INVESTIGATING NOVEL APPROACHES FOR THE INTEGRATED CONTROL OF
THE SOILBORNE STRAWBERRY PATHOGENS *MACROPHOMINA PHASEOLINA*
AND *FUSARIUM OXYSPORUM* f. sp. *FRAGARIAE*

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ABSTRACT

Investigating novel approaches for the integrated control of the soilborne strawberry pathogens *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae*

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Macrophomina phaseolina (*Mp*) and *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) are emerging soilborne pathogens causing crown rot and Fusarium wilt, respectively, in commercial strawberry production in California. Fungicides representing eight active ingredients from four different mode of action groups (FRAC groups 1, 3, 7 and 12) were evaluated for their efficacy against each pathogen *in vitro* and each disease *in planta*. Fungicide active ingredients were evaluated for their ability to inhibit mycelial growth of both pathogens *in vitro*. Half-strength potato dextrose agar was amended with six different concentrations (0.01, 0.1, 1.0, 5.0, 10, 50 µg a.i./ml) of seven fungicides in FRAC groups 3, 7 and 12. Concentrations that inhibited fungal growth by 75% (EC₇₅) compared to unamended media were determined for two different isolates each of *Mp* and *Fof* and were used to determine fungicide rates for subsequent *in planta* studies. Tebuconazole strongly inhibited the mycelial growth of both pathogens (average EC₇₅ for *Mp* was 2.4 ppm; average EC₇₅ for *Fof* was 7.48 ppm), as did metconazole (average EC₇₅ for *Mp* was 2.53 ppm; average EC₇₅ for *Fof* was 1.28 ppm). Fludioxonil strongly inhibited mycelial growth of *Mp*, but had no impact on the growth of *Fof*. Penthiopyrad, fluopyram, flutriafol, and flutolanil were less effective at inhibiting fungal growth of either fungus. Greenhouse *in planta* studies evaluated twenty-four fungicide treatments

(eight fungicides at low, med and high rates) that were drench applied to infested potting media two days prior to planting of pathogen susceptible strawberry cultivars (San Andreas for *Mp* and Monterey for *Fof*) and again at day 21. Controls were a non-inoculated and an inoculated water-drench treatment. Buried inoculum was recovered at days 2 and 23 and plated on selective media for colony forming unit (CFU) quantification. Plant disease assessments were made each week for 11 weeks. An analysis of variance (ANOVA) of CFUs revealed no significant differences ($p > 0.05$) among treatments and when compared to the non-treated control for both *Mp* and *Fof*, but showed significant decreases ($p < 0.05$) in CFUs between weeks 1 and 3 for both *Mp* and *Fof*. An ANOVA for disease assessments in the form of area under the disease progress curve (AUDPC) showed significant decreases of disease severity in treatments with penthiopyrad only (low, medium and high rates) ($p < 0.05$). There were no significant differences ($p > 0.05$) in AUDPC among treatments and when compared to the non-inoculated and no-fungicide controls for *Fof*. The data indicates that these fungicides used alone are not effective against these pathogens *in planta*.

A strawberry plant extract (germination stimulant) was assessed for its ability to stimulate germination of *Mp* microsclerotia *in vitro* and *in planta*. The germination stimulant was applied as a drench at six different concentrations (0, 10, 100, 1,000, 10,000 and 30,000 ppm) to soil containing filter disk packets of microsclerotia of *Mp* at day 0 and 14. Filter disk packets were retrieved three days after the drench and microsclerotia were observed microscopically for germination. Results showed that the number of germinating microsclerotia was significantly higher after the application of the germination stimulant compared to non-drench and 0 ppm controls ($p < 0.001$).

An integrated container trial was also conducted using the germination stimulant at 10,000 ppm applied three days prior to a fungicide drench with tebuconazole or thiophanate-methyl to determine the effect of fungicides on the germinated microsclerotia. The use of the germination stimulant with label rates of the fungicides lowered the number of germinated intact microsclerotia significantly ($p < 0.001$) especially after two drench applications. The use of the germination stimulant with fungicides could be investigated further as one method for controlling soilborne diseases of strawberry.

Key Words: disease management, *Fusarium oxysporum* f. sp. *fragariae*, germination stimulant, *Macrophomina phaseolina*, methyl bromide alternative, soilborne pathogen, strawberry

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CHAPTER 1: LITERATURE REVIEW

Introduction

Strawberry (*Fragaria x ananassa* Duchesne) production has relied heavily on soil fumigation to effectively control a wide range of economically devastating soilborne diseases. Emphasis on finding non-fumigant disease control measures has become a top priority due to the phase-out of methyl bromide and increasing regulations on other fumigants. Studies have shown that modern strawberry yields are dependent on the pre-plant reduction of soil pathogens (Fang et al., 2012). Pre-plant fumigation significantly reduces soilborne pathogen populations but does not eliminate them; repeated fumigation for each crop is often necessary to keep pathogens at acceptable levels in annual production systems (Wilhelm and Paulus, 1980).

California produces strawberries on approximately 40,000 acres with a value of over \$2.5 billion annually (California Strawberry Commission, 2015). Fresh berries are grown in three main growing regions: Santa Maria, Oxnard and Salinas/Watsonville, while transplants and genetic material are typically grown at low and high elevation nurseries in the central valley and interior of northern California (California Strawberry Commission, 2015). Fertile soils and mild climates benefit strawberry production in California. The success of the industry can be attributed to intensive breeding programs, both private and public, along with the optimization of planting systems and cultural practices (Voth and Bringhurst, 1990). Since the 1960s, methyl bromide mixed with chloropicrin has been used to control soilborne diseases, leading to an increase in production acreage of strawberries (Fravel et al., 2003). Due to its effect on the environment, methyl bromide has been phased-out in many countries and the U.S.

requires a critical use exemption as a result of the Montreal Protocol (Shennan et al., 2012; Fang et al., 2012). However, this chemical will be phased out in commercial strawberry production by the end of 2016 (Shennan et al., 2012).

Methyl-bromide & Other Fumigants

The implementation of methyl bromide has been the most cost effective tool for producers to control soilborne diseases, insects, and weeds (Shaw and Larson, 1996; Martin, 2003; Yuen et al., 1991). The largest consumers of methyl bromide have historically been tomato and strawberry producers in Florida and California (Shaw and Larson, 1996). The use of methyl bromide began in the 1960s and has been one of the top five most used pesticides in the U.S. (Fravel et al., 2003). The introduction of methyl bromide fumigation in 1961 resulted in consistently higher yields and changed grower practices with the use of drip irrigation and polyethylene films to prevent contact between fruit and soil (Voth and Bringhurst, 1990; Wilhelm and Paulus., 1980).

Methyl bromide mixed with chloropicrin has been used to fumigate most of California strawberry production soils until recently (Wilhelm and Paulus., 1980; De Cal et al., 2004). Methyl bromide is an odorless and colorless gas that is injected about two feet (0.61 m) below the soil surface as a pre-plant treatment (Leahy et al., 2013). Although the soil is covered with plastic tarps, some methyl bromide dissipates into the atmosphere. Methyl bromide has been implicated in the reduction of the ozone layer leading to increased ultraviolet radiation reaching the earth's surface (US EPA, 1999). The Montreal Protocol resulted in the gradual phase-out of methyl bromide, which began in 1991 and will end in 2016 (US EPA, 1999). On an annual basis, the protocol allows for

critical use exemptions, mainly applied for by tomato and strawberry producers (Martin, 2003). Since the phase-out, pathogens are becoming established in soil and contribute to reduced yields by lowering plant vigor and causing mortality (Martin, 2003; Shennan et al., 2012).

Alternative fumigants like metam sodium (Martin, 2003) and 1, 3-dichloropropene (1, 3-D) mixed with chloropicrin are less effective than methyl bromide-chloropicrin mixes (Shaw and Larson, 1999; Yuen et al., 1991). Flat or broadcast fumigation with methyl bromide, chloropicrin or methyl bromide-chloropicrin mixes are the most effective methods of fumigation and have reduced soilborne fungi such as *Macrophomina* and *Fusarium* between 79-100% (Martin, 2003). Due to increasing buffer zones and other regulations, growers are switching from flat fumigation to in-line fumigation under plastic beds. Inoculum can survive in furrows and bed shoulder areas if only the beds are fumigated (Matner, 2008). Biofumigants using isothiocyanates may also help to control Verticillium wilt and weeds (Matner, 2008; Gerald Holmes personal communication, 2016).

The efficacy of other available fumigants are limited resulting in the need for alternative pest control measures (Martin, 2003). Both chemical and non-chemical alternatives exist. For example, steam sterilization of soil and anaerobic soil disinfestation are alternatives to using chemical fumigants for certain pests and soil types, although the results are not always consistent (Shennan et al., 2012; Fennimore et al., 2012). Unless viable alternatives are found, the loss of methyl bromide will continue to have a severe impact on our nation's ability to meet the demands for strawberries. An integrated disease management approach is necessary for continued economically viable

strawberry production in pathogen infested soils (Fennimore and Daugovish, 2012; Koike, 2012).

Soilborne Pathogens

Macrophomina phaseolina (*Mp*) and *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) are emerging soilborne strawberry pathogens that cause vascular wilting and chlorosis and necrosis of leaves, often leading to significant reduction in yield (Koike & Gordon, 2015). *Mp* was first identified on strawberry in California (CA) in 2005 and is considered the most significant emerging soilborne threat to CA strawberry production because of its widespread distribution (Koike, 2012). Upon infection and plant death, *Mp* produces large numbers of microsclerotia, which are known to survive for many years in fallow fields. In other pathosystems, the survival of *Mp* in soil and on debris has been reported for up to 15 years (Baird et al., 2003). *Fof* was first confirmed in CA in 2006 and is considered the second most important soilborne threat to CA strawberry production (Koike et al., 2009). *Fof* is host specific to strawberry and survives in soil as chlamydospores for up to 15 years (Koike et al., 2009; Shennan et al., 2012).

Mp and *Fof* survive in infected crop debris and in soil as microsclerotia or chlamydospores, respectively. These survival structures serve as the primary source of inoculum in the subsequent strawberry crop in infested production fields and are resistant to desiccation and temperature extremes. Targeting the microsclerotia and chlamydospores of both strawberry pathogens in the soil will help in reducing primary inoculum and rate of secondary disease spread (Koike et al., 2013a; Leahy et al., 2013; Martin, 2003).

Macrophomina phaseolina

Macrophomina phaseolina has been reported as the causal agent of charcoal rot of strawberry in France (Baudry and Morzieres, 1993), Egypt (Maas, 1998), Florida (Merteley et al., 2005), Israel (Zveibil & Freeman, 2005), Spain (Aviles et al., 2008) and Argentina (Baino et al., 2011). *Mp* was limited to the southern part of the CA (Ventura and Orange counties) in 2005; by 2010 charcoal rot cases were documented in Santa Cruz, Santa Clara, Alameda, and Sacramento counties (Koike et al., 2013a).

Macrophomina phaseolina is in the family Botryosphaeriaceae. It is a polyphagous fungus that appears in growing regions worldwide on a number of crops and has a wide host range of more than 500 plant families, including monocots and dicots (Gupta et al., 2012; Kaur et al., 2012; Zveibil et al., 2012; Smith and Carvil, 1977). *Mp* produces large numbers of melanized, multicellular structures referred to as microsclerotia (Gupta et al., 2012; Kaur et al., 2012; Koike and Bolda, 2013). Microsclerotia have been documented in living plant tissue as well as necrotic tissue. An individual microsclerotium is an aggregation of 50-200 individual cells that can germinate repeatedly coupled by a melanin pigment (Gupta et al., 2012; Kaur et al., 2012). They are black in color and range from 50 to 150 μm in size (Gupta et al., 2012; Kaur et al., 2012). These microsclerotia are survival structures that allow the pathogen to persist in fallow fields (Kaur et al., 2012; Koike and Bolda, 2013), for up to fifteen years (Baird et al., 2003; Kaur et al., 2012). Olaya and Abawi (1996) documented the enhanced production of microsclerotia under low water potentials associated with drought; this is

especially disconcerting given drought conditions over the past few production seasons on the central coast of CA.

Plants infected with *Mp* do not show symptoms initially, however, once the plant undergoes stress (e.g. starts to produce fruit), symptoms develop (Koike, 2012).

Symptoms include discoloration of leaves, wilting, and overall plant decline and plant death (Gupta et al., 2012; Kaur et al., 2012; Koike, 2012; Koike and Bolda, 2013).

Charcoal rot symptoms develop slowly in fall and winter, but increase rapidly during the spring and summer due to the warm soil temperature requirements of *Mp* (optimal range 25-30°C) (Mihail, 1992a; Wyllie et al., 1984; Zveibil et al., 2012). Fruit production is not affected by onset of symptoms until the plant starts to collapse from infection (Mertely et al., 2010; Mertely et al., 2014). Because symptoms of other soilborne and crown diseases look identical in the field, isolation from diseased tissue is required for proper diagnostics in strawberry (Koike, 2012; Koike and Bolda, 2013; Koike et al., 2013c; Zveibil et al., 2012).

Fusarium oxysporum* f. sp. *fragariae

Fusarium wilt of strawberries was first reported in Australia in 1962 (Winks and Williams, 1965) and Japan in 1969 (Okamoto et al., 1970) and subsequently Korea in 1982 (Kim et al., 1982), USA in 2006 (Koike et al., 2009), and China and Spain in 2009 (Koike and Gordon, 2015; Fang et al., 2012). *Fusarium oxysporum* f. sp. *fragariae* was first confirmed in CA in 2006 and is considered to be the second most important soilborne threat to CA strawberry production behind *Macrophomina phaseolina* (Koike and Gordon, 2015; Koike et al., 2009; Islas et al., 2011). *Fof* was initially limited to

Ventura County, but has since been located throughout all production regions in California. (Islas et al., 2011).

Fusarium oxysporum is both a saprophyte and necrotroph and is commonly found in the fungal rhizosphere of many plants (Fang et al., 2012; Mihail, 1992b). Wilt inducing isolates of *F. oxysporum* are host-specific pathogens that have been divided into 120 different *formae speciales* (f.sp) (Fravel et al. 2003). *Fof* is host specific to strawberry and its vascular tissue (Brayford, 1996; Fang et al., 2012; Koike et al., 2009). *Fof* hyphae invade the inter-cellular spaces of the epidermis, hypodermis and then move through the cell layer of the cortex to infect vascular tissue (Fang et al., 2012). Strawberry plants can show initial symptoms such as stunting, wilt, and decline as early as 30 days after transplanting (Koike and Gordon, 2015).

As plants exhibit symptoms of Fusarium wilt, internal vascular tissues of the crown and petiole often show a brownish orange discoloration (Koike and Gordon, 2015). Plating pieces of infected crown and petiole on a semi-selective medium is necessary to make a proper diagnosis (Koike et al., 2009; Koike and Gordon, 2015).

Spread of the pathogens & management of the diseases

The movement of infested soil or plant material spreads both *Mp* and *Fof* (Brayford, 1996; Koike and Bolda, 2013; Koike and Gordon, 2015; Koike et al., 2013b & c).

Current management strategies involve the following: avoid planting in infested fields, rotate crops, apply pre-plant fumigants (methyl bromide plus chloropicrin flat fumigation is the most effective), grow cultivars that are tolerant to *Mp* or *Fof*, plant pathogen-free transplants, avoid planting into untreated buffer zones which may harbor soilborne

inoculum, and avoid the movement of infested soil (Brayford, 1996; Koike et al., 2013; Koike and Bolda, 2013; Koike and Gordon, 2015; Gupta et al., 2012). Very limited studies have focused on the use of fungicides in controlling these diseases. Studies in Florida have shown inconsistent results in controlling *Mp* using different fungicides applied to soil (Mertely et al., 2010; Mertely et al., 2014). At this time, there have been no published studies demonstrating the use of fungicides in soil to manage *Fof*.

Research Objectives

The overall goal of this research is to develop and optimize different approaches for managing the emerging soilborne pathogens *Macrophomina phaseolina* (*Mp*) and *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) of strawberry. The specific objectives of my research include:

1. to evaluate the efficacy of various rates and the timing of soil-applied fungicides for managing *Macrophomina* crown rot and *Fusarium* wilt;
2. to examine the influence of whole strawberry plant extracts on the germination of *Mp* microsclerotia and explore their use as germination stimulants; and
3. to evaluate the combined use of strawberry plant extracts (obj. 2) with the most effective soil-applied fungicides (obj. 1) for managing *Macrophomina* crown rot.

CHAPTER 2: EVALUATING THE EFFICACY OF VARIOUS RATES AND TIMING OF SOIL-APPLIED FUNGICIDES FOR MANAGING MACROPHOMINA CROWN ROT AND FUSARIUM WILT

Abstract

Macrophomina phaseolina (*Mp*) and *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) are emerging soilborne pathogens causing crown rot and Fusarium wilt (respectively) in commercial strawberry production in California. Fungicides representing eight active ingredients from four different mode of action (FRAC) groups (1, 3, 7 and 12) were evaluated for their relative efficacy in controlling each pathogen *in vitro* and *in planta*. For the *in vitro* studies, active ingredients were evaluated for their ability to inhibit mycelial growth of both fungi. Half-strength potato dextrose agar was amended with six different concentrations (0.01, 0.1, 1.0, 5.0, 10, 50 µg a.i./ml) of seven active ingredients in FRAC groups 3, 7 and 12. Concentrations that inhibited fungal growth by 75% (EC₇₅) compared to unamended media were determined for two different isolates each of *Mp* and *Fof*, and were then used to determine fungicide rates for *in planta* studies. Tebuconazole strongly inhibited the mycelial growth of both pathogens (average EC₇₅ for *Mp* was 2.4 ppm; average EC₇₅ for *Fof* was 7.48 ppm), as did metconazole (average EC₇₅ for *Mp* was 2.53 ppm; average EC₇₅ for *Fof* was 1.28 ppm). Fludioxonil strongly inhibited mycelial growth of *Mp*, but had no impact on the growth of *Fof*. Penthiopyrad, fluopyram, flutriafol, and flutolanil were less effective at inhibiting fungal growth of either fungus. Greenhouse *in planta* studies involved twenty-four fungicide treatments (eight fungicides at low, med and high rates) that were drench applied to infested potting media two days prior to planting of susceptible strawberry cultivars and twenty-one days

after planting. Buried inoculum was recovered at day two and 23 days after the first treatment application and plated on semi-selective media for colony forming unit (CFU) quantification. Plant disease assessments were made once a week for 11 weeks by calculating the area under the disease progress curve (AUDPC). An analysis of variance (ANOVA) of CFU counts of buried inoculum revealed no significant differences ($p > 0.05$) among treatments for both *Mp* and *Fof*, but showed significant decreases ($p < 0.05$) in CFU counts between one application (at week 1) and two applications (at weeks 1 and 3) for both *Mp* and *Fof*. An ANOVA of AUDPC showed significant decreases ($p < 0.001$) in disease severity in treatments with penthiopyrad for *Mp* only (low, medium and high rates). There were no significant differences ($p > 0.05$) in AUDPC between fungicide treatments and non-treated controls for *Fof*. This data indicates that these active ingredients did not provide effective control of these pathogens *in planta*.

Introduction

Currently, economically viable soilborne disease control alternatives to methyl bromide are lacking in commercial strawberry production. Strawberry producers will benefit from the development and optimization of novel ways to achieve soilborne disease control that can be integrated into existing conventional and organic production systems, or combined with other alternative approaches such as soil fungicides, anaerobic soil disinfestation (ASD), low rates of fumigants, or even steam.

Numerous fungicide chemistries have been utilized to manage soilborne crown and root diseases of other crops. The fungicides selected in this study were chosen due to success of pathogen control in other recent studies. Topguard[®] (flutriafol) was granted a

section 18 exemption in 2012 for cotton farmers in TX and has been granted a supplemental label through 2018. Studies by Tom Isakeit at Texas A&M have shown that one application of Topguard applied at planting demonstrated excellent potential as a fungicide for managing cotton root rot (Isakeit et al., 2011). Effectiveness of this product was associated with ample rain or overhead irrigation of cotton within one month of planting (Isakeit et al., 2011). However, there is more research that needs to be done to minimize the risk of phytotoxicity due to the long soil activity of this chemistry. In 2015, the US Environmental Protection Agency approved a supplemental flutriafol label in California for use on strawberries. The other five fungicides we selected to evaluate have been successfully used to control other soilborne diseases, and include Folicur (tebuconazole), Maxim (fludioxonil), Quash (metconazole), Fontelis (penthiopyrad), Moncut (flutolanil) and Topsin (thiophanate-methyl) (Ferry and Davis, 2012; Merteley et al. 2010; Merteley et al. 2014).

There is little information about fungicides currently used or evaluated for the management of *Mp* and *Fof* in strawberry. Florida studies showed that thiophanate-methyl has the ability to significantly reduce *Mp* disease incidence in raised-bed strawberries (Merteley et al. 2010; Merteley et al. 2014). These studies also showed that thiophanate-methyl and fluopyram were used together to increase fruit yield, but showed no significant difference in AUDPC caused by *Mp* (Merteley et al. 2014). There are no published studies pertaining to chemical control of *Fof* in strawberry at this point in time. Therefore, the goal of this research was to evaluate fungicides and to report their ability to manage both *Mp* and *Fof*.

Materials & Methods

In vitro fungicide assays

Two isolates of *Mp* (isolates 13 and 8), isolated from disease strawberry crowns from Irvine and Oxnard, CA, and two isolates of *Fof* (isolates 10.3 and 19), isolated from disease strawberry crowns from Santa Maria and Watsonville, CA, were used as test strains. The entire experiment was replicated twice. Trial I was conducted November 17, 2014 through January 6, 2015. Trial II was conducted December 2, 2014 through January 22, 2015. Seven fungicides from FRAC groups 3, 7 and 12 were selected for this experiment. Seven different concentrations (0, 0.01, 0.1, 1.0, 5.0, 10, 50 ppm) of technical grade active ingredient were added to molten half-strength potato dextrose agar. A 5.5 mm² plug of each actively growing *Mp* or *Fof* isolate was inverted (mycelium-side down) and placed in the center of each fungicide-amended and non-amended control plate; there were four replicates of each treatment combination for *Mp* and *Fof*. Radial mycelial growth was measured for *Mp* plates daily for the first two weeks and then twice per week through day 50. Radial mycelial growth was measured for *Fof* plates every other day through the third week and then twice per week through day 50 since *Fof* mycelium grows slower than *Mp*. Mycelial plugs that showed no growth at day 50 were transferred to unamended half strength PDA and monitored for growth over the course of two weeks to assess the viability of the fungus since it did not grow during these assays.

Radial mycelial growth (mm per day) was converted to the area under the growth progress curve (AUGPC) to quantify isolate growth over the entire time frame of each experiment. The formula is as follows:

$$\text{AUGPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Normalized growth rate of each treatment was regressed against the fungicide concentration, which was used to calculate the concentrations that suppress fungal growth by 75% (EC₇₅).

$$\text{Normalized growth rate} = \frac{\text{AUGPC of treatment}}{\text{AUGPC of control}}$$

Regression analyses were conducted using Minitab 16 Statistical Software (Minitab Inc., State College, PA).

In planta Macrophomina study

The same two isolates of *Mp* (*Mp* 8 and *Mp* 13) used in the *in vitro* experiments were grown on PDA and added to a cornmeal sand mixture to create inoculum (Mihail et al., 1992). Ten grams of inoculum was added to each trade one gallon pot (2.84 liter) with roughly 400 g potting media [7 ft³ (2.1 m³) peat moss, 7 ft³ (2.1 m³) coconut core, 7 ft³ (2.1 m³) perlite, 12 oz. (340 g) treble superphosphate, 18 oz. (510g) potassium nitrate, 59 oz. (1.67 kg) dolomite lime and 1 oz. (28 g) micromax plus] to both the inoculated control and to each of twenty-four treatments (eight fungicides at low, med and high rates) (Table 2.1). Three grams of inoculum were buried in cotton mesh teabags about one inch (25.4 mm) below the soil surface in each pot. Treatment drenches occurred 2 days prior to planting of San Andreas plants and 21 days after initial drench (450 ml drench per pot as identified through preliminary unpublished studies). Each treatment consisted of six single-plant reps. Teabags with inoculum were recovered after each drench (reps 1, 3, 5 two days after; reps 2, 4, 6 twenty-three days after) and plated on RB media (Cloud and Rupe, 1991) from serial dilutions of 100 mg inoculum/10 ml distilled

water for CFU quantification (Lin et al., 2010; Mihail et al., 1992; Mihail and Alcorn, 1982).

Plant disease assessments were made every week starting two weeks after planting. Plants were rated on a scale of 1-5; the rating of 1= no disease symptoms; 2=25% of plant wilted or necrotic; 3=50% of the plant wilted or necrotic; 4=75% of the plant wilted or necrotic; 5=dead plant (Figure 2.1). Scores were converted to percent disease (1= 0%, 2=25%, 3= 50%, 4=75% and 5=100%); these percentages were then used to determine the area under the disease progress curve (AUDPC). The AUDPC equation is commonly used to quantify disease intensity over time in plant pathology. The AUDPC is a method that uses time variables (hours, days, weeks, months, or years) and calculates the average disease intensity that has accumulated between each pair of adjacent time points (Madden *et al.* 1990; Simko, 2012). The equation is as follows:

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

To determine if *Mp* was the cause of plant mortality, half of all strawberry crowns from plants that died during these assays were plated on acidified PDA (APDA). Trial I was conducted June 22 through September 11 of 2015 in greenhouse B at the Cal Poly Crops Unit. Trial II was conducted September 28 through December 18 of 2015 in an outside hoop house at the Cal Poly Crops Unit.

In planta Fusarium study

The same two isolates of *Fof* (*Fof* 10.3 and 19) used in the *in vitro* experiments were grown on PDA for 21 days and added to sand to create inoculum according to the methods used by T. Gordon (Gordon et al., 2013). Approximately 70 grams of inoculum were added to approximately 400 g potting media to equal roughly 5,000 CFU/gram potting media. Teabags containing three grams of inoculum were buried in teabags one inch (25.4 mm) below soil surface. Drenches of 24 treatments (eight fungicides at low, med and high rates) (Table 2.1) and non-inoculated and inoculated controls occurred 2 days prior to planting of Monterey plants and 3 weeks after initial drench (450 ml drench per pot). Treatments consisted of five single-plant reps. Teabags of inoculum were recovered after each drench (reps 2 and 4 two days after; reps 1, 3, and 5 twenty three days after) and plated for quantification (Gordon et al., 2013). Plant disease assessments were made every week starting two weeks after planting as described in the above paragraph. Trial I was conducted December 2, 2015 through February 17, 2016. Trial II is currently being conducted. It began April 22 and will be completed July 8, 2016. Both trials have been conducted in greenhouse B at the Cal Poly Crops Unit.

Statistical analysis

A regression analysis using the normalized growth rate [area under the growth progress curve (AUGPC) quantitative summary] was used to generate an equation to determine effective concentrations (EC) of fungicides *in vitro*. An analysis of variance (ANOVA), was used to determine the significance of fungicide treatment effects on CFU counts. The GLM was used for both *Mp* and *Fof* bagged inoculum assays and AUDPC (plant disease

assessments). Friedman's test and Tukey's method were used to determine median AUDPC rankings and groupings for *Mp*. Dunnett's test was used to identify treatments significantly different than the inoculated control for *Fof*. Tukey's method was used to determine pairwise differences for *Fof*. The regression analysis and ANOVA were completed using Minitab 16 (Minitab Inc., State College, PA).

Results

In vitro fungicide assays

The data collected in both replicated *in vitro* trials consisted of radial mycelial growth measurements over time. Some of the treatments showed no measureable difference between the unamended control, while other treatments showed mycelial inhibition or little to no growth over time (Figure 2.2).

Mean fungicide concentrations that inhibited mycelial radial growth by 75% (EC₇₅) compared to unamended media over two trials were determined for two different isolates each of *Mp* and *Fof* (Table 2.2; see Appendix A.1 and Appendix A.2). Data was analyzed at day 34 and at day 50. Tebuconazole inhibited the mycelial growth of both *Mp* (the mean EC₇₅ at day 34 for *Mp* isolate 13 was 2.98 µg/ml and *Mp* isolate 8 was 1.7 µg/ml; the mean EC₇₅ at day 50 for *Mp* isolate 13 was 3.02 µg/ml and *Mp* isolate 8 was 1.71 µg/ml) and *Fof* (mean EC₇₅ at day 34 for *Fof* isolate 10.3 was 8.28 µg/ml and *Fof* isolate 19 was 6.23 µg/ml; the mean EC₇₅ at day 50 for *Fof* isolate 10.3 was 9.26 µg/ml and *Fof* isolate 19 was 5.7 µg/ml). Metconazole also inhibited mycelial growth of *Mp* (the mean EC₇₅ at day 34 for *Mp* isolate 13 was 2.97 µg/ml and *Mp* isolate 8 was 2.14 µg/ml; the mean EC₇₅ at day 50 for *Mp* isolate 13 was 2.9 µg/ml and *Mp* isolate 8 was

2.15 µg/ml) and *Fof* (mean EC₇₅ at day 34 for *Fof* isolate 10.3 was 1.11 µg/ml and *Fof* isolate 19 was 1.4 µg/ml; the mean EC₇₅ at day 50 for *Fof* isolate 10.3 was 1.14 µg/ml and *Fof* isolate 19 was 1.41 µg/ml). Fludioxonil strongly inhibited mycelial growth of *Mp* (the mean EC₇₅ at both day 34 and day 50 for *Mp* isolate 13 was 0.18 µg/ml and *Mp* isolate 8 was 0.15 µg/ml), but had no impact on the mycelial growth of *Fof* (EC₇₅ values greater than 1,000 µg/ml). *Mp* isolate 8 was more sensitive than *Mp* isolate 13 to the fungicide active ingredients. Penthiopyrad, fluopyram, flutriafol and flutolanil had EC₇₅ values greater than 1,000 µg a.i. /ml and were considered less effective at inhibiting fungal growth of either fungus. Overall, *Mp* was more sensitive than *Fof* to the active ingredients screened.

Mycelial plugs that did not show measureable growth on treatment plates (fludioxonil for *Mp* and metconazole for *Fof*) at day 50 were lifted and plated on unamended half strength PDA and monitored for growth over the course of two weeks to determine their viability after exposure to fungicide-amended media (Table 2.3). These plugs showed viability at 33% and 44% for *Mp* 13 and *Mp* 8 respectively and 62.5% for both *Fof* 10.3 and *Fof* 19.

In planta Macrophomina study

The data collected in both replicated trials consisted of CFU counts of buried inoculum and plant disease assessments over time. There was a significant difference ($p < 0.05$) between plating teabag inoculum after the first drench in week 1 (collected two days after first drench) and after the second drench in week 3 (collected 2 days after second drench) (Table 2.1, Table 2.4; see Appendix B.1) . Notably, there was a significant decrease of

CFU counts between week 1 and week 3 for both trial I and trial II. In both trials there were no significant differences in CFU counts among week 1 treatments and no significant differences between any of the treatments and the untreated control ($p > 0.05$). Both trials showed significant increases ($p < 0.05$) of CFU/plate of some treatments as compared to the inoculated control at week 3. In trial I, flutriafol at a low rate and fludioxonil at the low and high rates showed higher CFU counts as compared to the untreated control. In trial II, metconazole at the high rate showed a significant increases ($p < 0.05$) in the number of CFUs/plate compared to the untreated control.

Plant disease assessments (Figure 2.1) and corresponding AUDPC values did not meet the statistical requirements needed for a valid ANOVA for both *Mp* trials. Due to greenhouse temperatures (recorded in excess of 30°C in both soil and air) and pest pressure from powdery mildew (*Podosphaera aphanis*) and two spotted spider mite (*Tetranychus urticae*), disease assessments from the first trial of *Mp* were not used, as it was difficult to determine the cause of plant death/collapse. However, some plants were confirmed to have *Mp* through direct plating of crown tissue on APDA (Table 2.5). Less than 2% of all dead plant tissue from *Mp* trial I was positive for *Mp* while trial II resulted in roughly 12% of all dead plants being positive for the pathogen. However, I often waited too long to plate each plant after they died to get an accurate assessment for trial I.

The original AUDPC for *Mp* trial II data did not meet the statistical requirements for a valid ANOVA so a nonparametric analysis method, Friedman's test, of median AUDPC for each treatment was conducted instead (Figure 2.3, Table 2.1). The medians resulting from the Friedman's test were then used to perform an ANOVA and Tukey groupings with 90% simultaneous confidence intervals. There was a significant decrease ($p < 0.001$)

of median AUDPC with treatments containing the active ingredient penthiopyrad (treatments: J: low rate, K: med rate and L: high rate) (Figure 2.3, Table 2.1; see Appendix B.2) as compared to other treatments and to the inoculated control.

In planta Fusarium study

The data collected in both replicated *Fof* trials consisted of CFU counts of buried inoculum and plant disease assessments over time. There was a significant difference ($p < 0.05$) between CFU counts of buried inoculum after one drench in week 1 (collected 2 days after first drench) and after 2 drenches in week 3 (collected 2 days after second drench) (Table 2.1, Table 2.4). In trial I however, there were no significant differences ($p > 0.05$) among treatments when compared to each other or the inoculated control (see Appendix B.1).

Plant disease assessments (Figure 2.1) were used to calculate AUDPC for trial I; AUDPC values did show significant differences ($p < 0.001$) among treatments, but not when compared to the inoculated and non-inoculated control (Figure 2.4, Table 2.1; see Appendix B.2). Due to difficulties in producing inoculum at the required CFU counts needed for infection, a second trial of this study is in progress, but the data will not be included in this thesis.

Discussion

These results show that fungicides overall were ineffective at controlling the soilborne pathogens *Mp* and *Fof* *in planta*. *In vitro* trials showed that tebuconazole, metconazole, fludioxonil and, to a lesser extent, penthiopyrad were more effective at inhibiting

mycelial growth of *Mp*, while tebuconazole and metconazole were effective at inhibiting mycelial growth of *Fof*.

In planta greenhouse fungicide studies of *Mp* and *Fof* showed little to no control of both pathogens. Thiophanate-methyl was added to the study due to its ability to reduce disease incidence in raised-bed studies involving *Mp* of strawberries in Florida (Merteley et al. 2010; Merteley et al. 2014). These Florida studies also showed thiophanate-methyl and fluopyram were used together to increase fruit yield, but showed no significant difference in AUDPC (Merteley et al. 2014). The number of CFU counts per plate for both *Mp* and *Fof* were not significantly different from the control, showing that it is difficult to control these pathogens, in their resting state, using fungicides alone. An integrated approach will be needed to manage fields with existing inoculum and pathogen pressure.

The *in planta* data showed lower *Mp* disease incidence in treatments with penthiopyrad. Penthiopyrad also inhibited mycelial growth fairly well in the *in vitro* assays. Based on previous studies in Florida, thiophanate-methyl reduced disease severity in only one (2014) of the two field trials (Merteley et al. 2010). Our *in planta* pot trials showed no significant decrease in disease severity after thiophanate-methyl applications.

Due to the lack of research regarding chemical control of *Fof*, it was difficult to predict the outcome of the studies. These *in vitro* studies showed that many of the fungicide active ingredients that inhibited *Mp* were not as effective when used to treat *Fof* inoculated plants.

Conclusion

Of the chemical treatments evaluated in both *in vitro* and *in planta* studies, a limited number were effective at reducing radial mycelial growth, inoculum colony forming units and disease severity over time. In general, throughout the *in planta* studies, the number of colony forming units significantly decreased over time, but there were no significant differences among fungicide treatments and the control. Some of the fungicides tested reduced disease severity, but were not significant when compared to the control for both *Mp* and *Fof*. These studies show that tebuconazole and metconazole are effective at inhibiting mycelial growth for both pathogens *in vitro*, but not for controlling the disease *in planta*.

Table 2.1. List of all *in planta* fungicide treatments.

ID	Active Ingredient and Rate of Formulated Product
A	Tebuconazole ¹ -low rate (1.52 ml/1.426 gal H ₂ O)
B	Tebuconazole-med rate (3.04 ml/1.426 gal H ₂ O)
C	Tebuconazole-high rate (6.07 ml/1.426 gal H ₂ O)
D	Metconazole ² -low rate (0.81 g/1.426 gal H ₂ O)
E	Metconazole-med rate (1.62 g/1.426 gal H ₂ O)
F	Metconazole-high rate (2.43 g/1.426 gal H ₂ O)
G	Flutriafol ³ -low rate (3.41 ml/1.426 gal H ₂ O)
H	Flutriafol-med rate (6.81 ml/1.426 gal H ₂ O)
I	Flutriafol-high rate (13.63 ml/1.426 gal H ₂ O)
J	Penthiopyrad ⁴ -low rate (5.06 ml/1.426 gal H ₂ O)
K	Penthiopyrad-med rate (10.12 ml/1.426 gal H ₂ O)
L	Penthiopyrad-high rate (20.24 ml/1.426 gal H ₂ O)
M	Fluopyram ⁵ -low rate (1.44 ml/1.426 gal H ₂ O)
N	Fluopyram-med rate (2.88 ml/1.426 gal H ₂ O)
O	Fluopyram-high rate (5.77 ml/1.426 gal H ₂ O)
P	Flutolanil ⁶ -low rate (2.3 g/1.426 gal H ₂ O)
Q	Flutolanil-med rate (4.59 g/1.426 gal H ₂ O)
R	Flutolanil-high rate (7.12 g/1.426 gal H ₂ O)
S	Fludioxonil ⁷ -low rate (1.41 g/1.426 gal H ₂ O)
T	Fludioxonil-med rate (2.83 g/1.426 gal H ₂ O)
U	Fludioxonil-high rate (5.66 g/1.426 gal H ₂ O)
V	Thiophanate-methyl ⁸ -low rate (4.22 ml/1.426 gal H ₂ O)
W	Thiophanate-methyl-med rate (8.43 ml/1.426 gal H ₂ O)
X	Thiophanate-methyl-high rate (16.87 ml/1.426 gal H ₂ O)
Y	Inoculated control
Z	Non-inoculated control

¹ Tebuconazole is the active ingredient in Tebuzole

² Metconazole is the active ingredient in Quash

³ Flutriafol is the active ingredient in Rhyme

⁴ Penthiopyrad is the active ingredient in Fontelis

⁵ Fluopyram is the active ingredient in Velum

⁶ Flutolanil is the active ingredient in Moncut

⁷ Fludioxonil is the active ingredient in Cannonball

⁸ Thiophanate-methyl is the active ingredient in Topsin

Figure 2.1. Rating scale guide: (a) no symptoms = 1, (b) 25% wilt or necrosis = 2, (c) 50% wilt and necrosis = 3, (d) and (e) 75% wilt or necrosis = 4 and (f) 100% wilt or necrosis = 5.

Figure 2.2. Mycelial growth of *Macrophomina phaseolina* on (a) unamended media (maximum colony diameter at day 4), (b) Flutriafol at 1 µg/ml (1 ppm) (maximum colony diameter at day 4), (c) Tebuconazole at 1 µg/ml (1 ppm) (maximum colony diameter at day 15), and (d) Tebuconazole at 50 µg/ml (50 ppm) (minimal growth at day 50).

Table 2.2. Mean concentrations of seven fungicides inhibiting mycelial radial growth by 75% (EC₇₅) of *Macrophomina phaseolina* (Mp) and *Fusarium oxysporum* f. sp. *fragariae* (Fof) in Petri dishes.

Active Ingredient	Average EC ₇₅ Mp [*] (µg/ml)				Average EC ₇₅ Fof [*] (µg/ml)			
	Day 34		Day 50		Day 34		Day 50	
	Isolate ID		Isolate ID		Isolate ID		Isolate ID	
	<i>Mp</i> 13	<i>Mp</i> 8	<i>Mp</i> 13	<i>Mp</i> 8	<i>Fof</i> 10.3	<i>Fof</i> 19	<i>Fof</i> 10.3	<i>Fof</i> 19
Tebuconazole	2.98	1.70**	3.02	1.71**	8.28	6.23	9.26	5.7
Metconazole	2.97	2.14	2.9	2.15	1.11	1.4	1.14	1.41
Flutriafol	47.17**	55.96	50.77	55.96	301.44	460.36**	262.3	310.38**
Penthiopyrad	5.83	5.12	5.83	5.1	>***	>	>***	>
Fluopyram	9.24**	22.24	9.24**	22.24	>***	>**	>***	>**
Flutolanil	221.23***	150.05***	200.13***	150.05***	>***	>***	>***	>***
Fludioxonil	0.18**	0.15***	0.18**	0.15***	>***	>***	>***	>***

* Combined mean of Trial I & Trial II.

**r-squared value less than 85%.

*** r-squared value less than 75%.

> Data not shown; mean greater than 1,000 µg/ml.

See Appendix A.1 and Appendix A.2 for data analysis.

Table 2.3. Percent viable plugs^a after exposure to active ingredients at day 50.

Active Ingredient	Rate	Isolate ID			
		<i>Mp</i> 13	<i>Mp</i> 8	<i>Fof</i> 10.3	<i>Fof</i> 19
Fludioxonil	1 µg/ml	100% (1) ^b	50% (2)		
	5 µg/ml	0% (1)			
	10 µg/ml	0% (3)	33% (3)		
	50 µg/ml	50% (4)	50% (4)		
Metconazole	10 µg/ml			100% (4)	100% (4)
	50 µg/ml			25% (4)	25% (4)

^a Plugs from fungicide-amended media (*in vitro* study) that did not have measurable growth at day 50 were transferred to new half-strength PDA plates without active ingredients. Plugs were monitored for growth over 14 days.

^b Number in parentheses indicates number of plugs plated.

Table 2.4. Mean Colony Forming Units (CFUs) per plate of inoculum that was buried in bags and received fungicide treatments.

		Trial I				Trial II			
		<i>Macrophomina phaseolina</i>		<i>Fusarium oxysporum</i> f. sp. <i>fragariae</i>		<i>Macrophomina phaseolina</i>		<i>Fusarium oxysporum</i> f. sp. <i>fragariae</i>	
Treatment ^a		week 1	week 3	week 1	week 3	week 1	week 3	week 1	week 3
Tebuconazole	A	112.67	8.25	3.75	3	117.33	12.67	-	-
	B	100.33	10.25	3	1.17	64.67	14.33	-	-
	C	124.83	4.75	6	1.17	75.33	29.83	-	-
Metconazole	D	83	7.25	NA	1.67	122	18	-	-
	E	154.5	15.25	7.5	0.67	89.33	3.5	-	-
	F	127.83	5.5	2.75	1.67	122.67	59.5*	-	-
Flutriafol	G	97	24.75*	3.5	0.17	84.83	19.83	-	-
	H	106.17	9.5	5.5	0.17	101.5	12.5	-	-
	I	90.75	7.75	3	0.5	81	19.67	-	-
Penthiopyrad	J	153.17	10.25	13	0.5	84.83	3	-	-
	K	162.83	8.75	6	1.17	141.83	3.17	-	-
	L	133.17	5	9	0.17	112	1.83	-	-
Fluopyram	M	96.33	9	4	1	139.17	10.33	-	-
	N	121.83	4.75	8.25	1.33	124.17	5.33	-	-
	O	153.17	4.25	6	0.5	98.5	3.67	-	-
Flutolanil	P	95.33	15.25	4.75	1.33	94	11.17	-	-
	Q	187.83	9.75	7.5	0.5	103.83	9.5	-	-
	R	104.5	13.25	2.75	2.33	102	9.67	-	-
Fludioxonil	S	138	31.50*	10.5	0.67	126.5	8.83	-	-
	T	181.5	11.5	5	0.67	117.5	12.17	-	-
	U	186.33	38.75*	8	0.33	130.5	32.33	-	-
Thiophanate-Methyl	V	75.83	5.75	3	0	132.83	6.33	-	-
	W	84.33	8.25	6.5	0.5	102.33	9	-	-
	X	54.67	7	1.5	0.33	95.33	21	-	-
Inoculated Control		189	11.5	7.25	1	108.83	13.33	-	-

^a Treatment ID- see table 2.1 for descriptions of each treatment.

* Significantly higher than inoculated control.

CFUs counted on media: RB for *Mp* (6 reps of each treatment) & Komada for *Fof* (5 reps of each treatment) for two trials *in planta*.

See Appendix B.1 for data analysis.

Table 2.5. Number of dead plants confirmed with *Mp* or *Fof* by direct plating after fungicide treatment.

Treatment		Pathogen			
		<i>Macrophomina phaseolina</i> ^a		<i>Fusarium oxysporum</i> f. sp. <i>fragariae</i> ^a	
		Trial I	Trial II	Trial I	Trial II ^b
Tebuconazole	A	0 (4) ^c	0 (1)	75% (4)	-
	B	0 (4)	0 (1)	40% (5)	-
	C	0 (6)	0 (4)	40% (5)	-
Metconazole	D	0 (5)	0 (2)	0 (5)	-
	E	0 (6)	33% (3)	60% (5)	-
	F	0 (6)	0 (3)	20% (5)	-
Flutriafol	G	0 (6)	0 (6)	40% (5)	-
	H	0 (6)	0 (6)	0 (5)	-
	I	0 (6)	0 (6)	40% (5)	-
Penthiopyrad	J	0 (5)	NA ^d	0 (2)	-
	K	0 (6)	NA	NA	-
	L	0 (3)	NA	NA	-
Fluopyram	M	0 (4)	0 (1)	0 (2)	-
	N	0 (6)	0 (3)	0 (2)	-
	O	0 (3)	0 (1)	0 (2)	-
Flutolanil	P	0 (6)	66% (3)	0 (4)	-
	Q	16% (6)	40% (5)	66% (3)	-
	R	0 (6)	33% (3)	20% (5)	-
Fludioxonil	S	0 (6)	NA	0 (4)	-
	T	0 (6)	0 (1)	0 (3)	-
	U	0 (6)	0 (3)	75% (4)	-
Thiophanate-methyl	V	0 (6)	25% (4)	50% (2)	-
	W	0 (6)	NA	NA	-
	X	0 (5)	0 (2)	50% (2)	-
Inoculated control	Y	33% (6)	60% (5)	50% (2)	-
Non-inoculated control	Z	0 (5)	0 (1)	0 (2)	-

^a Half of all dead plants per treatment were plated on acidified potato dextrose agar for the detection of *Mp* or *Fof*.

^b Trial currently taking place.

^c Number in parentheses is the total number of crowns plated.

^d NA None of the plants in these treatments died.

Figure 2.3. Median AUDPC for *Macrophomina phaseolina* in planta fungicide trial II.

¹Tukey grouping in block ranked AUDPC median and 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings based on six reps of each treatment. See Appendix B.2 for data analysis.

Figure 2.4. Mean AUDPC for *Fusarium oxysporum* f. sp. *fragariae* in planta fungicide trial I.

¹Tukey grouping based on 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings based on six reps of each treatment.

See Appendix B.2 for data analysis.

CHAPTER 3: EXAMINING THE INFLUENCE OF STRAWBERRY PLANT
EXTRACTS ON GERMINATION OF *MACROPHOMINA PHASEOLINA*
MICROSCLEROTIA

Abstract

Macrophomina phaseolina (*Mp*) is an emerging soilborne pathogen causing crown rot in commercial strawberry fields in California. A strawberry whole plant extract was assessed for its ability to stimulate the germination of *Mp* microsclerotia *in vitro* and *in planta*. For the *in vitro* trials, the plant extract was applied as a drench at six different concentrations (0, 10, 100, 1,000, 10,000 and 30,000 ppm) at day 0 and day 14 in Petri dishes containing filter packets of *Mp* microsclerotia. Filter packets were retrieved 3 days after the drench and processed by microscopy to determine the number of germinated microsclerotia. Results showed that the number of germinated microsclerotia was significantly higher ($p < 0.001$) after the application of the strawberry plant extracts compared to non-drench and water (0 ppm) controls. An *in planta* container trial was also conducted using a strawberry extract at 10,000 ppm applied 3 days prior to a fungicide drench (tebuconazole or thiophanate-methyl) to assess the effect of fungicides on germinated microsclerotia. The use of strawberry plant extracts as germination stimulants with label rates of both fungicides lowered the number of living germinated microsclerotia significantly ($p < 0.001$), especially after two drench applications of the strawberry plant extract and fungicide combinations. In the future, field trials will be necessary to determine if strawberry plant extracts could be coupled with fungicides as a potential control measure for *Macrophomina* crown rot of strawberry.

Introduction

A novel approach targeting sclerotia of *Sclerotium cepivorum* has been developed which shows potential as an integrated control measure for managing white rot of garlic and onion in central California (Coventry et al., 2002; Davis et al., 2007; Hovius and McDonald, 2002). This method involves the application of host plant extracts (i.e. onion and garlic extractions) to white rot infested fields in the absence of a crop in order to “trick” or stimulate pathogen sclerotia to germinate. These host plant extracts are commonly referred to as germination stimulants. Numerous studies involving this approach in garlic production have reported white rot control similar to results achieved with methyl bromide (Hovius and McDonald, 2002).

Garlic and onion bulbs and roots continuously leak exudates at low levels naturally, which induce germination of sclerotia produced by *S. cepivorum* at very high rates (Hovius and McDonald, 2002). One such stimulatory substance isolated from onion and garlic is diallyl disulfide, often referred to as DADS (Crowe et al., 1993). When DADS was applied by injection or irrigation throughout field soil in the absence of an actual onion crop, 90-99% of the pathogen sclerotia germinated (Coley-Smith and Parfitt, 1986). Numerous studies involving DADS, as well as food grade garlic powder, garlic juice, and garlic bulb exudates have shown similar results. Control of garlic white rot was similar to control achieved with methyl bromide (Davis et al., 2007; Hovius and McDonald, 2002). Field trials conducted in Kern and San Benito Counties in California garlic fields reported over 90% death of sclerotia of *S. cepivorum* within 3 months after application of these germination stimulants (Davis et al., 2007;).

There are limitations regarding the use of germination stimulants for controlling white rot of garlic in California; these products cannot be applied when the host crop is growing in the field and further studies showed that the best control of white rot was achieved when fields lay fallow for 3-6 months after treatment. Additional studies determined that this method is most effective when combined with soil-applied fungicides (Ferry and Davis, 2012). None of the five fungicides tested by Ferry and Davis significantly reduced white rot when applied alone. However, when combined as a treatment one week after the application of DADS, all fungicides significantly reduced disease severity without requiring a fallow period. This technique could be utilized by strawberry producers as a pre-plant treatment in fallow fields before the application of soil fungicides, ASD, low rates of fumigants, or even steam as an integrated program for managing soilborne pathogens. It is widely documented that sclerotia are more tolerant of extreme conditions and that the vegetative mycelium of fungi is much more sensitive to treatments such as fumigants, fungicides, and temperature extremes (Davis et al., 2007; Hovius and McDonald, 2002).

The objectives of this study were 1) to evaluate the use of strawberry whole plant extracts as potential microsclerotia germination stimulants for *Mp*, and 2) to evaluate the combined use of strawberry plant extracts with the most effective soil-applied fungicides for managing *Macrophomina* crown rot.

Materials & Methods

Strawberry plant extract production

Twenty pounds of mature Albion strawberry plants were collected from a production field. The plants were washed free of soil, and fruit and necrotic tissue were removed. Leaf, stem, crown and root tissue was finely chopped using a 14-cup Cuisinart food processor (Cuisinart, E Windsor, New Jersey). Strawberry plant material was added to sterilized deionized water to achieve a 7:3 (700 ml distilled water: 300 g plant material) volume-to-volume ratio. Enzymes from California Safe Soil were added per 500 g strawberry plant material as follows: Enzyme A at 0.5 ml, enzyme B at 3 µl and enzyme C at 1.2 g. In order to activate enzymes, the solution was placed in glass jars and submerged in a water bath at 55° C under constant agitation for 3.5 hours. The heat was then increased to 77° C for 30 minutes under constant agitation in order to deactivate the enzymes. This solution was stored in the refrigerator for up to four weeks. The resulting slurry was passed through sterile cheesecloth to remove solids, and only the filtrate was used. The initial concentration (stock) of the germination stimulant was 300,000 ppm. All concentrations were made using this stock and diluted using sterile deionized water.

***Macrophomina phaseolina* microsclerotia packets**

Three isolates of *Mp* (*Mp* 8, *Mp* 13 and *Mp* 20; isolated from diseased strawberry crowns from Irvine and Oxnard, CA) were grown on wooden toothpicks (Mihail, 1992) for two weeks then air dried and stored in the dark at room temperature. Microsclerotia were transferred to filter disks (Fisherbrand® filter #20, 86 mm diameter) by scraping them off

the toothpicks. Filter disks were folded and glued shut using Elmer's Glue. These microsclerotia packets were used in both *in vitro* and *in planta* studies.

***In vitro* germination trials**

This *in vitro* study was repeated twice. Trial I was conducted February 19, 2016 through March 7, 2016 and trial II was conducted March 11, 2016 through March 28, 2016. A clay-loam soil from a pre-plant fumigated strawberry field was sterilized by autoclaving twice for 60 minutes, amended with vermiculite (80:20, soil: vermiculite, volume to volume) to prevent aggregate blocking and improve water mobility. Two tablespoons of this soil mix was added to each deep 86 mm diameter Petri dish. One microsclerotia packet was added and then covered with another tablespoon of soil mix. On day 0, 10 ml of five concentrations of the germination stimulant (0, 10, 100, 1,000, 10,000 and 30,000 ppm) were applied evenly to the soil. There were four controls: no drench and no microsclerotia packet, no drench with a microsclerotia packet, 30,000 ppm germination stimulant with no microsclerotia packet, and sterile distilled water only with microsclerotia packet. Petri dishes were then stored in the dark. Each treatment and drench combination had six replicates.

On day 3, one set of the six replicates of each treatment was evaluated. Microsclerotia packets were removed from the soil and placed on standard glass slides to quantify the number of germinated microsclerotia. Each slide was treated with a 2% lactophenol blue and distilled water then covered with a glass cover slip. Slides were evaluated under the compound microscope at 20X magnification to visualize

microsclerotia. The number of germinated and non-germinated microsclerotia were counted in the area of one cover slip (22 mm²).

A second drench of each treatment was applied to one set of the six replicate dishes on day 14. On day 17, microsclerotia packets were removed from the set of six replicate dishes that received the second drench, as well as the remaining set of six replicate dishes that only received the first drench on day 0. The microsclerotia packets were prepared and analyzed as described in the above paragraph.

***In planta* germination trials**

This experiment was designed to assess differences in the number of plant extract drenches (one or two) for their ability to induce the germination of *Mp* microsclerotia. This experiment was only conducted once (from March 1, 2016 through June 11, 2016) due to time limitations and is still in progress (Table 3.1).

The same three isolates of *Mp* used in the *in vitro* experiments were also used in these *in planta* trials. Isolates were grown on potato dextrose agar (PDA) and then added to a cornmeal sand mixture to create inoculum (Mihail et al., 1992b). Inoculum was incubated for two weeks at 30°C. Ten grams of inoculum was added to each one gallon pot (2.84 liter) with roughly 400 g potting media [7 ft³ (2.1 m³) peat moss, 7 ft³ (2.1 m³) coconut core, 7 ft³ (2.1 m³) perlite, 12 oz. (340 g) treble superphosphate, 18 oz. (510g) potassium nitrate, 59 oz. (1.67 kg) dolomite lime and 1 oz. (28 g) micromax plus] for inoculated controls (0 ppm plant extract and no drench) and to all treatment pots receiving the five different concentrations of the plant extract (0, 10, 100, 1,000, 10,000 and 30,000 ppm). Microsclerotia packets were placed in cotton mesh teabags that were

buried approximately 25.4 mm below soil surface in each pot. Drenches of treatments and non-inoculated and inoculated controls occurred two days prior to the planting of cultivar San Andreas transplants and two weeks after initial drench (450 ml drench per pot at day 0). Treatments consisted of six single-plant replications; there were two sets of replications. The first replicate set of pots contained two individual teabags with a microsclerotia packet; the second set of replicate pots contained only one teabag with a microsclerotia packet. Bare root plants were planted in the first set of replicates on day 14 and assessed starting day 28 (two weeks after planting) by rating plants for disease symptoms (Figure 2.1). Bare root plants were planted two weeks after the second drench treatment in the second set of replicates (day 28) and disease assessments started two weeks (day 42) after planting date (Table 3.1).

One teabag containing the microsclerotia packet was recovered on day three after the first drench of plant extract and mounted on slides as described above. A second drench of the plant extract was applied to the pots on day 14. Three days after this extract drench on day 17, the second teabag containing the microsclerotia packet from the first replicate set the only teabag from the second replicate set was recovered. The microsclerotia packet was mounted on slides as described above and microsclerotia were assessed for germination (Table 3.1).

Plant disease assessments were made every week starting two weeks after planting. Plants were rated on a scale of 1-5; the rating of 1= no disease symptoms; 2=25% of plant wilted or necrotic; 3=50% of the plant wilted or necrotic; 4=75% of the plant wilted or necrotic; 5=dead plant (Figure 2.1). Scores were used to determine the area under the disease progress curve (AUDPC). Half of all strawberry crowns from plants that died

during these assays were plated on APDA to determine if *Mp* was the cause of plant mortality.

The second part of this experiment was designed to assess whether the combination of plant extracts and fungicide drenches were more effective at reducing plant mortality than plant extract drenches alone. Drenches of two different fungicides were also included in this *in planta* trial. Plant extract drench applications were applied (450 ml drench per pot) at the 10,000 ppm rate at day 0 to two replicate sets of pots; inoculated and non-inoculated controls pots were also included. All fungicide treatment pots had two teabags each containing a microsclerotia packet. One teabag was recovered on day 3 after the first plant extract drench and before the first fungicide drench. A treatment of either tebuconazole (Tebuzole) or thiophanate-methyl (Topsin) was then applied at the label rate (tebuconazole applied at 3.04 ml per 1.426 gallons of water and thiophanate-methyl applied at 8.43 ml per 1.426 gallons of water) as a 450 ml drench on day 3 to all fungicide pots. The second teabag containing a microsclerotia packet was recovered from the first replicate set of pots three days (day 6) after the application of the first fungicide drench. Microsclerotia packets from all retrieved teabags were slide mounted and analyzed as described earlier. Bare root plants were planted in the first replicate set of pots on day 14; disease assessments starting two weeks after planting on day 28 using the rating system described earlier (Table 3.1).

A second drench of the plant extract (10,000 ppm) was applied to the second set of replicate pots on day 14. On day 17, teabags from both the first and second replicate sets of pots was recovered and analyzed as described above. A second fungicide drench of either fungicide was applied to a third replicate set on day 17, three days after the

second plant extract application. Teabags from the third replicate set of pots was recovered on day 20 and analyzed as described earlier. Transplants were planted in the third set of replicated pots on day 28; disease assessments started on day 42 (Table 3.1).

Statistical analysis

An analysis of variance (ANOVA) was used to determine the significance of plant extract and fungicide treatment effects on the germination of microsclerotia of *Mp* both *in vitro* and *in planta*. Data was transformed using a square root transformation to ensure valid statistical analysis when determining significant differences among treatments. All statistical analyses were conducted using Minitab 16 (Minitab Inc., State College, PA). Plant disease assessment data is currently being collected and will be used to calculate the area under the disease progress curve (AUDPC).

Results

***In vitro* germination study**

The number of germinated and non-germinated microsclerotia of *Mp* were recorded for each buried teabag retrieved during this study. Lactophenol blue was used to stain and visualize mycelium (Figure 3.1). Treatment dishes that received a plant extract drench showed a significant increase ($p < 0.001$) in the percent of microsclerotia that germinated (Figure 3.2; see Appendix C.1). Treatment dishes that only received the water had an increase in the percent of germinated microsclerotia as compared to the dishes that received no drenches (see Appendix C.2). The rates of plant extract concentration had an impact on the percent of germinated microsclerotia with 10 ppm being significantly less

($p < 0.001$) effective, but still significantly higher than both the water and no drench controls. All other extract concentrations showed no significant difference ($p > 0.005$) among rates (Figure 3.2, Table 3.2; see Appendix C.1).

The number of plant extract drenches (one drench vs two drenches) over time (day 3 vs day 17) were also significant ($p < 0.05$) regarding the mean percent of germinated microsclerotia (Table 3.2; see Appendix C.1). Germination was lower when treatments received only one drench at day 3, but were processed and visualized at day 17 (Table 3.2). Treatments that received two plant extract drenches at day 3 and day 17 had a higher percent germination than those which only received one drench at day 0 and were processed at day 3 (Table 3.2; see Appendix C.2).

***In planta* germination study**

In planta results were similar to *in vitro* results. Treatments that received one or more plant extract drenches showed a significant increase ($p < 0.001$) in the percent of microsclerotia that germinated (Figure 3.3; see Appendix C.3). Treatments that only received a water drench had an increase in the percent of germinated microsclerotia compared to treatments that received no drench. The plant extract concentration had an impact on the percent of germinated microsclerotia, with 10 ppm being less effective, but still significantly higher ($p < 0.05$) than both water and no drench controls (Figure 3.3). Extract concentrations of 10,000 ppm, 1,000 ppm and 100 ppm had higher germination rates than controls and the 10 ppm treatments, but were not significantly different ($p > 0.05$) among themselves (Figure 3.3).

The number of drenches (one vs. two) over time (day 3 vs. day 17) was not significant ($p > 0.05$) regarding a change in percent germinated microsclerotia (Table 3.3). All treatments that received a plant extract drench had higher percentages of germinated microsclerotia, but were statistically similar when compared to each other (Table 3.3).

For the second part of this trial, microsclerotia of *Mp* were also visualized after the application of both the plant extract and fungicide drenches. Non-living mycelium (from germinated microsclerotia) was differentiated from living intact mycelium by staining mycelium with a 2% lactophenol blue solution (Figure 3.4). The number of drenches of either fungicide following a drench of plant extract at the concentration of 10,000 ppm significantly lowered the number of living germinated microsclerotia with intact mycelium ($p < 0.001$) (Figure 3.5, Table 3.4; see Appendix C.3). If only one fungicide drench was applied on day 3, thiophanate-methyl would be the optimal fungicide as it had a lower percent of germinated microsclerotia with intact mycelium than the 10,000 ppm extract alone (Table 3.4). If two drenches of fungicide are applied following drenches of the plant extract, both tebuconazole and thiophanate-methyl worked as effectively and were not significantly different ($p > 0.05$) from each other, but were significantly lower ($p < 0.05$) than the 10,000 ppm extract alone (Table 3.4; see Appendix C.3).

Plant disease assessments are currently being collected, but are not complete at this time. The project will be concluded June 11, 2016 at which time the data will be entered and analyzed.

Discussion

The use of whole strawberry plant extracts can be used to induce the germination of microsclerotia of *Mp*. In both *in vitro* and *in planta* trials, an increase in the amount of germinated microsclerotia was observed. Every concentration tested had higher germination rates than the water control, with the 10 ppm extract showing the least amount of microsclerotia germination.

It is unclear whether two drenches of the plant extracts are preferable to a single drench. *In vitro* experiments show a significant increase in the number of germinated microsclerotia after the second extract drench, but *in planta* data showed no significant differences between the numbers of extract drenches. Regarding the integration of fungicide drenches with plant extract drenches, it seems clear that a fungicide drench following a plant extract drench is more effective when the combination is applied twice rather than just a single application of each. The integrated portion of the trial shows that using plant extracts and fungicide drenches in combination may help to reduce the number of viable microsclerotia in the soil.

The use of germination stimulants made from strawberry plant tissue has the potential to assist fungicides for the control of soilborne pathogens of strawberry if coupled with the most effective fungicides (tebuconazole and thiophanate-methyl). Results from our earlier fungicide trials (Chapter 2 of this thesis) showed that soil-applied fungicides alone were not effective at controlling both *Macrophomina* crown rot and *Fusarium* wilt. It will be necessary to integrate multiple tactics in order to effectively manage these two emerging soilborne threats to California strawberry production.

Due to the time restraints of completing this thesis, data is still being collected from the *in planta* trial, but will be analyzed after June 11, 2016 when the study is completed. In order for all *in planta* data to be more relevant, the entire experiment needs to be repeated in field settings, where the extracts can be applied through drip irrigation at different timings. It would also be important to show whether the pathogens respond to extracts from a variety of strawberry cultivars and whether or not an alternative crop may be used in place of strawberry in making the germination stimulant, i.e. one that is easier to extract or from a plant that is easier to harvest.

Table 3.1. Timeline of various treatment applications and data collection during the *in planta* trial.

	Treatment					
	Time 1		Time 2			
Day	1 extract drench	1 extract drench, 1 fungicide drench	1 extract drench	1 extract drench, 1 fungicide drench	2 extract drenches	2 extract drenches, 2 fungicide drenches
0*	Drench Extract	Drench Extract	Drench Extract	Drench Extract	Drench Extract	Drench Extract
3	Pull <i>Mp</i> Packets	Pull <i>Mp</i> Packets Apply Fungicides		Apply Fungicides		Apply Fungicides
6		Pull <i>Mp</i> Disks				
14	Plant Strawberries	Plant Strawberries	Plant Strawberries	Plant Strawberries	Drench Extract	Drench Extract
17			Pull <i>Mp</i> Packets	Pull <i>Mp</i> Packets	Pull <i>Mp</i> Packets	Pull <i>Mp</i> Packets Apply Fungicides
20						Pull <i>Mp</i> Packets
28	Start Weekly Disease Assessments	Start Weekly Disease Assessments	Start Weekly Disease Assessments	Start Weekly Disease Assessments	Plant Strawberries	Plant Strawberries
42					Start Weekly Disease Assessments	Start Weekly Disease Assessments
91	End Weekly Disease Assessments	End Weekly Disease Assessments	End Weekly Disease Assessments	End Weekly Disease Assessments		
105**					End Weekly Disease Assessments	End Weekly Disease Assessments

*Day 0 corresponds to February 27, 2016.

** Day 105 corresponds to June 11, 2016.

Figure 3.1. Microsclerotia of *Macrophomina phaseolina* that (a) did not germinate, and (b) did germinate.

The mycelium has been stained with 2% lactophenol blue.

Figure 3.2. Percent of *Macrophomina phaseolina* microsclerotia that germinated after treatment drench *in vitro*.

*Days based on day microsclerotia were counted.

¹Grouping based on 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings are based on the means of six reps of each treatment over two repeated trials.

See Appendix C.1 for data analysis.

Table 3.2. Percent of *Macrophomina phaseolina* microsclerotia that germinated after treatment drench for two repeated trials *in vitro*.

Treatment	Trial I			Trial II		
	Day 3, 1 Drench	Day 21, 1 Drench	Day 21, 2 Drenches	Day 3, 1 Drench	Day 21, 1 Drench	Day 21, 2 Drenches
No drench	2.87	9.53	11.96	4.74	11.98	16.25
Water drench	16.89	31.70	24.35	14.25	30.60	27.72
10 ppm extract	46.53	30.30	41.59	51.65	57.63	55.48
100 ppm extract	60.52	42.61	64.46	55.96	62.71	58.15
1,000 ppm extract	71.00	56.80	60.00	68.03	58.32	62.01
10,000 ppm extract	71.05	52.54	61.95	76.04	62.87	71.74
30,000 ppm extract	57.91	53.80	64.22	66.52	63.81	63.86

Figure 3.3. Percent of *Macrophomina phaseolina* microsclerotia that germinated after treatment drench *in planta*.

*Days based on day microsclerotia were counted.

¹Grouping based on 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings are based on the mean of six reps of each treatment.

See Appendix C.3 for data analysis.

Table 3.3. Percent of *Macrophomina phaseolina* microsclerotia that germinated after specific *in planta* trial treatments.

	Day 3*, 1 drench	Day 17, 1 drench	Day 17, 2 drenches
Treatment	% germinated	% germinated	% germinated
No drench	23.53 g ¹	9.21 i	9.89 i
Water drench	24.73 g	19.77 gh	12.59 hi
10 ppm extract	62.01 b-f	47.39 f	57.01 c-f
100 ppm extract	71.43 a-c	52.87 d-f	64.13 a-e
1,000 ppm extract	73.87 ab	53.09 d-f	63.87 a-e
10,000 ppm extract	79.77 a	53.46 d-f	59.96 b-f
30,000 ppm extract	76.44 ab	49.39 ef	53.44 d-f

*Days based on day microsclerotia were counted.

¹Grouping based on a square root transformation and 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings are based on the mean of six reps of each treatment.

See Appendix C.3 for data analysis.

Figure 3.4. Microsclerotia of *Macrophomina phaseolina* that (a) did not germinate, and (b) did germinate. Microsclerotia in (c) and (d) did germinate, but the mycelium is no longer intact (red arrows). The mycelium has been stained with 2% lactophenol blue.

Figure 3.5. Percent of germinated *Macrophomina phaseolina* microsclerotia with intact mycelium after drenches of plant extracts and fungicides *in planta*.

*Days based on day microsclerotia were counted.

¹Grouping based on a square root transformation with 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings are based on the mean of six reps of each treatment.

See Appendix C.3 for data analysis.

Table 3.4. Percent of germinated *Macrophomina phaseolina* microsclerotia with intact mycelium after drenches of plant extracts and fungicides *in planta*.

Treatment	Day 3*, 1 Drench	Day 17, 2 Drenches
	% germinated microsclerotia	% germinated microsclerotia
10,000 ppm extract	74.86 a ¹	65.52 b
Tebuconazole + 10,000 ppm extract	70.63 ab	8.35 c
Thiophanate-methyl + 10,000 ppm extract	66.98 b	9.69 c

*Days based on day microsclerotia were counted.

¹Grouping based on 90% simultaneous confidence intervals. Means that do not share the same letter are significantly different. Groupings are based the mean of six reps of each treatment.

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APPENDICES

Appendix A. Regression Analysis for *In Vitro* Fungicide Assays

A 1. *In vitro* fungicide assays for *Macrophomina phaseolina* (Mp) isolates

Table A 1.1. Regression equations for *in vitro* fungicide assays Mp 13 day 34 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=63.32 + 24.61 lograte	92.4
Metconazole	100-Y5 by Log ₁₀ rate	EC value=62.71 + 25.97 lograte	93.7
Flutriafol	100-Y5 by rate	EC value=10.05 + 1.377 rate	76.9
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=62.27 + 16.62 lograte	89.4
Fluopyram	100-Y5 by rate	EC value=33.43 + 4.498 rate	72.2
Flutolanil	100-Y5 ² by rate	EC value ² =0.490 + 0.3368 lograte	29.3
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=87.13 + 16.24 lograte	80.3

Table A 1.2. Regression equations for *in vitro* fungicide assays Mp 13 day 50 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=63.24 + 24.53 lograte	92.6
Metconazole	100-Y5 by Log ₁₀ rate	EC value=62.95 + 26.10 lograte	93.7
Flutriafol	100-Y5 ² by rate	EC value ² =248.7 + 105.9 rate	95.7
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=62.27 + 16.62 lograte	89.4
Fluopyram	100-Y5 ² by rate	EC value ² =33.43 + 4.498 rate	72.2
Flutolanil	100-Y5 ² by rate	EC value ² =0.077 + 0.0585 lograte	26
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=87.13 + 16.24 lograte	80.2

Table A 1.3. Regression equations for *in vitro* fungicide assays Mp 8 day 34 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=69.68 + 22.96 lograte	80.7
Metconazole	100-Y5 by Log ₁₀ rate	EC value=66.72 + 25.00 lograte	88.1
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=34.71 + 23.05 lograte	92.2
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=55.85 + 26.99 lograte	96.4
Fluopyram	100-Y5 by Log ₁₀ rate	EC value=40.62 + 25.52 lograte	90.5
Flutolanil	100-Y5 by rate	EC value=1.925 + 0.487 lograte	58.1
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=86.96 + 14.39 lograte	73.7

Table A 1.4. Regression equations for *in vitro* fungicide assays *Mp* 8 day 50 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=69.64 + 22.95 lograte	80.6
Metconazole	100-Y5 by Log ₁₀ rate	EC value=66.69 + 24.95 lograte	88
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=34.71 + 23.05 lograte	92.2
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=55.88 + 27.02 lograte	96.5
Fluopyram	100-Y5 by Log ₁₀ rate	EC value=40.62 + 25.52 lograte	90.5
Flutolanil	100-Y5 by rate	EC value=1.925 + 0.487 lograte	58.1
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=86.96 + 14.39 lograte	73.6

A 2. *In vitro* fungicide assays for *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) isolates

Table A 1.5. Regression equations for *in vitro* fungicide assays *Fof* 10.3 day 34 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=50.09 + 27.14 lograte	92.1
Metconazole	100-Y5 by Log ₁₀ rate	EC value=73.86 + 24.28 lograte	88.3
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=11.16 + 25.75 lograte	89.1
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=1.775 + 4.18 lograte	22.1
Fluopyram	100-Y5 by Log ₁₀ rate	EC value=3.452 + 2.644 lograte	17.2
Flutolanil	100-Y5 by Log ₁₀ rate	EC value=5.882 + 5.595 lograte	46.7
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=13.36 + 3.221 lograte	18.3

Table A 1.6. Regression equations for *in vitro* fungicide assays *Fof* 10.3 day 50 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=49.24 + 26.65 lograte	92.5
Metconazole	100-Y5 by Log ₁₀ rate	EC value=73.60 + 24.14 lograte	88.2
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=11.58 + 26.22 lograte	88.5
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=0.925 + 3.223 lograte	22.1
Fluopyram	100-Y5 by Log ₁₀ rate	EC value=3.452 + 2.644 lograte	17.2
Flutolanil	100-Y5 by Log ₁₀ rate	EC value=5.882 + 5.595 lograte	46.7
Fludioxonil	100-Y5 by Log ₁₀ rate	EC value=13.36 + 3.221 lograte	18.3

Table A 1.7. Regression equations for *in vitro* fungicide assays *Fof* 19 day 34 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=52.98 + 27.71 lograte	96.2
Metconazole	100-Y5 by Log ₁₀ rate	EC value=71.11 + 26.62 lograte	86.4
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=24.72 + 18.88 lograte	79.4
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=12.15 + 7.461 lograte	87.8
Fluopyram	100-Y5 ² by rate	EC value ² =0.1058 + 0.4364 rate	82.3
Flutolanil	100-Y5 by Log ₁₀ rate	EC value=8.056 + 4.189 lograte	55.1
Fludioxonil	100-Y5 ² by Log ₁₀ rate	EC value=5.745 + 0.2106 lograte	33

Table A 1.8. Regression equations for *in vitro* fungicide assays *Fof* 19 day 50 (Table 2.2)

Fungicide	Transformation	Equation	r-squared
Tebuconazole	100-Y5 by Log ₁₀ rate	EC value=53.81 + 28.04 lograte	95.7
Metconazole	100-Y5 by Log ₁₀ rate	EC value=71.02 + 26.62 lograte	86.6
Flutriafol	100-Y5 by Log ₁₀ rate	EC value=25.56 + 19.84 lograte	77.9
Penthiopyrad	100-Y5 by Log ₁₀ rate	EC value=12.15 + 7.461 lograte	87.8
Fluopyram	100-Y5 by rate	EC value ² =0.1058 + 0.4364 rate	82.3
Flutolanil	100-Y5 by Log ₁₀ rate	EC value=8.056 + 4.189 lograte	55.1
Fludioxonil	100-Y5 ² by Log ₁₀ rate	EC value=5.745 + 0.2106 lograte	33.4

Appendix B. Variance Tables for *In Planta* Fungicide Studies

B 1. Variance tables for *in planta* CFU counts

Table B 1.1. ANOVA for CFU counts *Mp* trial I week 1 (Table 2.4)

Source	DF	SS	MS	F	P
Block	2	72584	36292	15.46	0.000
Treatment	24	105558	4398	1.78	0.032
Error	48	112692	2348		
Total	74	290833			

Table B 1.2. ANOVA for CFU counts *Mp* trial I week 3 (Table 2.4)

Source	DF	SS	MS	F	P
Block	2	1711.1	855.56	52.15	0.000
Treatment	24	5105.4	212.73	12.97	0.000
Error	48	787.5	16.41		
Total	74	7604.1			

Table B 1.3. ANOVA for in CFU counts *Mp* trial II week 1 (Table 2.4)

Source	DF	SS	MS	F	P
Block	2	16743	8371.3	13.6	0.000
Treatment	24	30265	1261	2.05	0.017
Error	48	29544	615.5		
Total	74	76552			

Table B 1.4. ANOVA for CFU counts *Mp* trial II week 3 (Table 2.4)

Source	DF	SS	MS	F	P
Block	2	922.6	461.3	3.26	0.047
Treatment	24	8782.3	365.9	2.59	0.003
Error	48	6652.8	141.5		
Total	74	16696.8			

Table B 1.5. ANOVA for in CFU counts *Fof* trial I week 1 (Table 2.4)

Source	DF	SS	MS	F	P
Block	1	28.88	28.88	0.59	0.451
Treatment	24	1440.25	60.01	1.22	0.316
Error	24	1181.37	49.22		
Total	49	2650.5			

Table B 1.6. ANOVA for CFU counts *Fof* trial I week 3 (Table 2.4)

Source	DF	SS	MS	F	P
Block	2	0.14	0.07	0.04	0.958
Treatment	24	36.83	1.53	0.95	0.541
Error	48	77.53	1.62		
Total	74	114.5			

B 2. Variance tables for *in planta* AUDPC analysis

Table B 2.1. ANOVA with in block ranked AUDPC for *Mp* trial II (Figure 2.3)

Source	DF	SS	MS	F	P
Block	5	0.00	0.00	0.00	1.000
Treatment	24	3221.5	134.229	3.62	0.000
Error	120	4447.5	37.063		
Total	149	7669			

Table B 2.2. ANOVA for AUDPC for *Fof* trial I (Figure 2.4)

Source	DF	SS	MS	F	P
Block	4	4787476	1196869	1.19	0.320
Treatment	24	120372691	5015529	4.99	0.000
Error	96	96526257	1005482		
Total	124	221686424			

Appendix C. Data Analysis for Plant Extract Studies

C 1. Variance tables for *in vitro* germination study

Table C 1.1. ANOVA using square root transformation germinated microsclerotia (Figure 3.2)

Source	DF	SS	MS	F	P
Treatment	6	833.79	138.964	177.72	0.000
Block	5	4.6	0.92	1.18	0.319
# of Drenches	1	5.9	5.90	7.55	0.006
Trial	1	9.9	9.94	12.71	0.000
Error	238	186.1	0.78		
Total	251	1040.34			

C 2. Main effects plot for *in vitro* germination study

C 3. Variance tables for *in planta* germination studies

Table C 3.1. ANOVA using square root transformation germinated microsclerotia (Figure 3.3, Table 3.3)

Source	DF	Seq SS	Contribution	Adj SS	MS	F	P
Treatment	6	384.47	83.24%	384.47	64.08	250.16	0.000
# Drenches	1	357	0%	357	357.1	3.11	0.080
Drench Time	2	40.80	8.83%	40.80	20.40	79.64	0.000
Treatment* Drench Time	12	9.73	2.11%	9.73	0.81	3.17	0.001
Error	105	26.90	5.82%	26.90	0.26		
Total	125	461.89	100.00%				

Table C 3.2. ANOVA using square root transformation germinated microsclerotia integrated trial (Figure 3.5, Table 3.4)

Source	DF	Seq SS	Contribution	Adj SS	MS	F	P
Treatment	2	92.56	35.74%	85.04	42.52	404.12	0.000
Drench Time	1	97.68	37.71%	142.25	142.25	1351.92	0.000
Treatment* Drench Time	2	64.54	24.92%	64.54	32.27	306.67	0.000
Error	40	4.21	1.63%	4.21	0.11		
Total	45	258.98	100.00%				