, among Monterey control plants, charcoal rot severity was significantly higher in both the low and high soil moisture treatments compared to optimal. Among Monterey inoculated plants, contrast statements showed that charcoal rot severity was significantly higher in the low soil moisture treatment compared to the optimal, but there was no difference between optimal and high.

Analysis of disease incidence (severity rating = 3, 4, or 5) was comparable to analysis of mortality and severity ratings (data not shown). In both the 2019–2020 and

2020–2021 seasons there was a significant irrigation × cultivar × inoculum interaction with an influence of irrigation on Monterey control, Monterey inoculated, and Fronteras inoculated plants.

Field experiment – mite damage data. In the 2020–2021 season, a mite outbreak occurred in the study area in mid-April and was concentrated in one corner of the field in a main plot of low soil moisture and two main plots of high soil moisture treatments. On 17 Apr. 2021, 23% and 11% of plants were assigned a mite severity rating of 2 and 3 respectively. Following the applications of miticides made on 15 Apr. 2021 and 29 Apr. 2021, by 31 May 2021 mite activity had declined and 97% of living plants were rated as having no mite damage (data not shown). Therefore, data analysis was performed for the 17 Apr. 2021 rating date only.

Analysis of main effects revealed a significant soil moisture × cultivar × inoculum interaction (Table A-10). Slicing this interaction by each cultivar × inoculum combination revealed an influence of soil moisture on mite severity among Fronteras inoculated and Monterey control plants, with weak evidence for significance among Monterey inoculated plants (Table A-10). There was not a consistent trend, however, among the soil moisture treatments. Among both Fronteras inoculated and Monterey control plants, mite severity was significantly higher in the high soil moisture treatment compared to the optimal (Fig. A-10), which is in line with the visual observation of more severe damage in two high soil moisture main plots. However, there was no difference in mite severity between optimal and low (Table A-10). Though the evidence for an influence of soil moisture on mite severity was statistically weak among Monterey

inoculated plants, severity was slightly higher in the low soil moisture treatment compared to the optimal, but there was no difference in mite severity between optimal and high (Fig. A-10). Overall, the trends observed in analysis of mite damage were not similar to those of charcoal rot.

Field experiment – diagnostic samples. Among the plants sampled for mite diagnosis, across all treatments *M. phaseolina* was isolated from 83% of plants sampled from the area with high mite damage and from 100% of the plants sampled from the area with low mite damage (data not shown). A further exploratory examination of *M. phaseolina* isolation incidence from plants randomly and nonrandomly sampled after the mite outbreak began revealed no consistent association between isolation incidence and mite severity (Fig. A-11).

DISCUSSION

We expected that low soil moisture would affect disease severity among Monterey plants more than Fronteras due to its classification as highly susceptible (Table A-4; Knapp and Cole 2018b, 2018a; Ivors et al. 2018; Winslow et al. 2017). Contrary to that expectation, low soil moisture significantly exacerbated charcoal rot severity on the short-day cultivars Petaluma and Fronteras in all three seasons, whereas low soil moisture significantly affected charcoal rot severity among Monterey plants only in the 2020–2021 season. In addition to the effect of low soil moisture, we expected that absolute levels of disease would be higher in Monterey compared to Fronteras. We could not statistically compare cultivars due to the presence of significant three-way interactions including cultivar and our choice to examine soil moisture within these interactions. However,

among inoculated plants, the numerical trends of much higher mortality in Fronteras in the 2019–2020 season and similar mortality in the 2020–2021 season when compared to Monterey was also unexpected.

A major difference between Monterey and Petaluma or Fronteras is photoperiodism, or the length of daylight required for the plant to induce flower production. Short-day cultivars begin to produce flowers when exposed to 14 hours or less of day light, although temperatures above 15°C can help shorten this required period. Day-neutral cultivars begin to produce flowers regardless of day length if temperatures remain favorable (Strand 2008). For example, yield curves show that Petaluma yield peaks from March to May and Fronteras yield peaks from April to May, while Monterey yield peaks from May to June (Knapp and Cole 2018a, 2018b, 2022b, 2022c, 2022a). Flowering and fruit development are a source of stress on strawberry. The different timing of fruit production-related stress may cause short-day cultivars to be more susceptible to other stresses, such as soil moisture and pathogen infection, earlier in the season than day-neutral cultivars.

In our study, disease symptoms appeared in mid-April to early May which coincides with peak fruit yield for both Petaluma and Fronteras, but is before the peak for Monterey. This may have accelerated disease development in Petaluma or Fronteras compared to Monterey, thus potentially explaining why Fronteras appeared more sensitive to low soil moisture. Future research should continue to investigate these factors in relation to charcoal rot severity to determine which cultivars are best suited for different environments and levels of disease pressure.

When these cultivars were chosen for this study, data indicated that Monterey was much more susceptible to charcoal rot than either Petaluma or Fronteras. This is not what we observed in our study, however our findings align with more recent data from cultivar resistance screening trials conducted by the Strawberry Center at California Polytechnic State University (Cal Poly), San Luis Obispo (Table i). Over five consecutive years, the Cal Poly trials showed that susceptibility of Monterey, Petaluma, and Fronteras to charcoal rot varies from year to year. Similar to our observation of numerically higher mortality of Fronteras compared to Monterey in the 2019–2020 season, the Cal Poly trial that same season also reported that mortality was higher in Fronteras than Monterey, however we cannot identify a consistent trend between the two locations that may explain this result. Though related research found a strong positive correlation between soil temperature at planting or mid-season air temperature and final plant mortality (Wang et al., *unpublished data*), this same correlation was not found with our results. It is possible factors related to the specific nursery that provided transplants or weather conditions affecting all nurseries may have affected susceptibility of Fronteras relative to Monterey.

The outbreak of another pest in the study area has the potential to affect charcoal rot results, for example by increasing stress and possibly plant susceptibility to charcoal rot. However, analysis of mite damage did not reveal a consistent trend among soil moisture treatments and the trends observed in analysis of mite damage were not similar to those of charcoal rot severity or mortality.

Table A-1. Dates of planting, irrigation treatment initiation, and season termination.

Season	Study	Planting	Drip Irrigation Initiation[a]	Soil Moisture Treatment Initiation	Season Termination
2018–2019	Field	October 26, 2018	November 14, 2018	January 26, 2019	June 26, 2019
2019–2020	Field	October 23, 2019	December 5, 2019	December 5, 2019	June 24, 2020
2019–2020	Greenhouse	October 24, 2019	November 7, 2019	November 7, 2019	July 27, 2020
2020–2021	Field	October 23, 2020	November 20, 2020	November 23, 2020	June 28, 2021
2020–2021	Greenhouse	October 26, 2020	October 26, 2020	November 18, 2020	July 6, 2021

[a] For greenhouse plants, this date indicates the date the plants began being irrigated with the spray stakes. Any irrigation done before this date was by hand.

Table A-2. Fertilizer Applications

Season	Application Date	Product Name	Manufacturer	Company Location	Product Contents (N-P-K + any micronutrients)	Soil Moisture Treatment(s) Treated	Application Rate (lbs/acre/week unless otherwise noted)
All	Pre-plant	Slow release standard strawberry blend			22-7-10	High, optimal, and low	800 lbs/acre
2018-2019	NA	Biomin Booster 11	JH Biotech Inc	Ventura, CA, USA	1-0-0 (1% Ca and Mg; 0.5% Cu, Fe, Mn, Zn)	High, optimal, and low	as directed
2018-2019	NA	Ultra	AgriGro	Doniphan, MO, USA	0-0-1	High, optimal, and low	as directed
2018-2019	NA	Biomin Zinc	JH Biotech Inc	Ventura, CA, USA	1-0-0 (7% Zn)	High, optimal, and low	as directed
2018-2019	NA	Biomin Manganese	JH Biotech Inc	Ventura, CA, USA	1-0-0 (5% Mn)	High, optimal, and low	as directed
2018-2019	NA	100% Liquid Seaweed Concentrate	Acadian Plant Health	Dartmouth, Nova Scotia, Canada	0.1-0.0-5.0	High, optimal, and low	as directed
2019-2020	1/29/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High	10.5
2019-2020	1/31/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High, optimal, and low	31.5
2019-2020	2/13/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and optimal	10
2019-2020	2/28/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and optimal	10
2019-2020	3/21/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High, optimal, and low	10
2019-2020	4/3/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and optimal	10
2019-2020	4/13/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	Low	10
2019-2020	4/17/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and optimal	10
2019-2020	4/29/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High, optimal, and low	10
2019-2020	5/15/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and optimal	10
2019-2020	5/31/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High, optimal, and low	10
2019-2020	6/16/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	High and low	10
2019-2020	6/17/2020	AN20	Nutrien	Carlgary, Alberta, Canada	20-0-0	Optimal	10
2020-2021	2/18/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High and optimal	8
2020-2021	2/24/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	2/26/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal and low	8
2020-2021	3/1/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High and optimal	8
2020-2021	3/8/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	3/16/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High, optimal, and low	8
2020-2021	3/24/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	3/25/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	3/31/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	4/1/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal and low	8
2020-2021	4/6/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	4/8/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	4/15/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	4/19/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	4/21/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	4/27/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	4/28/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	5/3/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	5/4/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	5/5/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Low	8
2020-2021	5/10/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Low	8
2020-2021	5/18/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8

Season	Application Date	Product Name	Manufacturer	Company Location	Product Contents (N-P-K + any micronutrients)	Soil Moisture Treatment(s) Treated	Application Rate (lbs/acre/week unless otherwise noted)
2020-2021	5/19/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	5/26/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	5/28/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	6/1/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	6/2/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	6/4/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Low	8
2020-2021	6/7/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	6/9/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	6/16/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8
2020-2021	6/18/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	Optimal	8
2020-2021	6/23/2021	N Phuric	Loveland Products Inc.	Greeley, CO	15-0-0	High	8

Table A-3. Pesticide Applications

Date	Product Name	Company	Active Ingredient	Application Rate
2018-10-20	Admire Pro	Bayer Crop Sci	Imidacloprid, 1-[(6-Chloro-3-pyridinyl)methyl]- *N*-nitro-2-imidazolidinimine.	14.00 Oz/BroadCt Ac
2018-11-20	Intrepid 2 F	Dow Agroscience	Methoxyfenozide: Benzoic acid, 3-methoxy- 2-methyl-,2-(3,5-dimethylbenzoyl)-2- (1,1-dimethylethyl) hydrazide	12.00 Oz/Treated Ac
2018-11-20	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2018-11-20	Silwet L-77 Surfactant	Helena	Polyalkyleneoxide modified heptamethyltrisiloxane	4.00 Oz/100 Gal
2018-12-11	Elevate 50 WDG Fungicide	Arysta	Fenhexamid	1.50 Lbs/Treated Ac
2018-12-11	Javelin-WG	Certis	*Bacillus thuringiensis*, subspecies *kurstaki* strain SA-11 solids, spores, and Lepidopteran active toxins	1.50 Lbs/Treated Ac
2018-12-11	Hi-Wett	Loveland	Alcohol ethoxylate, Polysiloxane Polyether Copolymer, Polyoxyethylene-polyoxypropylene copolymer	4.00 Oz/Treated Ac
2019-01-03	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-01-03	Fontelis	Dupont	Penthiopyrad	24.00 Oz/Treated Ac
2019-01-03	Dipel DF Insecticide	Valent Bio	Bacillus thuringiensis subspecies kurstaki (Btk)	2.00 Lbs/Treated Ac
2019-01-03	Acramite 50WS	Macdermid	Bifenazate	1.00 Lb/Treated Ac
2019-01-03	Tactic Sticker Surfactant	Loveland	Alcohol ethoxylates (C11), silicone polyether copolymer, 1,2-propanediol	8.00 Oz/ 100 Gal
2019-01-30	Luna Sensation	Bayer CropSci	Fluopyram 250 g/L, Trifloxystrobin 250 g/L	7.60 Oz/Treated Ac
2019-01-30	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-01-30	Dipel DF Insecticide	Valent Bio	Bacillus thuringiensis subspecies kurstaki (Btk)	2.00 Lbs/Treated Ac
2019-01-30	Tactic Sticker Surfactant	Loveland	Alcohol ethoxylates (C11), silicone polyether copolymer, 1,2-propanediol	8.00 Oz/ 100 Gal
2019-02-12	Switch 62.5WG	Syngenta	Cyprodinil, Fludioxonil	0.88 Lbs/Treated Ac
2019-02-12	Elevate 50 WDG Fungicide	Arysta	Fenhexamid	0.66 Lbs/Treated Ac
2019-02-12	Dipel DF Insecticide	Valent Bio	Bacillus thuringiensis subspecies kurstaki (Btk)	2.00 Lbs/Treated Ac
2019-02-12	Hi-Wett	Loveland	Alcohol ethoxylate, Polysiloxane Polyether Copolymer, Polyoxyethylene-polyoxypropylene copolymer	4.00 Oz/Treated Ac
2019-02-19	Actara	Syngenta	Thiamethoxam	0.25 Lbs/Treated Ac
2019-02-19	Luna Sensation	Bayer CropSci	Fluopyram 250 g/L, Trifloxystrobin 250 g/L	7.60 Oz/Treated Ac
2019-02-19	Javelin-WG	Certis	*Bacillus thuringiensis*, subspecies *kurstaki* strain SA-11 solids, spores, and Lepidopteran active toxins	1.50 Lbs/Treated Ac
2019-02-19	Tactic Sticker Surfactant	Loveland	Alcohol ethoxylates (C11), silicone polyether copolymer, 1,2-propanediol	8.00 Oz/ 100 Gal

Date	Product Name	Company	Active Ingredient	Application Rate
2019-02-19	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-03-05	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-03-05	Elevate 50 WDG Fungicide	Arysta	Fenhexamid	1.50 Lbs/Treated Ac
2019-03-05	Javelin-WG	Certis	*Bacillus thuringiensis*, subspecies *kurstaki* strain SA-11 solids, spores, and Lepidopteran active toxins	1.50 Lbs/Treated Ac
2019-03-05	Silwet L-77 Surfactant	Helena	Polyalkyleneoxide modified heptamethyltrisiloxane	7.00 Oz/100 Gal
2019-03-13	PH-D WDG	Arysta	Group M4 Fungicide	0.39 Lbs/Treated Ac
2019-03-13	Dipel DF Insecticide	Valent Bio	*Bacillus thuringiensis* subspecies *kurstaki* (Btk)	2.00 Lbs/Treated Ac
2019-03-13	Switch 62.5WG	Syngenta	Cyprodinil, Fludioxonil	0.88 Lbs/Treated Ac
2019-04-06	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-04-06	Elevate 50 WDG Fungicide	Arysta	Fenhexamid	1.50 Lbs/Treated Ac
2019-04-06	Dipel DF Insecticide	Valent Bio	Bacillus thuringiensis subspecies kurstaki (Btk)	2.00 Lbs/Treated Ac
2019-04-06	Silwet L-77 Surfactant	Helena	Polyalkyleneoxide modified heptamethyltrisiloxane	4.00 Oz/100 Gal
2019-04-20	Fontelis	Dupont	Penthiopyrad	24.00 Oz/Treated Ac
2019-04-20	Javelin-WG	Certis	*Bacillus thuringiensis*, subspecies *kurstaki* strain SA-11 solids, spores, and Lepidopteran active toxins	1.50 Lbs/Treated Ac
2019-04-20	BroadSpred Green	Custom Ag	Siloxane Polyalkyleneoxide Copolymer and Polyalkyleneoxide	4.00 Oz/100 Gal
2019-05-30	Captan 80 WDG Fungicide	Albaugh	Captan	3.75 Lbs/Treated Ac
2019-05-30	Javelin-WG	Certis	*Bacillus thuringiensis*, subspecies *kurstaki* strain SA-11 solids, spores, and Lepidopteran active toxins	1.50 Lbs/Treated Ac
2019-05-30	BroadSpred Green	Custom Ag	Siloxane Polyalkyleneoxide Copolymer and Polyalkyleneoxide	16.00 Oz/100 Gal
2021-02-17	Monterey Horticultural Oil	Lawn and Garden Products Inc.	Mineral oil with petroleum distillates	As directed
2021-04-15	Nealta	BASF	Cyflumetofen	As directed
2021-04-29	Acramite-50WS	MacDermid Agricultural Solutions, Inc.	Bifenazate: hydrazine carboxylic acid, 2-(4-methoxy-[1,1'-biphenyl]-3-yl) 1-methylethyl ester	As directed

Table A-4. Characteristics of strawberry cultivars used in the study.

	Source Nursery			Cultivar Traits	
Cultivar	2018–2019	2019–2020	2020–2021	Photoperiodism	Susceptibility to *M. phaseolina*[a]
Monterey	Crown Nursery LLC	Planasa	Lassen Canyon Nursery	Day-neutral	Highly susceptible
Petaluma	Crown Nursery LLC	–	–	Short-day	Moderately susceptible
Fronteras	–	Planasa	Lassen Canyon Nursery	Short-day	Moderately susceptible

[a] Knapp and Cole 2018a; Knapp and Cole 2018b; Ivors et al. 2018; Winslow et al. 2017

Table A-5. Results from the irrigation water analysis completed by Fruit Growers Laboratory, Inc. Santa Paula, CA in the 2018–2019 and 2020–2021 seasons.

Test Description[a]	2018–2019[b]	2020–2021[c]
pH	7.9	7.9
EC (dS/m)	1.12	1.14
SAR	3.9	4.5
Boron (mg/L)	0.30	0.30
Chloride (mg/L)	126	138
Sulfate (mg/L)	156	144

[a] EC = electro conductivity. SAR = sodium absorption ratio.
[b] Sample was collected on 28 Sep. 2018.
[c] Sample was collected on 25 Jan. 2021.

Table A-6. Summary of soil moisture treatments in each season of the field study.

Season	Treatment	# of Events	Average Depth of Water Administered per Irrigation Event (mm)	Total Depth of Water Administered (mm)[a]
2018–2019	Low	25	22.6	565
	Optimal	54	10.5	567
	High	92	6.9	635
2019–2020	Low	26	27.4	713
	Optimal	68	10.9	738
	High	169	4.3	721
2020–2021	Low	21	25.6	538
	Optimal	59	11.3	665
	High	114	5.7	648

[a] Total depth of water administered was obtained from fertilizer meters installed in each soil moisture treatment main line.

Table A-7. The total rainfall (mm) and average air temperature (°C) at planting and symptom development during each season.

Season	Total Rainfall (mm)	Average Soil Temperature at Planting (°C)[a]	Average Air Temperature at Symptom Development (C°)[a]
2018–2019	256	20.5	16.2
2019–2020	350	18.5	18.5
2020–2021	142	20.3	15.9

[a] The average was calculated from a range of data collected by CIMIS Station 75 located 0.40 km from the study site for the whole month of October, when planting occurred, and from 14 days before to 14 days after the date that disease severity ratings were first taken, indicating the onset of symptom development.

Table A-8. Examination of the influence of inoculum or soil moisture on strawberry mortality caused by charcoal rot within the significant inoculum × rating date and soil moisture × rating date interactions in field studies in Irvine, CA.

Effect					
2019–2020	May 12	May 26	June 9	June 23	
Inoculum × Rating Date[z]	0.0544	<.0001	<.0001	<.0001	
			Mortality (%)		
Control	1.2	4.7	11.4	21.7	
Inoculated	6.0	27.3	41.2	53.1	
2020–2021	May 3	May 17	May 31	June 14	June 28
Soil Moisture × Rating Date[z]	0.1264	0.0035	<.0001	<.0001	<.0001
			Mortality (%)		
Low	13.2	23.2 a[y]	35.4 a	48.7 a	72.1 a
Optimal	6.9	11.3 b	16.6 b	26.3 b	40.6 b
High	11.9	17.0 ab	21.6 b	30.9 b	44.4 b

[z] The first rating date was removed from each season in order for the model to run.

[y] Within each rating date, soil moisture treatment means followed by the same letter are not significantly different according to Tukey's honestly significant test at $P \leq 0.05$. Data without letters indicate a non-significant influence of soil moisture on mortality.

Table A-9. The influence of soil moisture, cultivar, and inoculum on charcoal rot severity in strawberry in field trials in Irvine, CA.

	2018–2019				2019–2020				2020–2021			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	9	0.66	0.5407	2	9	2.52	0.1355	2	9	3.98	0.0577
Cultivar	1	5130	244.16	<.0001	1	4916	51.69	<.0001	1	6385	2.85	0.0915
Soil Moisture x Cultivar	2	5130	43.93	<.0001	2	4916	4.59	0.0102	2	6385	4.22	0.0148
Inoculum	1	5130	1.86	0.1732	1	4916	206.28	<.0001	1	6385	212.28	<.0001
Soil Moisture x Inoculum	2	5130	4.52	0.0110	2	4916	7.75	0.0004	2	6385	47.67	<.0001
Cultivar x Inoculum	1	5130	0.02	0.9003	1	4916	44.04	<.0001	1	6385	31.16	<.0001
Soil Moisture x Cultivar x Inoculum	2	5130	13.40	<.0001	2	4916	4.42	0.0121	2	6385	14.47	<.0001
Monterey Control	2	5130	1.20	0.2998	2	4916	0.99	0.3722	2	6385	7.42	0.0006
Optimal vs High	–	–	–	–	–	–	–	–	–	–	–	0.0002
Optimal vs Low	–	–	–	–	–	–	–	–	–	–	–	0.0065
Monterey Inoculated	2	5130	0.72	0.4859	2	4916	1.44	0.2363	2	6385	10.11	<.0001
Optimal vs High	–	–	–	–	–	–	–	–	–	–	–	0.6612
Optimal vs Low	–	–	–	–	–	–	–	–	–	–	–	0.0002
Petaluma/Fronteras Control	2	5130	0.74	0.4770	2	4916	0.79	0.4533	2	6385	1.30	0.2728
Petaluma/Fronteras Inoculated	2	5130	6.00	0.0025	2	4916	8.95	0.0001	2	6385	4.36	0.0128
Optimal vs High	–	–	–	0.1753	–	–	–	0.5195	–	–	–	0.3820
Optimal vs Low	–	–	–	0.0006	–	–	–	0.0010	–	–	–	0.0040
Rating Date	3	5130	27.03	<.0001	4	4916	539.21	<.0001	5	6385	288.12	<.0001

Table A-10. Influence of soil moisture, cultivar, and inoculum on spider mite severity on 17 Apr. 2021 in the 2020–2021 season.

Effect	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	1130	8.70	0.0002
Cultivar	1	1130	25.81	<.0001
Soil Moisture × Cultivar	2	1130	0.03	0.9708
Inoculum	1	1130	14.35	0.0002
Soil Moisture × Inoculum	2	1130	5.87	0.0029
Cultivar × Inoculum	1	1130	2.03	0.1549
Soil Moisture × Cultivar × Inoculum	2	1130	10.34	<.0001
Fronteras Control	2	1144	1.31	0.2696
Fronteras Inoculated	2	1144	3.26	0.0387
Optimal vs High				0.0315
Optimal vs Low				0.9482
Monterey Control	2	1144	15.77	<.0001
Optimal vs High				<.0001
Optimal vs Low				0.3844
Monterey Inoculated	2	1144	2.96	0.0520
Optimal vs High				0.6423
Optimal vs Low				0.0648

Table A-11. The influence of soil moisture, cultivar, and inoculum on charcoal rot severity in greenhouse-grown strawberries.

	2019–2020				2020–2021			
Effect	Num DF	Den DF	F Value	Pr > F	Num DF	Den DF	F Value	Pr > F
Soil Moisture	2	748	62.01	<.0001	a	453	133.59	<.0001
Cultivar	1	748	23.87	<.0001	1	453	19.39	<.0001
Soil Moisture × Cultivar	2	748	3.90	0.0206	2	453	2.30	0.1012
Fronteras	2	748	23.38	<.0001	–	–	–	–
High vs Moderate	–	–	–	<.0001	–	–	–	–
Low vs Moderate	–	–	–	0.0640	–	–	–	–
Monterey	2	748	45.68	<.0001	–	–	–	–
High vs Moderate	–	–	–	<.0001	–	–	–	–
Low vs Moderate	–	–	–	0.8836	–	–	–	–
Inoculum	1	748	11.74	0.0006	1	453	71.88	<.0001
Soil Moisture × Inoculum	2	748	0.92	0.3994	2	453	17.58	<.0001
Cultivar × Inoculum	1	748	7.81	0.0053	1	453	0.44	0.5071
Fronteras	1	748	0.22	0.6399	–	–	–	–
Monterey	1	748	18.14	<.0001	–	–	–	–
Soil Moisture × Cultivar × Inoculum	2	748	0.26	0.7673	2	453	4.28	0.0144
Fronteras Control	–	–	–	–	2	453	40.93	<.0001
High vs Moderate	–	–	–	–	–	–	–	<.0001
Low vs Moderate	–	–	–	–	–	–	–	<.0001
Fronteras Inoculated	–	–	–	–	2	453	85.80	<.0001
High vs Moderate	–	–	–	–	–	–	–	<.0001
Low vs Moderate	–	–	–	–	–	–	–	0.0820
Monterey Control	–	–	–	–	2	453	63.10	<.0001
High vs Moderate	–	–	–	–	–	–	–	<.0001
Low vs Moderate	–	–	–	–	–	–	–	0.0093
Monterey Inoculated	–	–	–	–	2	453	90.50	<.0001
High vs Moderate	–	–	–	–	–	–	–	<.0001
Low vs Moderate	–	–	–	–	–	–	–	<.0001
Rating Date	12	748	33.30	<.0001	7	453	21.14	<.0001

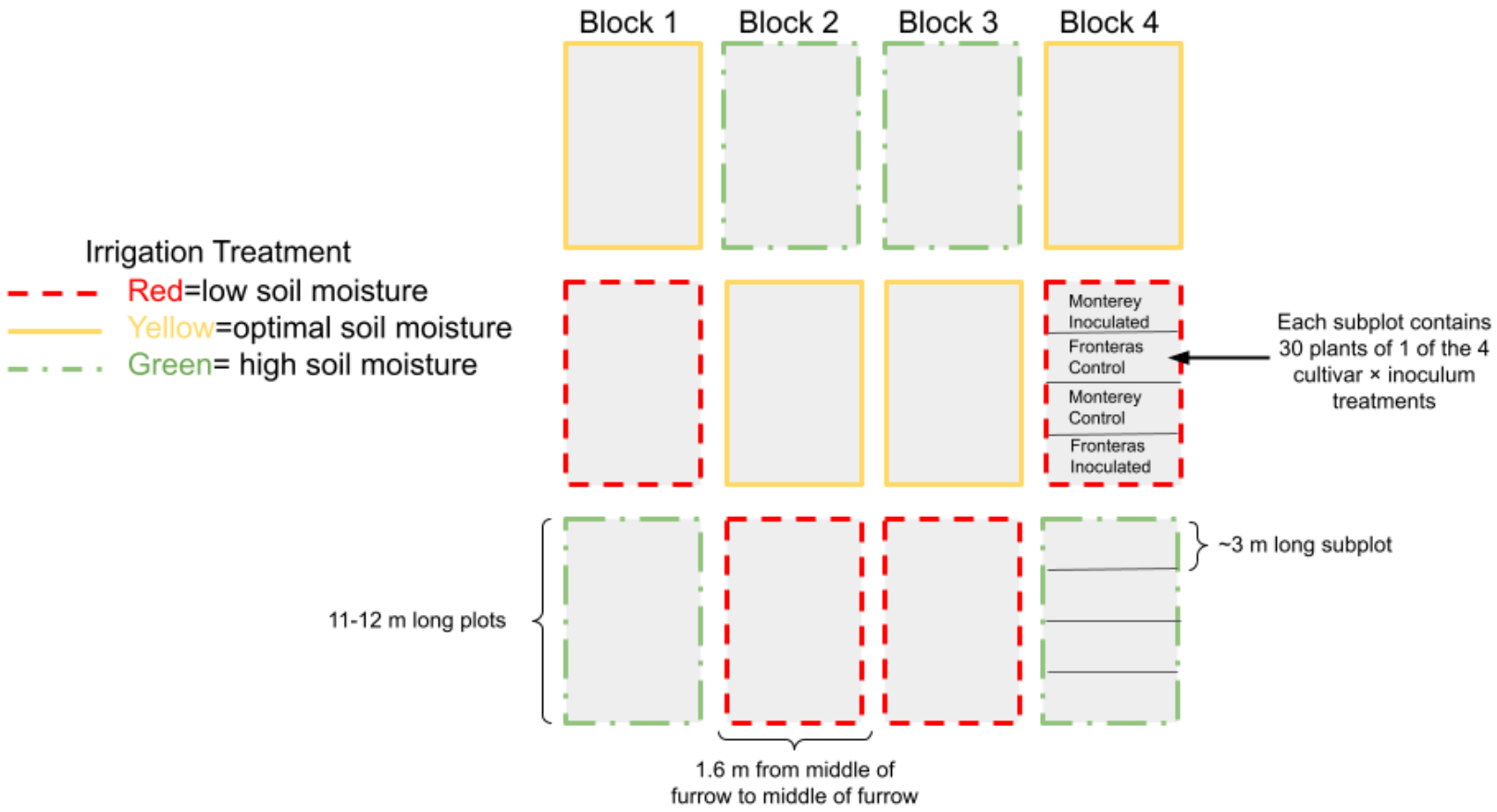

Fig. A-1. Diagram of field treatments. The randomization of the soil moisture treatments within each block and the cultivar × inoculum combinations within each main plot were different for each year of the study and the randomization for a single year is shown here.

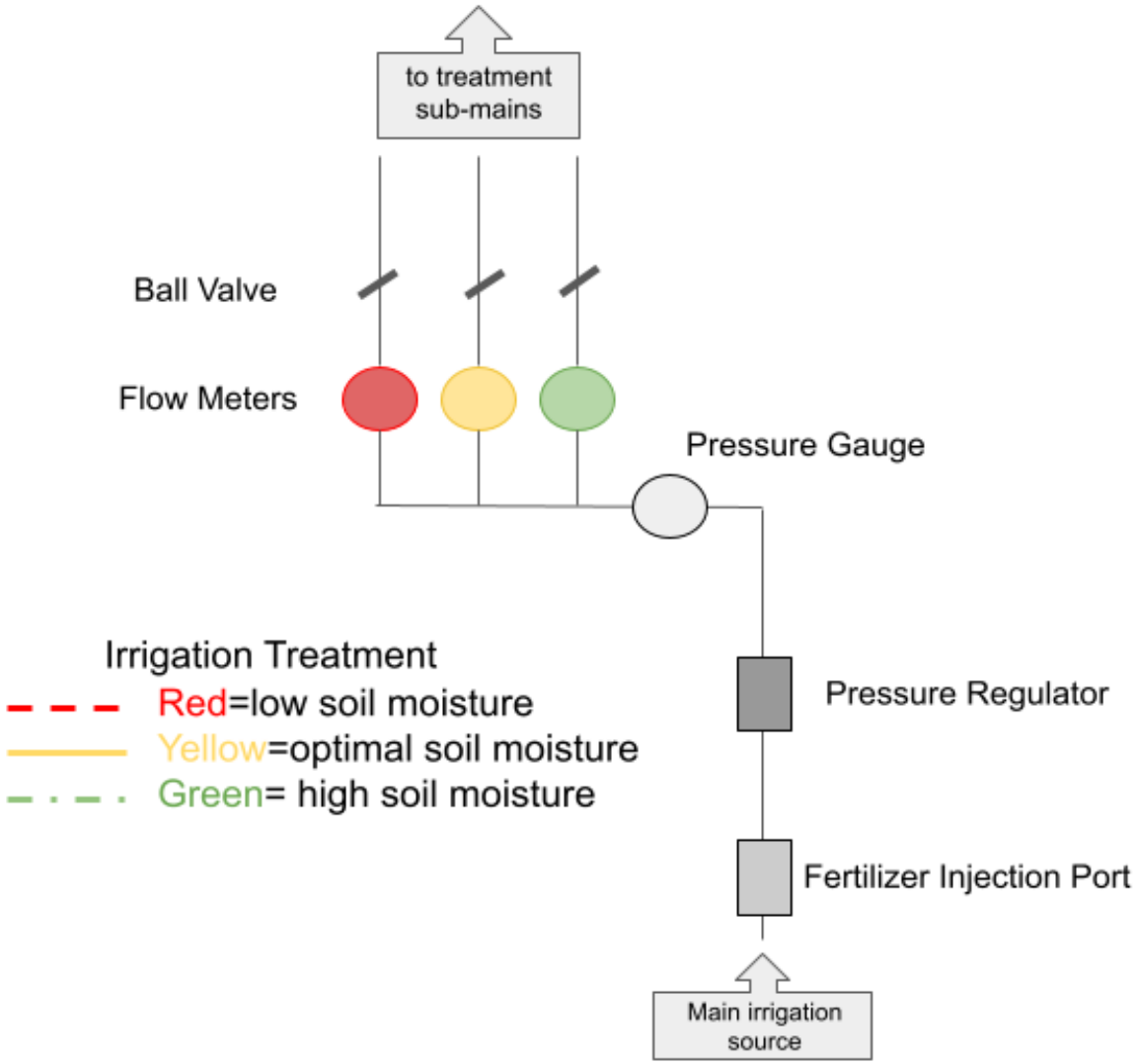

Fig. A-2. Diagram of the irrigation manifold.

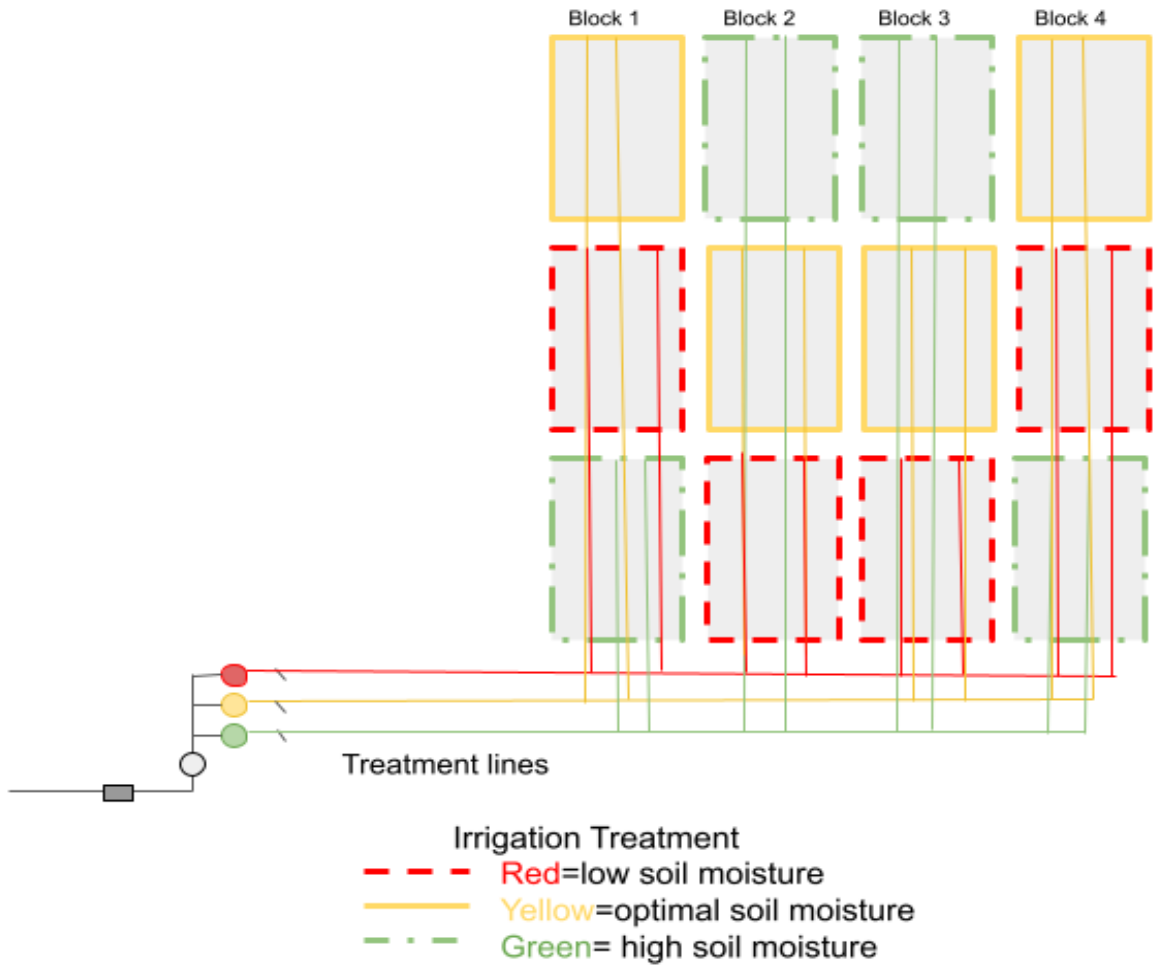

Fig. A-3. Diagram of irrigation main and drip lines for the 2018–2019 and 2019–2020 seasons. Main lines for each treatment ran from the manifold along one end of the beds, and tubing was used as laterals to connect each main line to the drip line in each main plot. Drip lines and tubing were fed under the plastic. The main plot randomization shown is for the 2018–2019 season, a different randomization was used for the 2019–2020 season.

Fig. A-4. Diagram of irrigation main and drip lines for the 2020–2021 season. Treatment main lines diverged at the manifold and ran along two sides of the study area to directly connect to the drip lines within each treatment main plot. Lines along the end of the bed were connected to an extra bed, but the valve remained closed.

Fig. A-5. Tensiometer readings for each soil moisture treatment (high, optimal, and low) throughout each season. Displayed are the readings of the two (2018–2019 season) or four tensiometers (2019–2020 and 2020–2021 seasons) per soil moisture treatment for each cultivar, which were used to determine when to irrigate that treatment.

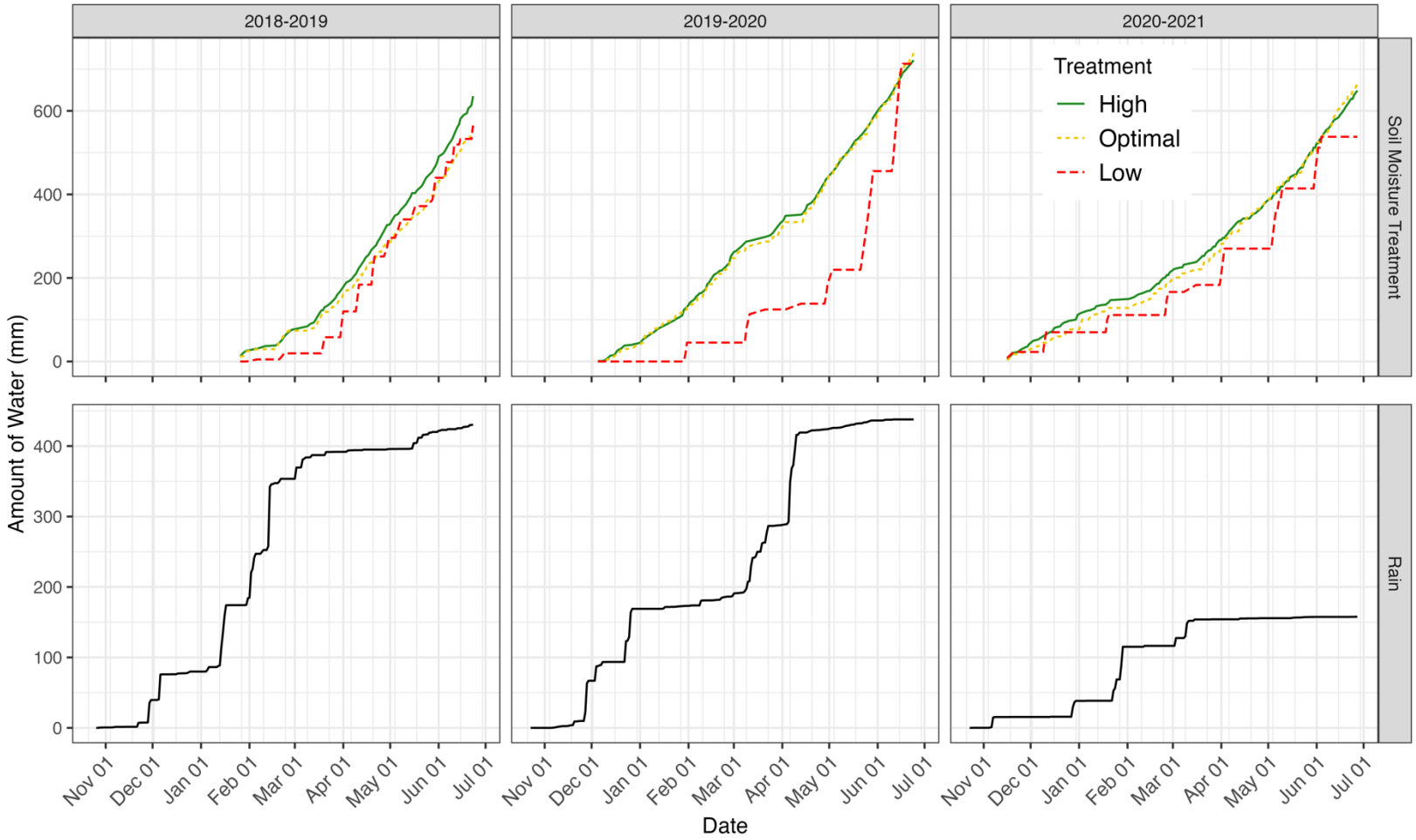

Fig. A-6. Cumulative amount of water (mm) received in each soil moisture treatment from irrigation as measured by flow meters or from rain as measured by sensors of the CIMIS station 0.4 km from the study site.

Fig. A-7. Disease severity rating scale for plant assessment in the field where: 0 = completely healthy; 1 = slight discoloration on the leaves; and a rating of 2, 3, 4, or 5 indicated that 0 to 25%, >25 to 50%, >50 to 75%, or >75 to 100% of the leaves were necrotic, respectively.

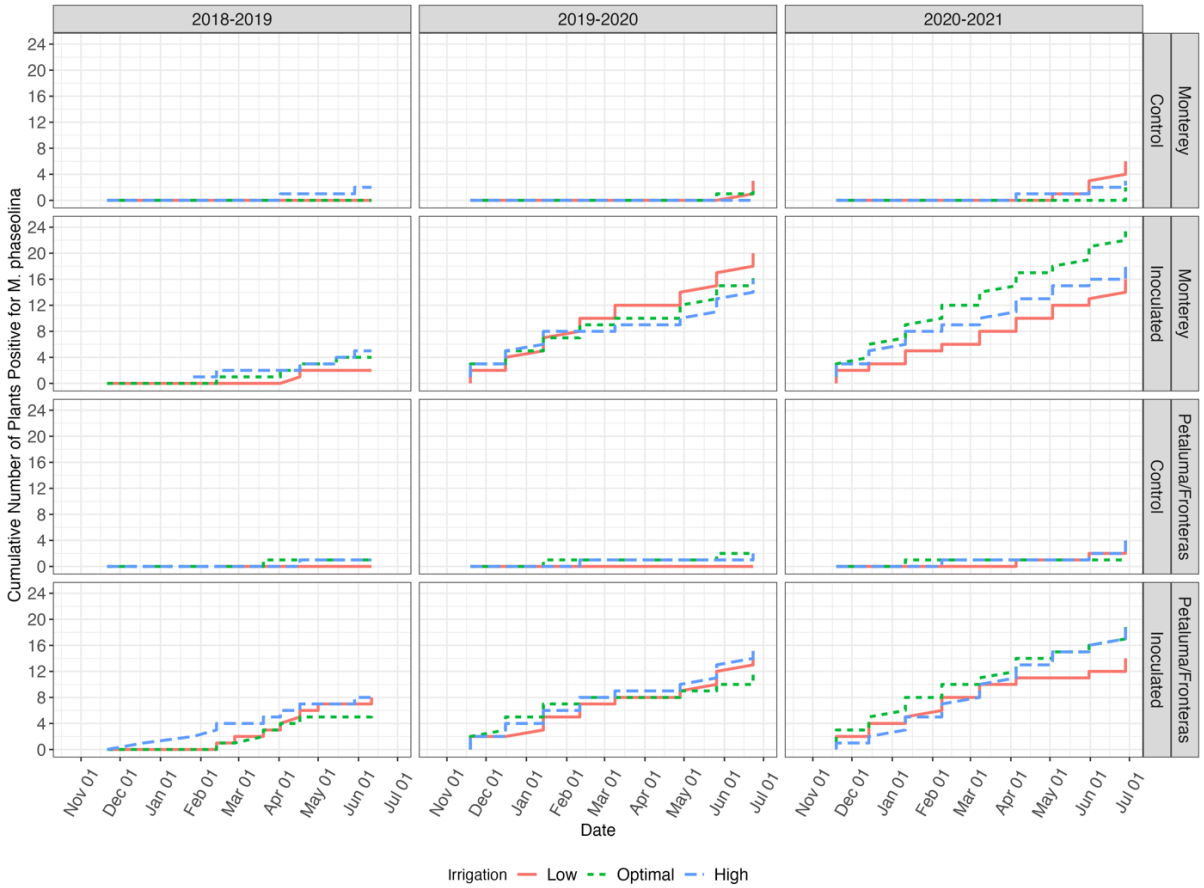

Fig. A-8. Summary of the cumulative number of positive *M. phaseolina* isolations from randomly sampled plants from each treatment during each season. In the 2018–2019 season, one plant per treatment in two replicate blocks was sampled every two weeks, and in the 2019–2020 and 2020–2021 seasons, one plant per treatment in three replicate blocks was sampled every four weeks. A total of 252 plants in 2018–2019 (red), 288 plants in 2019–2020 (green), and 324 plants in 2020–2021 (blue) were sampled. 20 root and 8 crown pieces were cut from each plant, surface sterilized, rinsed, and dried. The plant was considered positive if *M. phaseolina* grew from at least one piece of either root or crown tissue.

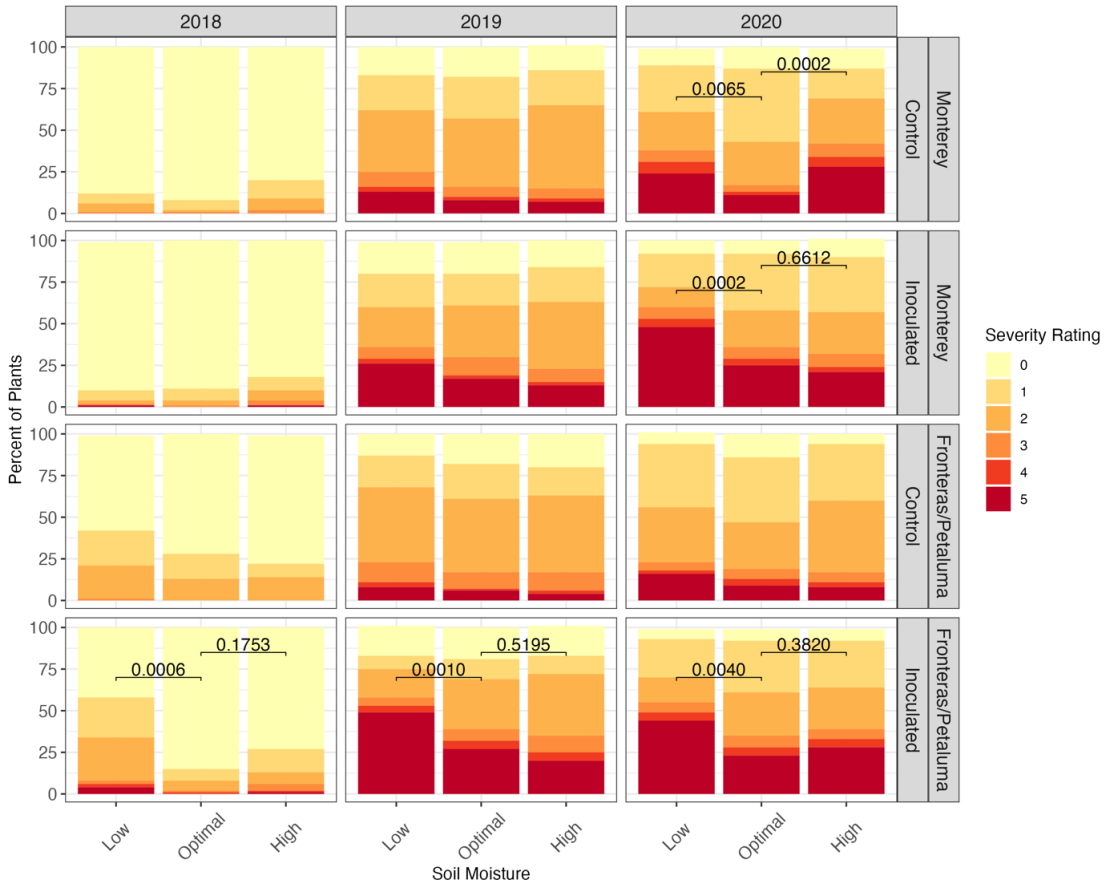

Fig. A-9. Influence of soil moisture, cultivar, and inoculum on severity of charcoal rot of strawberry in field studies in Irvine, CA. Each plant was rated on a scale from 0 to 5, where 0 is a healthy plant and 5 = >75 to 100% of the total number of leaves were completely necrotic. Data represent averages across all rating dates and are presented as stacked bars with the percent of plants with each disease severity rating. Brackets and adjacent *P*-values indicate results of contrast statements comparing soil moisture treatments. Absence of a bracket indicates there was not a significant influence of soil moisture on the cultivar × inoculum combination. Plants that died from other causes (such as transplant) or that were sampled prior to taking severity ratings are not included.

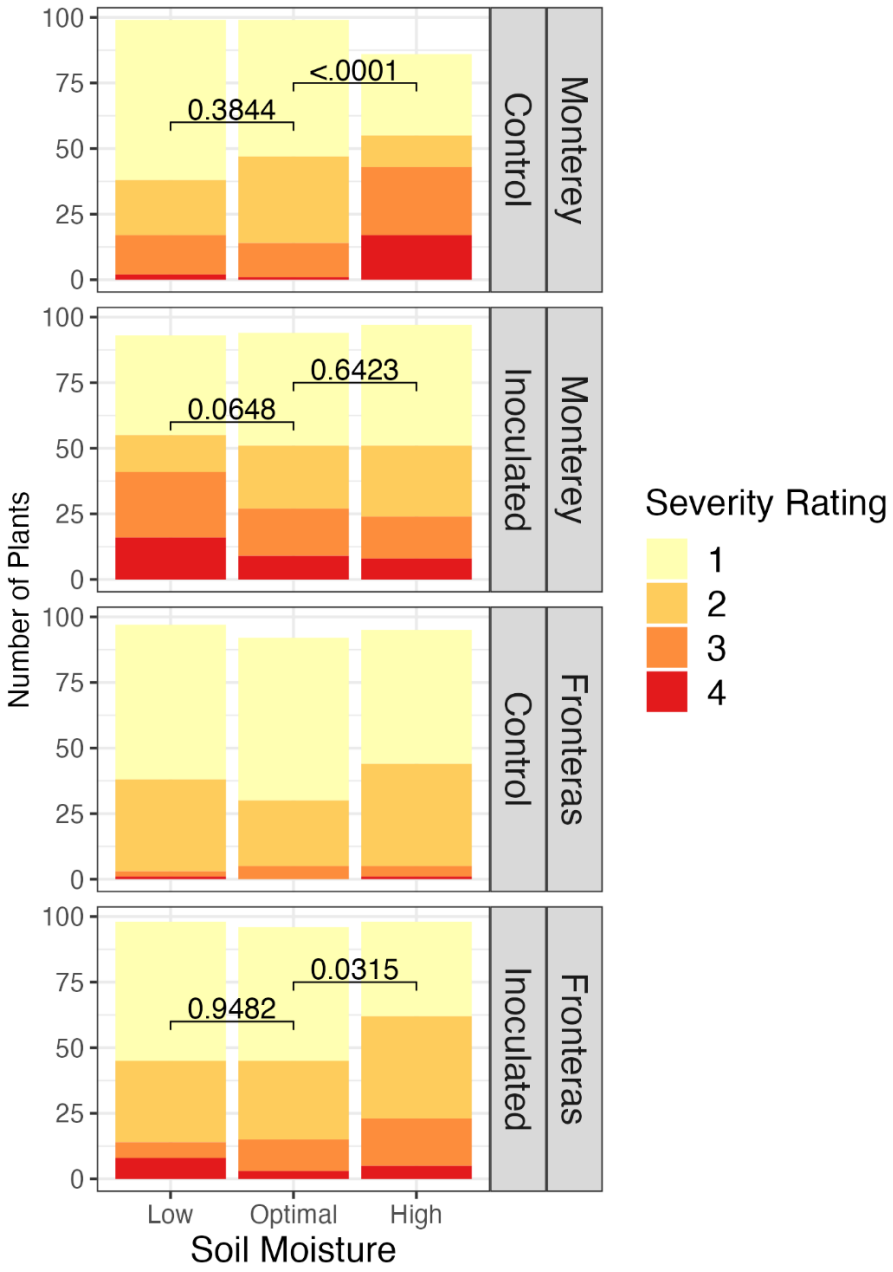

Fig. A-10. The number of plants with each mite severity rating on 17 Apr. 2021 in the 2020–2021 season. Brackets and adjacent *P*-values indicate results of contrast statements comparing soil moisture treatments. Absence of a bracket indicates there was not a significant influence of soil moisture on the cultivar x inoculum combination. Plants were rated based on photos taken on 17 Apr. 2021 for mite damage using a scale from 1 to 4 where 1 = no mite damage, 2 = 0-50% of leaves exhibiting mite damage, 3 = 50% or more of the leaves exhibit mite damage, and 4 = the plant has died. Plants that died from other causes (such as transplant) or that were sampled prior to taking severity ratings are not included.

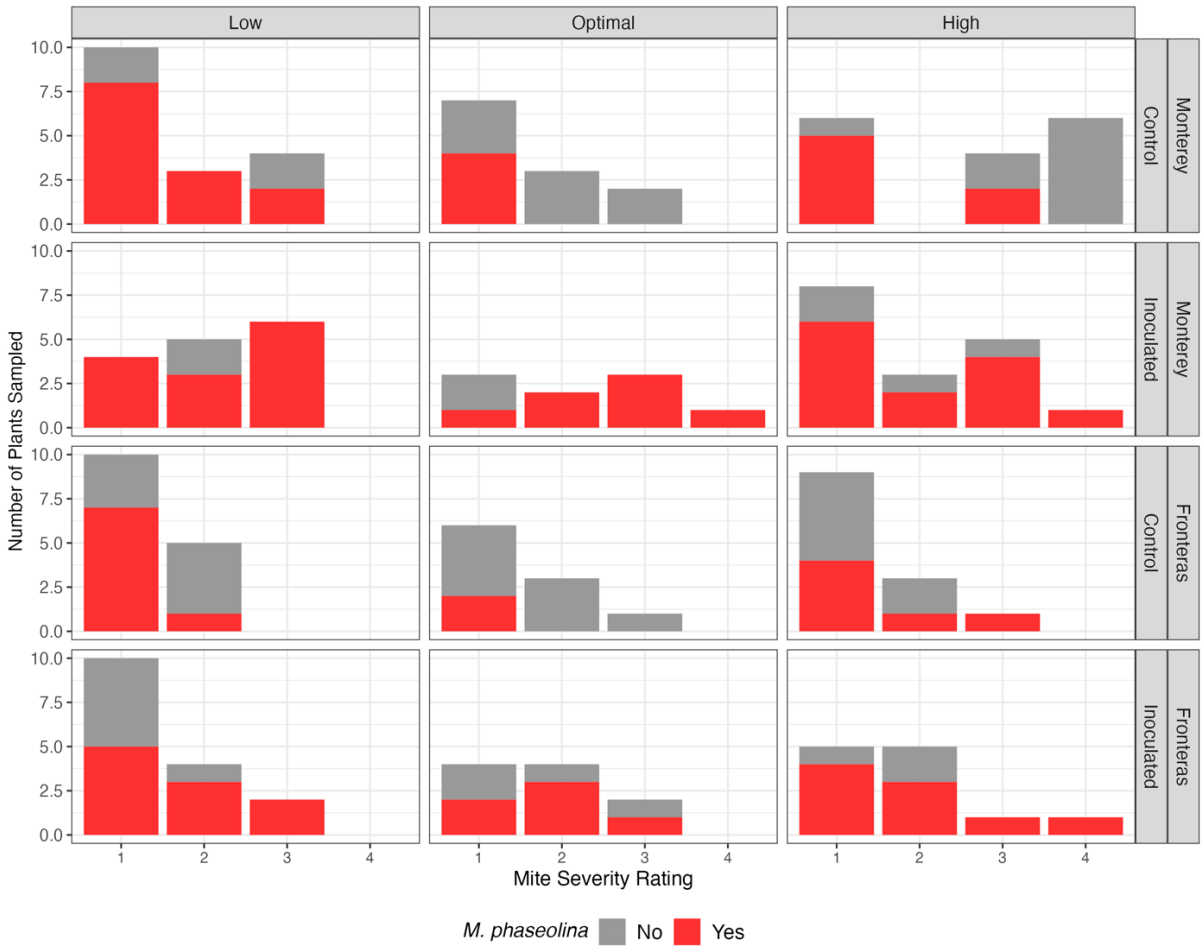

Fig. A-11. Positive (red) and negative (gray) isolations of *M. phaseolina* from all randomly and nonrandomly sampled plants taken after mite damage was first seen on 17 Apr. 2021. The plant was considered positive if *M. phaseolina* grew from at least one piece of crown or root tissue sampled. Plants were rated based on photos taken on 17 Apr. 2021 for mite damage using a scale from 1 to 4 where 1 = no mite damage, 2 = 0-50% of leaves exhibiting mite damage, 3 = 50% or more of the leaves exhibit mite damage, and 4 = the plant has died.

APPENDIX B

Supplemental Figures to Chapter 2

Fig. B-1. Pre-plant grouping of strawberry transplants into groups 1 through 5 where group 5 contains the largest plant with a crown width between 2 and 2.5 cm and group 1 contains the smallest plant with a crown width between 0.6 to 1.3 cm.

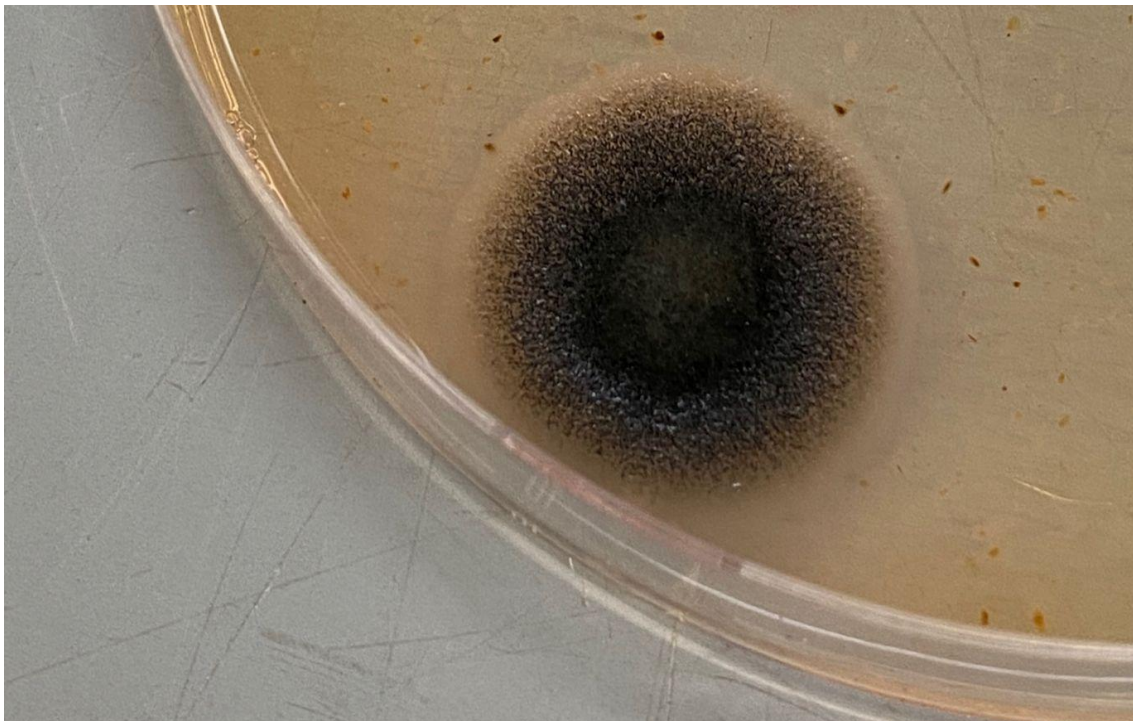

Fig. B-2. Morphology of a *M. phaseolina* colony on amended PDA after 7 days of growth at 28-30°C in the dark. Microsclerotia were visible throughout the colony with darker coloring at the center of the colony than at the edge. Fluffy gray to black mycelium was at the center of the colony. Colonies were typically 2.5 cm in diameter at this stage.

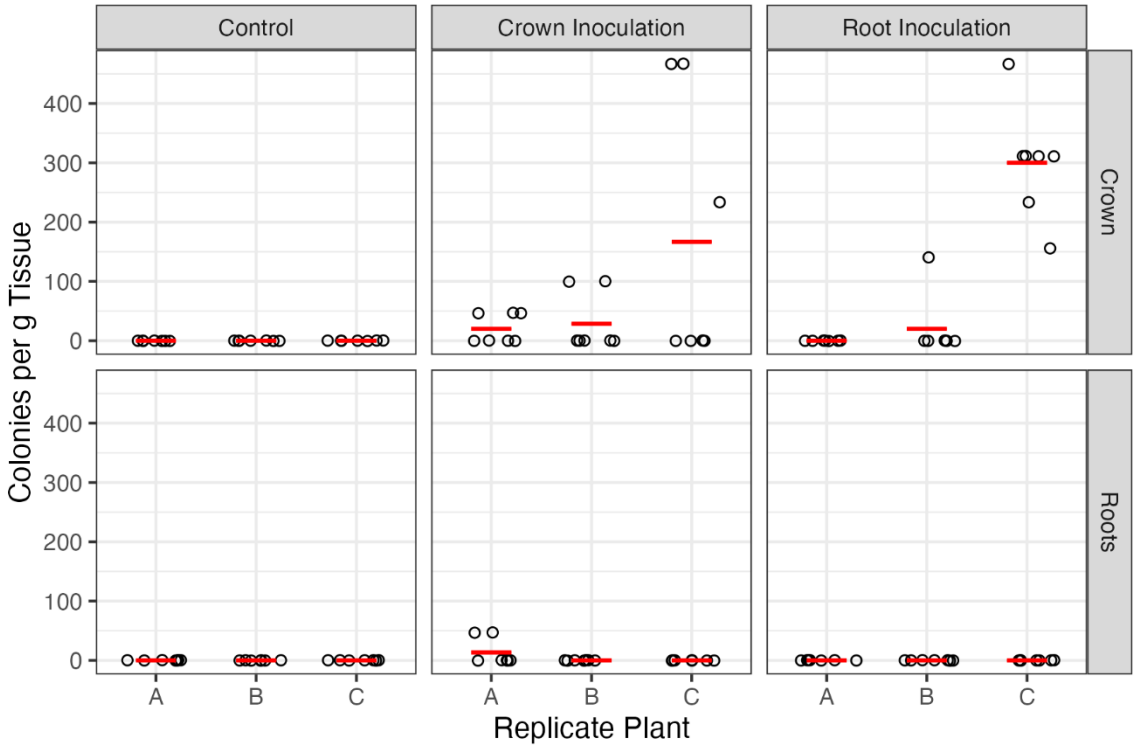

Fig. B-3. The number of *Macrophomina phaseolina* colonies detected in the roots and crown of control, crown-, and root-inoculated plants in the post-hoc inoculum washing test. Each data point represents each of seven Petri dishes as subsamples. The red bar represents the average number of colonies across the seven Petri dishes for each of 3 replicate plants (A, B, and C).

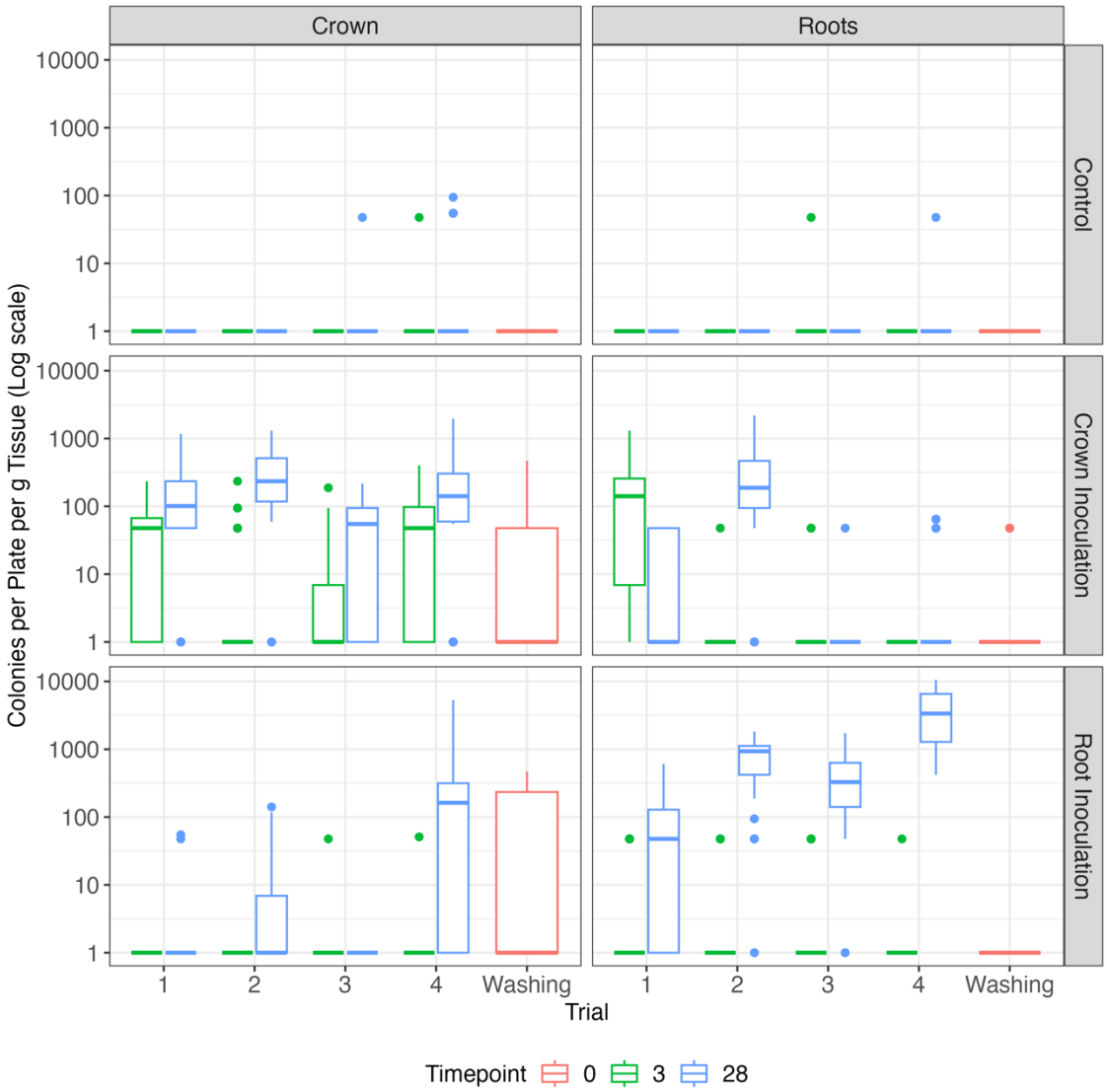

Fig. B-4. To compare the washing test to the experiment data, raw data shows colonization of strawberry cultivar 'Monterey' roots and crown as determined by inoculation location at 3 dpi (green) and 28 dpi (blue) across four experimental trials: 1, 2, 3, and 4, and in the inoculum washing test (red; timepoint = 0). Boxplots summarize raw data from five replicate plants in the experimental trials and 3 replicate plants in the inoculum washing test, and seven Petri dishes per plant for a total of 35 and 21 data points, respectively. To account for zeros on the log scale, the data were adjusted by adding 1 to each value. These data cannot be compared statistically but can be compared visually.

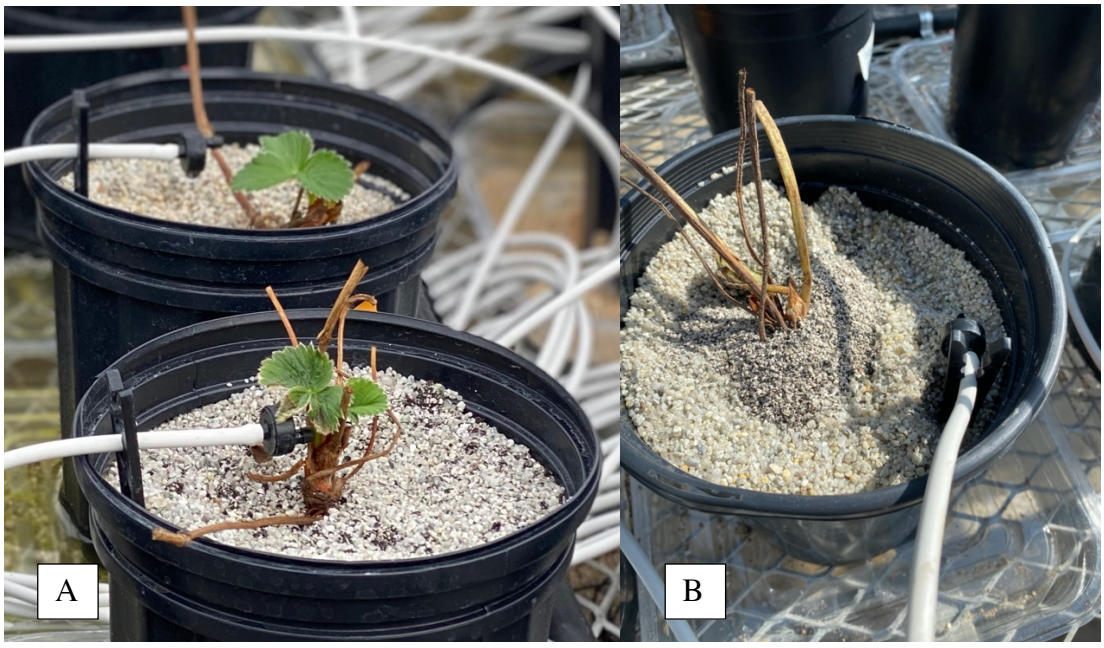

Fig. B-5. In trials 1 and 2 the drip emitter was placed directly next to the crown such that water was dripping on or directly next to the crown (A). In trials 3 and 4 the drip emitter was placed near the edge of the pot, approximately 7.6 cm from the plant (B).

APPENDIX C

Characterization of *Macrophomina phaseolina* Isolates from Uninoculated Plants in the Field Soil Moisture Study

INTRODUCTION

In a field study investigating the role of soil moisture in disease development and management of Macrophomina charcoal rot on strawberry, 'Monterey', 'Petaluma', and 'Fronteras' bare-root strawberry transplants were either inoculated with a slurry of *Macrophomina phaseolina* microsclerotia or uninoculated (control), and provided high, optimal, or low soil moisture throughout each of three seasons. Both inoculated and uninoculated control plants were sampled throughout each season to observe pathogen colonization. Unexpectedly, *M. phaseolina* was isolated from several uninoculated control plants during each season. The objectives of this study were to: i) confirm the identity of each isolate obtained from an uninoculated control plant, ii) analyze and compare the genomes of select isolates from the study and from diagnostic samples, and iii) test the pathogenicity of isolates genetically identified as *M. phaseolina* not within the strawberry clade.

MATERIALS AND METHODS

Fungal storage. *M. phaseolina* was isolated from a total of 220 inoculated plants and 68 uninoculated control plants in the field soil moisture study: 5 control plants in the 2018–2019 season, 19 control plants in the 2019–2020 season, and 44 control plants in the 2020–2021 season. 4 isolates, 14 isolates, and 40 isolates from each season, respectively, were stored on autoclaved toothpicks and filter paper in the dark at 19°C to 22°C, as described in Chapter 1 (Table C-1). All isolates included in this study that were obtained in the field soil moisture study were isolated in the latter part of the season

except for VSP-0572, the isolate obtained from the pre-plant soil sample in the 2020–2021 season.

Molecular identification. All 58 stored isolates from control plants were grown from storage on a Petri dish of PDA and subcultured onto a square of cellophane placed on a Petri dish of PDA. One of the colonies obtained from a pre-plant soil sample, VSP-0572, was also grown and the isolate used as the inoculum, GL1310, was grown as a positive control. Once the cellophane was fully covered in fungal growth, a sample of mycelium was aseptically scraped off the cellophane and placed in a microcentrifuge tube. The DNA was extracted from the fungal tissue using the DNeasy Plant Mini Kit (69106, Qiagen, Hilden, Germany) following the manufacturer's instructions with the following modifications: Steps 1 through 7 were modified such that, for homogenization, a single 3.2 mm stainless steel bead (11079132SS, BioSpec Products, Bartlesville, OK) was added to a microcentrifuge tube with ≤ 90 mg freshly harvested fungal tissue and then 400 µL Buffer AP1 was added to the tube. The tube was placed in the FastPrep-24 Instrument (116004500, MP Biomedicals, Irvine, CA) and the tissue was homogenized in two rounds at 6.5 m/s for 60 s. After homogenization, 4 µL RNase A was added to each tube and steps 8, 9, and 10 were followed according to the manufacturer's instructions. The centrifugation in step 10 was done at the highest setting (13,300 rpm = 16,300 x g) for 6 min. The lysate was moved to a new tube and the ice incubation and centrifugation in steps 9 and 10 were repeated once to remove additional precipitates. In step 11 the tubes were centrifuged for 3 min at 13,300 rpm. Steps 12 through 16 were followed according to the manufacturer's instructions. In step 17 the tubes were centrifuged for 3

min at 13,300 rpm to dry the membrane. In steps 18 and 19 the DNA was eluted with 50 µL of Buffer AE twice into the same tube. DNA was measured on a Nanodrop Spectrophotometer and stored at -20°C.

Identification of these isolates was performed with the *M. phaseolina* strawberry (*Mps*) genotype-specific quantitative real time polymerase chain reaction (qPCR) assay (Burkhardt et al. 2018). The 25 µL reaction volume consisted of 400 nM each of the Mps_TaqMan_F and Mps_TaqMan_R primers, 20 nM each of the Mps_TaqMan_External_F and Mps_TaqMan_External_R primers, 200 nM of the Mps_TaqMan_Probe, 400 nM each of the IC_F and IC_R primers, 40 nM of the IC_Probe, 20 fg of internal control DNA, and 1X Perfecta Multiplex qPCR ToughMix (95147, Quantabio, Beverly, MA) (Table C-2). 1 µL of each DNA sample was added to each duplicate reaction. Isolate GL1310, which had been used to inoculate plants in the field study, was included as a positive control. The cycling parameters were as follows: 1 cycle of 95°C for 3 min, 20 cycles of 95°C for 15 s and 70°C for 30 s with a plate read, followed by 50 cycles of 95°C for 15 s and 62°C for 30 s with a plate read.

Isolates exhibiting a negative reaction in the qPCR assay were further investigated by amplifying the same *Mps* fragment and the internal transcribed spacer (ITS) region in conventional PCR (White et al. 1990; Burkhardt et al. 2018). Three isolates that amplified in one of two technical replicates in the qPCR assay were also included in conventional PCR: VSP-0614, VSP-0616, and VSP-0627. For *Mps,* 25 µL reactions consisted of 400 nM each of the Mps_PCR_F and Mps_PCR_R primers (Table C-2), 1X Applied Biosystems PCR Buffer (100020474, Thermo Fisher Scientific, Waltham, MA),

2 mM MgCl2 (100020476, Invitrogen by Thermo Fisher Scientific, Waltham, MA), 200 µM dNTPs (R0192, Thermo Scientific, Waltham, MA) and 1 unit of AmpliTaq (100020477, Invitrogen by Thermo Fisher Scientific, Waltham, MA). 2.5 µL of each DNA sample was added to each reaction. Isolate GL1310 was included as a positive control. The cycling parameters were as follows: one cycle of 95°C for 2 min, 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min.

For ITS, 25 µL reactions consisted of 500 nM each of the ITS4 and ITS5 primers (Table C-2), 1X GoTaq G2 Green Taq MasterMix (M782A, ProMega, Madison, WI), and 2.5 µL DNA sample. Isolate GL1310 was included as a positive control. The cycling parameters were as follows: 1 min at 94°C followed by 35 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min, followed by 2 min at 72°C.

The PCR product from both the *Mps* and ITS PCR reactions were visualized using 5% RedSafe™ Nucleic Acid Staining Solution (21141, Bulldog Bio Inc, Portsmouth, NH) on a 1.5% agarose gel run with a 100 bp DNA Ladder (SM0243, Thermo Scientific, Waltham, MA) and 1X Tris-borate-EDTA running buffer at 120V for 40 min. The PCR product from the ITS reaction was purified using the ExoSAP-IT PCR product cleanup reagent (78201, Affymetrix, USB Products, Thermo Fisher Scientific, Waltham, MA). 7.5 µL of PCR product was mixed with 2 µL ExoSAP-IT reagent mixture prepared by mixing ExoSAP-IT cleanup reagent with nuclease-free water in a 50:50 ratio by volume. The PCR product and ExoSAP-IT mixture was incubated in the thermalcycler at 37°C for 30 min followed by 80°C for 20 min. The purified DNA was sequenced at the UCR Genomics Core via Sanger sequencing on an Applied Biosystems

3730xl with BigDye v3.1 using the same ITS4 and ITS5 primers. Sequence analysis was conducted in CLC Main Workbench 22. Sequences ends were trimmed and searched in NCBI BLAST.

To investigate the origin of the isolates obtained from uninoculated control plants in the field study, whole genome sequencing was performed on a subset of the 58 isolates. The subset consisted of twelve isolates from control strawberry plants selected because of their results in the qPCR assay, including the three identified to not be in the strawberry clade, one isolate from the soil, and two isolates selected from the 220 isolates obtained from inoculated plants. For comparison, seven isolates obtained from strawberry plants grown in the Oxnard, Santa Maria, and South Coast production districts submitted to our lab and diagnosed as Macrophomina charcoal rot were included. In addition, two isolates obtained from non-strawberry hosts, one from tomato and one from basil, from geographic areas where strawberry is not grown were included. DNA was extracted as described above and samples were submitted to the UCR Genomics Core for Illumina NextSeq High output 150 bp Paired End sequencing (Table C-1).

Pathogenicity trial. Three *M. phaseolina* isolates were strawberry clade-negative but identified as *M. phaseolina* via NCBI BLAST search of the ITS region: VSP-0343, VSP-0354, and VSP-0577. To evaluate the pathogenicity of these isolates, ‘Monterey’ low elevation ‘frigo’ strawberry plants were inoculated via toothpick inoculation (Koike 2008; Mertely et al. 2005). The pathogenicity trial included five treatments: the three strawberry clade-negative isolates, GL1310 as a positive control, and a mock-inoculated negative control. Monterey ‘frigo’ plants that had been stored at 2°C for 47 days were

planted in 2.65 liter injection molded nursery containers (CN-NCIM, Greenhouse Megastore, Danville, IL) with a custom soil mixture composed of 25% sand, 25% peat, 25% redwood mulch, and 25% perlite by volume with 2.48 kg per m^3 dolomitic lime and 3.97 kg per m^3 Osmocote Smart-Release Plant Food Plus (15-9-12, Item Number 274850, The Scotts Company LLC, Marysville, OH). Each pot was placed in a 20 cm square plant saucer (B093L1TGW2, UltraOutlet) to collect water flow-through. Treatments were arranged in a randomized complete block design with ten replicates. Plants were watered by hand the day they were planted. The day after planting, each pot received a single 3.79 liter per hour drip emitter (XB-10PC, Rainbird, Azusa, CA) that was programmed to automatically irrigate the plants 3 times a day every day for 1 min each time. Four days later, the trays were overflowing with water so the irrigation was changed to twice a day, and then 13 days after planting the irrigation was changed to once a day. Jack's Classic All Purpose plant food (20-20-20, #77010, JR Peters Inc. Allentown, PA) was added to all plants 73 days after planting by mixing approximately 2.5 ml of powder per 3.8 liters of water and pouring approximately 60 ml into each pot.

Toothpicks were autoclaved in a glass beaker covered loosely with aluminum foil for 30 min with fast exhaust and then cooled to 19°C to 22°C. Four Petri dishes containing PDA were used for each of the four isolates. Approximately ten autoclaved toothpicks were placed on each plate and then a PDA plug taken from actively growing mycelium was placed near the center of the plate. All Petri dishes were double wrapped in Parafilm and incubated at 19°C to 22°C in the dark with growth side facing up. Cultures grew for 41 days before some of them were aseptically opened to remove some

toothpicks from the dish. Autoclaved toothpicks without fungus were taken directly from the autoclaved beaker and used as the negative control. Some toothpick cultures became contaminated so to have enough for each plant, toothpicks were aseptically cut in half and then tape was wrapped around the toothpick approximately 1 cm from the pointed end. The tape helped to ensure that each toothpick would be poked into the crown of the plant at approximately the same depth. Plants were inoculated 13 and 27 days after planting for experimental repeats 1 and 2, respectively, by stabbing toothpicks into the crown of the plants to a depth of approximately 1 cm. Control plants were mock inoculated with autoclaved toothpicks and then all other plants were inoculated with their designated toothpicks. Charcoal rot severity was assessed every 7 to 12 days on an ordinal 0 to 4 scale where: 0 = healthy; 1 = >1-25% necrotic leaves; 2 = >25-50% necrotic leaves; 3 = >50-75% necrotic leaves; and 4 = dead plant with 100% necrotic leaves.

To confirm the cause of mortality in the experiment, all plants that were dead (severity rating = 4) were sampled from both experimental repeats 83 days after planting. The plant was gently removed from the potting soil and placed in a labeled Ziploc bag. The roots and crown of each plant were washed in a beaker of deionized water to remove the potting soil. The crown of each plant was cut open and eight 5 mm cubed pieces were cut from both the stele and cortex of the crown. Crown pieces were surface disinfested in a 0.5% sodium hypochlorite solution for 2 min and then rinsed three times for 60 s each in autoclaved deionized water, submerging and swirling the pieces to ensure pieces were thoroughly disinfested and rinsed, and replacing the water after each rinse. Pieces were

air dried on an autoclaved paper towel and then 4 pieces each were aseptically placed on a Petri dish of PDA+++ and a Petri dish of NP-10. PDA+++ and NP-10 were prepared as described in Chapter I. All Petri dishes were wrapped in a double layer of Parafilm M (PM992, Bemis Company Inc., Sheboygan Falls, WI) and placed in a square crisper box (295C, Pioneer Plastics, Dixon, KY) kept at 20°C to 26°C in a day–night cycle of artificial fluorescent light during working hours. Plates were observed for the presence of *M. phaseolina* after 7 days.

The pathogenicity trial was terminated 107 days after planting. All remaining plants that had been inoculated with GL1310 were sampled along with 2 plants per treatment per experimental repeat from each other treatment for a total of 24 plants. All plants were processed as previously described for isolation of *M. phaseolina*.

RESULTS

Molecular identification. Of the 58 isolates obtained from non-inoculated control plants and soil in the irrigation field trial, 55 were identified as belonging to the *M. phaseolina* strawberry genotype by the genotype-specific qPCR assay (Table C-1). The 3 isolates that were negative in the qPCR assay were also negative in the conventional PCR assay. However, these three isolates each had 98-100% sequence identity at the ITS region with *M. phaseolina* via a NCBI BLAST search and were thus identified as *M. phaseolina*.

Analysis of the whole genome sequencing data is pending.

Pathogenicity trial. Mortality was first observed 21 and 31 days after inoculation in experimental repeats 1 and 2, respectively (Fig. C-1). Disease severity increased in

plants inoculated with GL1310 until 36 days after inoculation in repeat 1 and 59 days after inoculation in repeat 2, at which point six plants had died in each repeat. Disease severity was very low in plants inoculated with VSP-0343, VSP-0354, and VSP-0577 and the mock for the length of the experiment. Among non-GL1310 treatments no plants died except for those inoculated with VSP-0343, in which one plant died in each repeat either 21 or 31 days after inoculation, respectively.

M. phaseolina was isolated from all dead plants sampled from both repeats of the pathogenicity trial. The crown of each dead plant was either partially or fully necrotic; necrosis commonly spanned the entire width of the crown but may not have spanned the entire length. Crowns that were partially necrotic had necrosis around the site where the toothpick entered the crown.

None of the plants sampled at the final rating date of both trials were dead, but some were symptomatic with necrotic older leaves. The crown of the control plants internally had brown discoloration where the toothpick had entered the crown but were otherwise healthy. Most of the inoculated plants only had a small area of brown discoloration near the inoculation site that did not span the entire width or length of the crown, but the crown was otherwise healthy. *M. phaseolina* was isolated from 0 of the 4 mock-inoculated plants and all 20 of the inoculated plants sampled on the final date.

LITERATURE CITED

Bilodeau, G. J., Koike, S. T., Uribe, P., and Martin, F. N. 2012. Development of an assay for rapid detection and quantification of *Verticillium dahliae* in soil. Phytopathology. 102:331–343.

Burkhardt, A., Ramon, M. L., Smith, B., Koike, S. T., and Martin, F. 2018. Development of molecular methods to detect *Macrophomina phaseolina* from strawberry plants and soil. Phytopathology. 108:1386–1394.

Koike, S. T. 2008. Crown rot of strawberry caused by *Macrophomina phaseolina* in California. Plant Dis. 92:1253.

Mertely, J., Seijo, T., and Peres, N. 2005. First report of *Macrophomina phaseolina* causing a crown rot of strawberry in Florida. Plant Dis. 89:434 Available at: http://dx.doi.org/10.1094/PD-89-0434A.

White, T. J., Bruns, T. D., Lee, S. B., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications. p. 315–322.

Table C-1. *Macrophomina phaseolina* isolates included in this study.

Isolate Number	Host[a]	Source	Cultivar	Field Study Info[b]		Collection Year	County	*Mps* qPCR	*Mps* PCR	ITS ID[c]	WGS[d]
				Inoculum	Soil Moisture						
VSP-0338	*F.* × *ananassa*	roots	Monterey	C	H	2019	Orange	(+)	NA	NA	Y
VSP-0343[e]	*F.* × *ananassa*	roots	Petaluma	C	H	2019	Orange	(-)	(-)	*M. p.*	Y
VSP-0354[e]	*F.* × *ananassa*	roots	Petaluma	C	O	2019	Orange	(-)	(-)	*M. p.*	Y
VSP-0519	*F.* × *ananassa*	crown	Monterey	C	H	2019	Orange	(+)	NA	NA	Y
VSP-0535	*F.* × *ananassa*	crown	Monterey	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0536	*F.* × *ananassa*	crown	Monterey	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0538	*F.* × *ananassa*	crown	Monterey	C	H	2020	Orange	(+)	NA	NA	NA
VSP-0539	*F.* × *ananassa*	crown	Fronteras	C	O	2020	Orange	(+)	NA	NA	NA
VSP-0541	*F.* × *ananassa*	crown	Fronteras	C	H	2020	Orange	(+)	NA	NA	NA
VSP-0542	*F.* × *ananassa*	crown	Fronteras	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0543	*F.* × *ananassa*	roots	Monterey	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0544	*F.* × *ananassa*	crown	Monterey	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0545	*F.* × *ananassa*	crown	Fronteras	C	H	2020	Orange	(+)	NA	NA	NA
VSP-0548	*F.* × *ananassa*	crown	Monterey	C	O	2020	Orange	(+)	NA	NA	NA
VSP-0549	*F.* × *ananassa*	roots	Monterey	C	L	2020	Orange	(+)	NA	NA	NA
VSP-0552	*F.* × *ananassa*	crown	Fronteras	C	O	2020	Orange	(+)	NA	NA	Y
VSP-0554	*F.* × *ananassa*	crown	Monterey	C	O	2020	Orange	(+)	NA	NA	NA
VSP-0555	*F.* × *ananassa*	crown	Fronteras	C	O	2020	Orange	(+)	NA	NA	Y
VSP-0572	*F.* × *ananassa*	soil	NA	NA	NA	2020	Orange	(+)	NA	NA	Y
VSP-0577[e]	*F.* × *ananassa*	roots	Fronteras	C	O	2021	Orange	(-)	(-)	*M. p.*	Y
VSP-0581	*F.* × *ananassa*	roots	Fronteras	C	H	2021	Orange	(+)	NA	NA	Y
VSP-0603	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0607	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0608	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0609	*F.* × *ananassa*	root	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0610	*F.* × *ananassa*	crown	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0611	*F.* × *ananassa*	crown	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0612	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0613	*F.* × *ananassa*	crown	Fronteras	C	O	2021	Orange	(+)	NA	NA	NA
VSP-0614	*F.* × *ananassa*	crown	Fronteras	C	O	2021	Orange	(+)	(-)[f]	*M. p.*	NA
VSP-0615	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0616	*F.* × *ananassa*	crown	Monterey	C	O	2021	Orange	(+)	(+)	*M. p.*	Y
VSP-0617	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0618	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0619	*F.* × *ananassa*	crown	Monterey	C	O	2021	Orange	(+)	NA	NA	NA
VSP-0620	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0621	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA

Isolate Number	Host[a]	Source	Cultivar	Field Study Info[b]		Collection Year	County	*Mps* qPCR	*Mps* PCR	ITS ID[c]	WGS[d]
				Inoculum	Soil Moisture						
VSP-0622	*F.* × *ananassa*	root	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0623	*F.* × *ananassa*	crown	Monterey	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0624	*F.* × *ananassa*	root	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0627	*F.* × *ananassa*	root	Monterey	C	H	2021	Orange	(+)	(+)	M. p.	Y
VSP-0655	*F.* × *ananassa*	crown	Monterey	C	O	2021	Orange	(+)	NA	NA	NA
VSP-0697	*F.* × *ananassa*	crown	Monterey	C	O	2021	Orange	(+)	NA	NA	Y
VSP-0698	*F.* × *ananassa*	root	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0701	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0702	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0703	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0704	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0705	*F.* × *ananassa*	crown	Monterey	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0706	*F.* × *ananassa*	crown	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0707	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0708	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0709	*F.* × *ananassa*	crown	Monterey	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0710	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0711	*F.* × *ananassa*	crown	Monterey	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0712	*F.* × *ananassa*	crown	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0713	*F.* × *ananassa*	crown	Fronteras	C	L	2021	Orange	(+)	NA	NA	NA
VSP-0715	*F.* × *ananassa*	crown	Fronteras	C	H	2021	Orange	(+)	NA	NA	NA
VSP-0716	*F.* × *ananassa*	crown	Monterey	C	H	2021	Orange	(+)	NA	NA	NA
GL1310[e]	*F.* × *ananassa*	NA	NA	NA	NA	NA	Orange	(+)	(+)	M. p.	Y
VSP-0256	*S. lycopersicum*	roots	NA	NA	NA	2018	Kern	NA	NA	NA	Y
VSP-0288	*F.* × *ananassa*	crown	Portola	NA	NA	2018	Ventura	NA	NA	NA	Y
VSP-0497	*F.* × *ananassa*	crown	Monterey	NA	NA	2019	Santa Barbara	NA	NA	NA	Y
VSP-0521	*F.* × *ananassa*	crown	Monterey	I	L	2019	Orange	NA	NA	NA	Y
VSP-0591	*O. basilicum*	stem/root	NA	NA	NA	2020	Riverside	NA	NA	NA	Y
VSP-0641	*F.* × *ananassa*	crown	NA	NA	NA	2021	Santa Barbara	NA	NA	NA	Y
VSP-0677	*F.* × *ananassa*	crown	San Andreas	NA	NA	2021	Orange	NA	NA	NA	Y
VSP-0684	*F.* × *ananassa*	crown	San Andreas	NA	NA	2021	Orange	NA	NA	NA	Y
VSP-0690	*F.* × *ananassa*	crown	San Andreas	NA	NA	2021	Orange	NA	NA	NA	Y
VSP-0714	*F.* × *ananassa*	root	Monterey	I	H	2021	Orange	NA	NA	NA	Y
VSP-0741	*F.* × *ananassa*	crown	NA	NA	NA	2021	Ventura	NA	NA	NA	Y

[a] Isolates were obtained from 3 hosts: *Fragaria* x *ananassa*, *Solanum lycopersicum*, and *Ocimum basilicum*.

[b] Field study info includes the inoculation treatment: inoculated (I) or control (C); and the soil moisture treatment: high (H), optimal (O), or low (L) soil moisture.

[c] *M.p. = Macrophomina phaseolina*

[d] WGS = whole genome sequencing

[e] Isolates included in the pathogenicity trial.

[f] DNA yield was very low for this isolate due to heavy contamination in storage, which affected the *Mps* conventional PCR assay.

Table C-2. Sequences of primers and probes used in the study.

Name	Sequence (5' to 3')	Reference
Mps_TaqMan_F[a]	CCT CGG CAA ATC CCT ATA G	Burkhardt et al. (2018)
Mps_TaqMan_External_F[a]	CTA AAG TGG CTT AAT ACT AAT TTA GCG CCG GCG AAT C	Burkhardt et al. (2018)
Mps_TaqMan_R[a]	GTT TAC CCT CTG TCT ATT CC	Burkhardt et al. (2018)
Mps_TaqMan_External_R[a]	GTA AGC CTT ACC GCA CTA GAA GTA AGG GTA AGA TCG	Burkhardt et al. (2018)
Mps_TaqMan_Probe[b]	Quasar670-CTA TTT GGT TAA CCC CTA CTC GCT TAG ACT-BHQ2	Burkhardt et al. (2018)
IC_F[a]	CGT TTC CCG TTA CTC TTC T	Bilodeau et al. (2012)
IC_R[a]	GGA TTT CGG CCC AGA AAC T	Bilodeau et al. (2012)
IC_Probe[b]	Cal Flour Red 610-AAA GTA AGC TTA TCG ATA CCG TCG ACC T-BHQ2	Bilodeau et al. (2012)
Mps_PCR_F[a]	CCT CGG CAA ATC CCT ATA G	Burkhardt et al. (2018)
Mps_PCR_R[a]	GTT TAC CCT CTG TCT ATT C	Burkhardt et al. (2018)
ITS4[a]	TCC TCC GCT TAT TGA TAT GC	White et al. (1990)
ITS5[a]	GGA AGT AAA AGT CGT AAC AAG G	White et al. (1990)

[a] Primers were synthesized by Integrated DNA Technologies, Inc., Coralville, IA.

[b] BHQ2 = Black Hole Quencher 2. Probes were synthesized by Biosearch Technologies, Inc., Novato, CA

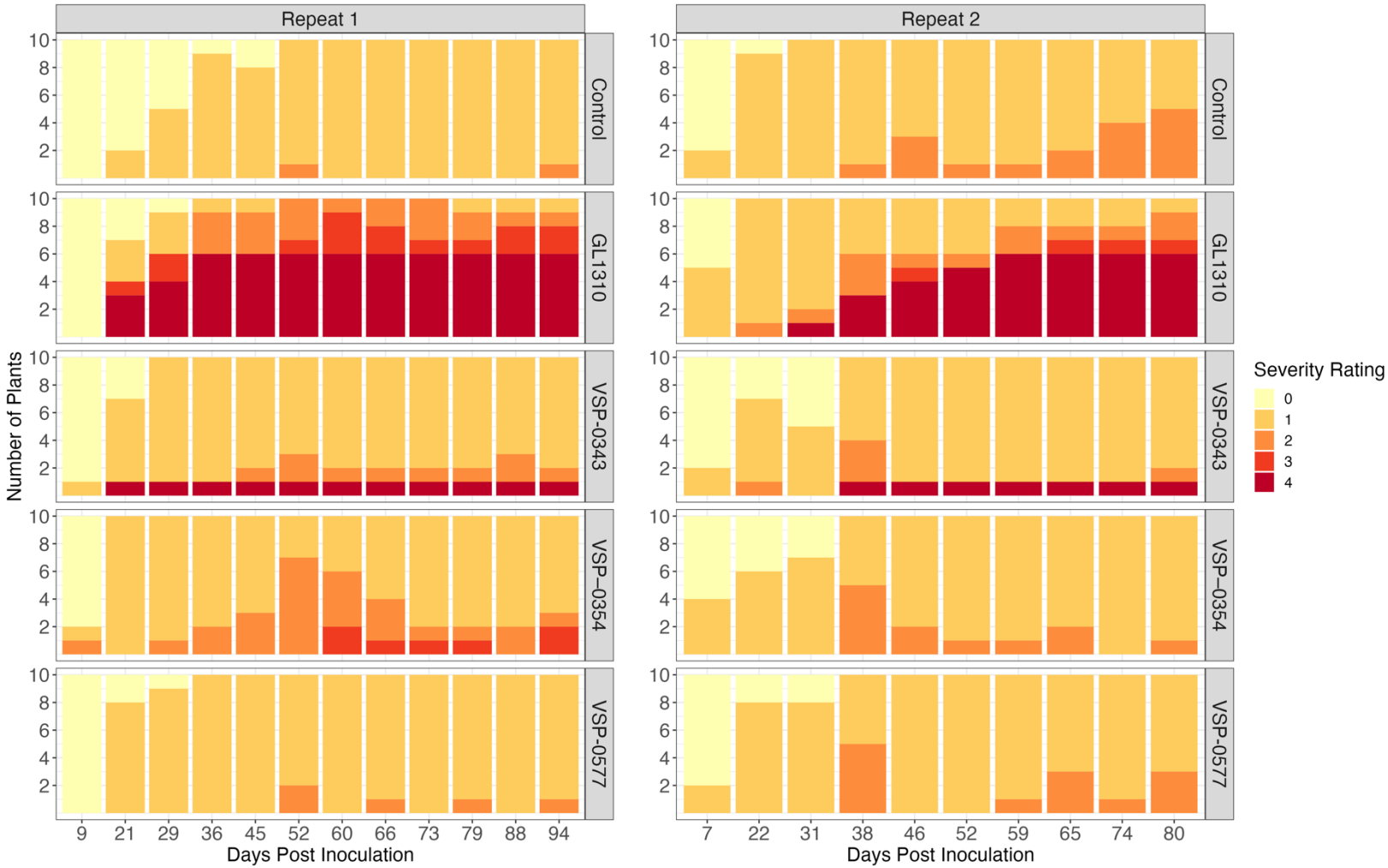

Fig. C-1. Disease severity ratings for plants in repeats 1 and 2 of the pathogenicity trial. Plants were rated on a scale from 0 (healthy plant, no necrotic foliage) to 4 (dead plant, 100% necrotic foliage)

GENERAL CONCLUSIONS

The disease cycle of Macrophomina charcoal rot on strawberries must be well understood to develop management strategies that are effective, accessible to growers, and sustainable for the industry. To my knowledge, this research is the first to document the temporal dynamics of *M. phaseolina* colonization of strawberry plants, and the influence of soil moisture on this process, in a field setting. This research is also the first to my knowledge to report direct, asymptomatic *M. phaseolina* colonization of strawberry roots and crown within 28 days of inoculation.

Results from both studies show that *M. phaseolina* asymptomatically colonizes the roots and crown of the strawberry plant. In the field study, plants were inoculated and planted in October but were asymptomatic until late April or early May when they began wilting, becoming necrotic, and collapsing. However, *M. phaseolina* was isolated from the roots and crown of randomly selected plants as early as six months before symptom development. In the inoculation study, *M. phaseolina* was isolated from roots and crowns of asymptomatic plants within 28 days of inoculation. These data align with new evidence in non-strawberry hosts that *M. phaseolina* may be a hemibiotroph, though this should be investigated further on strawberry through histopathological observations and expression of biotrophy and necrotrophy marker genes.

The inoculation study begins to illustrate the infection process of *M. phaseolina* on strawberry. Though direct inoculation of the crown via toothpick inoculation has been shown to cause disease, direct infection of the crown without the aid of wounding had not been described on strawberry. Our results show that *M. phaseolina* can directly infect

both the roots and crown of the plant, but infection remains asymptomatic within 28 days of inoculation. However, the inoculation study was not designed to determine the depth of pathogen growth into the crown. Future studies should continue to investigate the depth and extent of pathogen colonization in association with symptom development.

Our field study showed that symptoms of charcoal rot on strawberries and plant mortality due to charcoal rot are exacerbated by low soil moisture conditions but are not significantly improved by high soil moisture. The effect of soil moisture on pathogen colonization of the crown was not consistent between cultivars, suggesting this effect may be cultivar-dependent. In the field study, low soil moisture led to increased mortality in Monterey and Fronteras inoculated plants in 2 years of the study. In contrast, high soil moisture led to decreased colonization of inoculated Monterey crowns, but no effect was observed for Fronteras or Petaluma. These findings highlight the importance of proper irrigation management as well as the need for further research on the differences in susceptibility of strawberry cultivars to *M. phaseolina* as influenced by soil moisture.

This research contributes to our understanding of the infection process and influence of soil moisture on development of Macrophomina charcoal rot on strawberries. Overall, strawberry growers are recommended to maintain the soil moisture at an optimal level if *M. phaseolina* is present. These results will aid in developing management strategies for charcoal rot on strawberries that will benefit strawberry growers throughout California and the world.