Investigating Novel Approaches for the Integrated Control of Soilborne Pathogens *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *Fragariae*

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Summary

Macrophomina phaseolina (Mp) and *Fusarium oxysporum* f. sp. *fragariae (Fof)* are emerging soilborne pathogens causing crown rot and Fusarium wilt in commercial strawberry production in California. Fungicides representing seven active ingredients from four different mode of action groups (FRAC 1, 3, 7 and 12) were evaluated for their efficacy against each pathogen in vitro and each disease in planta. Active ingredients were evaluated for their ability to inhibit mycelial growth of both pathogens in vitro. Potato dextrose agar was amended with six different concentrations of seven fungicides. Concentrations that inhibited growth by 75% (EC75) compared to unamended media were determined for two isolates each of *Mp* and *Fof.* Tebuconazole strongly inhibited the growth of both pathogens (average EC_{75} for *Mp* was 2.4 ppm; average EC_{75} for Fof was 7.48 ppm), as did metconazole (average EC_{75} 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 growth of either fungus.

Greenhouse *in planta* studies evaluated twenty-four fungicide treatments (eight fungicides at three rates) that were drench applied to infested potting media two days prior to planting of susceptible cultivars ('San Andreas' for *Mp*; 'Monterey' for *Fof*) and again at day 21. Buried inoculum was recovered at day 2 and 23 and colony forming units (CFUs) were quantified. Plant assessments were made weekly 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 week 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 both pathogens *in planta*.

Finally, a strawberry plant extract was assessed for the ability to stimulate germination of *Mp* microsclerotia *in vitro* and *in planta*. The germination stimulant was drench applied at six different concentrations to soil containing packets of microsclerotia of *Mp* at day 0 and 14. Packets were retrieved three days after the drench and microsclerotia were observed for germination. Results indicated the number of germinating microsclerotia was significantly higher after application of the germination stimulant compared to non-drench and 0 ppm controls ($p < 0.001$). A container trial was conducted using the germination stimulant at 10,000 ppm applied three days prior to a drench with tebuconazole or thiophanate-methyl to determine the effect of fungicides on germinated microscleotia. 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 applications.

Introduction

Crown rot, caused by *Macrophomina phaseolina (Mp)*, and Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *fragariae (Fof)*, are emerging soilborne fungal pathogens in commercial strawberry production in California (CA). *Macrophomina phaseolina* and *Fof* survive in infected crop debris and in soil as microsclerotia or chlamydospores, respectively. These survival structures serve as the primary inoculum in the subsequent strawberry crop and are resistant to environmental extremes. Recently, a novel approach targeting sclerotia produced by another soilborne fungus has been developed which shows potential as an integrated control measure for managing white rot of garlic and onion in central CA. This method involves the application of host plant extracts (i.e., onion and garlic extracts) to white rot infested fields in the absence of a crop in order to "trick" or stimulate the pathogen sclerotia to germinate. After germinating, the pathogen eventually dies when there is no suitable host to infect. Numerous studies have reported increased efficacy of fungicides when combining them with the application of germination stimulants.

The specific objectives of this research were: 1) to evaluate the efficacy of various rates and the timing of soil-applied fungicides; 2) to examine the influence of whole strawberry plant extracts on the germination of *Mp*; and 3) to evaluate the combined use of strawberry plant extracts with the most effective fungicides for managing Macrophomina crown rot.

Objective 1. Evaluating the Efficacy of Various Rates and Timing of Soil-Applied Fungicides for Managing Macrophomina Crown Rot and Fusarium Wilt

Materials and Methods

In Vitro Fungicide Assays

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.

In Planta Fungicide Trials

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 weekly for 11 weeks by calculating the area under the disease progress curve (AUDPC).

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 1). Tebuconazole strongly inhibited the mycelial growth of both pathogens (average EC_{75} for *Mp* was 2.4 ppm; average EC_{75} for *Fof* was 7.48 ppm), as did metconazole (average EC_{75} for *Mp* was 2.53 ppm; average EC_{75} 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. Overall, *Mp* was more sensitive than *Fof* to the active ingredients screened.

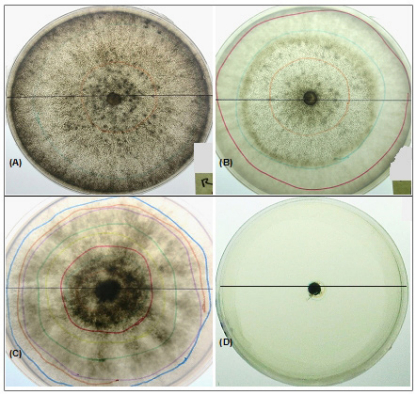


Figure 1. Mycelial growth of *Macrophomina phaseolina* on (A) unamended media (maximum colony diameter at day 4), (B) flutriafol at 1 µg/ml (1 ppm) (maximum colony diameter at day 4), (C) tebuconazole at 1 µg/ml (1 ppm) (maximum colony diameter at day 15), and (D) tebuconazole at 50 µg/ml (50 ppm) (minimal growth at day 50).

In Planta Fungicide Trials

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) (Figure 2). There were no significant differences ($p > 0.05$) in AUDPC between fungicide treatments and non-treated controls for *Fof* (Figure 3). This data indicates that these active ingredients did not provide effective control of these pathogens *in planta*.

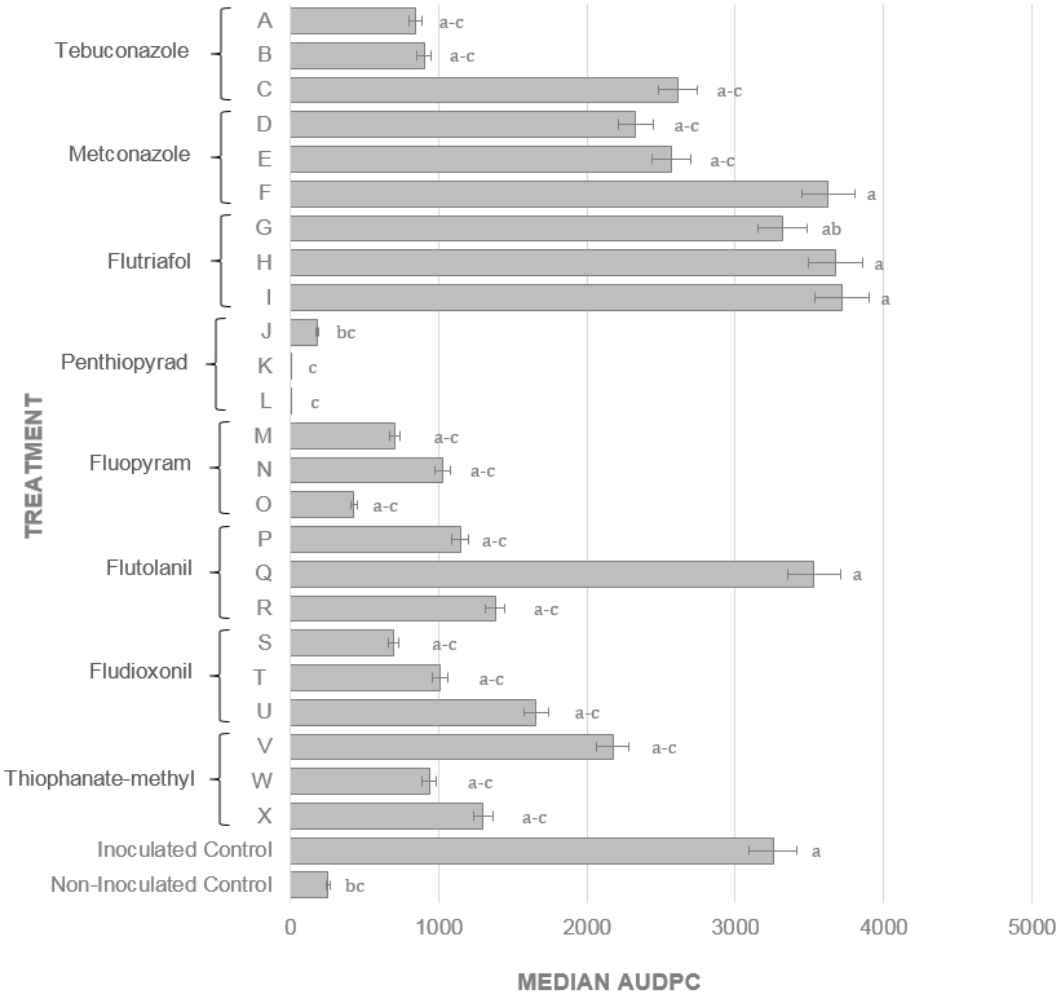


Figure 2. Median Area Under the Disease Progress Curve (AUDPC) values for *Macrophomina phaseolina* in planta fungicide trial II.

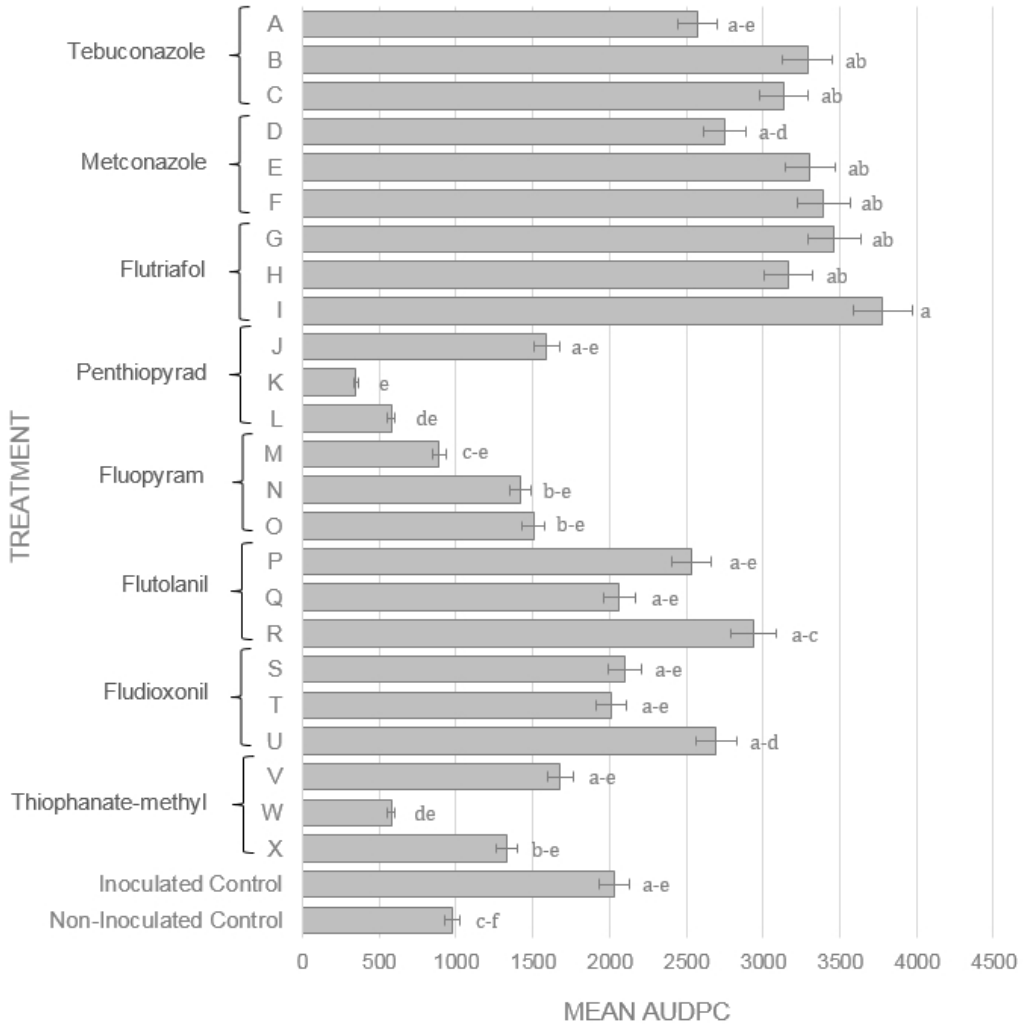


Figure 3. Mean Area Under the Disease Progress Curve (AUDPC) values for *Fusarium oxysporum* f. sp. *fragariae in planta* fungicide trial I.

Objectives 2 & 3: Examining the Influence of Strawberry Plant Extracts on Germination of Macrophomina phaseolina Microsclerotia.

Materials and 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 (not disclosed) from California Safe Soil were added per 500 g strawberry plant material according to their recommendations. The solution was heated and agitated to activate enzymes. 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.

Macrophomina phaseolina Microsclerotia Packets

Three isolates of *Mp* 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 by scraping them from the toothpicks. Filter disks were folded and glued shut. These microsclerotia packets were used in both *in vitro* and *in planta* studies.

In Vitro Plant Extract Studies

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 three days after the drench and processed by microscopy to determine the number of germinated microsclerotia (Figure 4).

In Planta Plant Extract Studies

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 packets of microsclerotia of *Mp* at day 0 and 14. Packets were retrieved three days after the drench and microsclerotia were observed microscopically for germination. 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 microscleotia.

Results

In Vitro Plant Extract Studies

Results showed that the number of germinated microsclerotia (Figure 4) was significantly higher ($p < 0.001$) after the application of the strawberry plant extract compared to non-drench and water (0 ppm) controls.

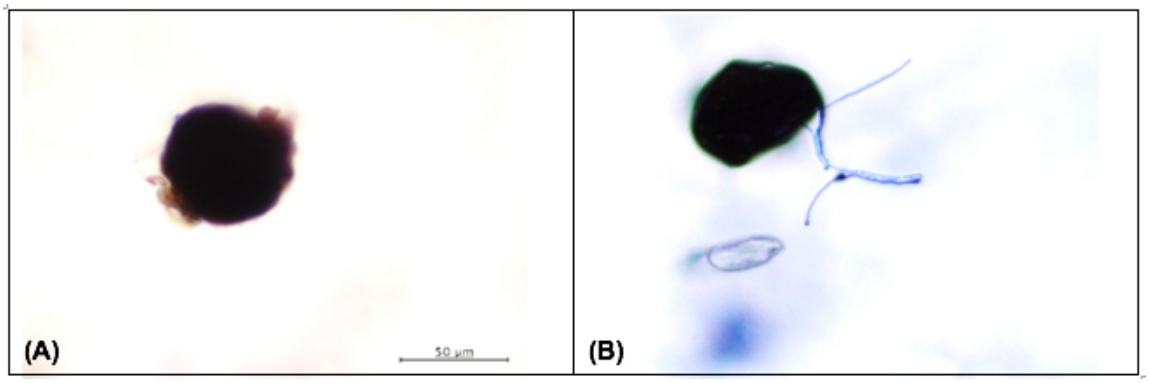
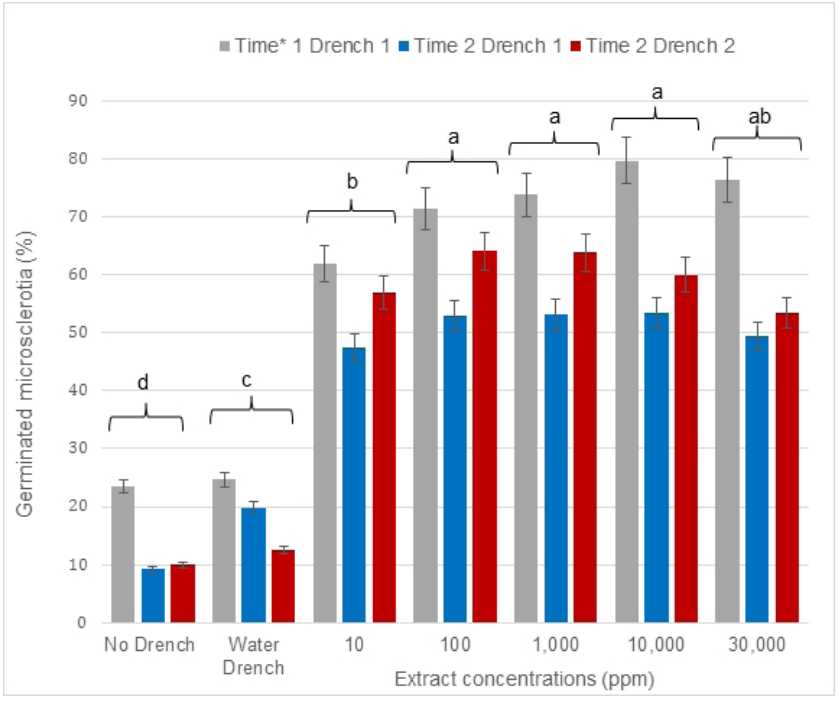


Figure 4. Microsclerotia of *Macrophomina phaseolina* that (A) did not germinate, and (B) did germinate.

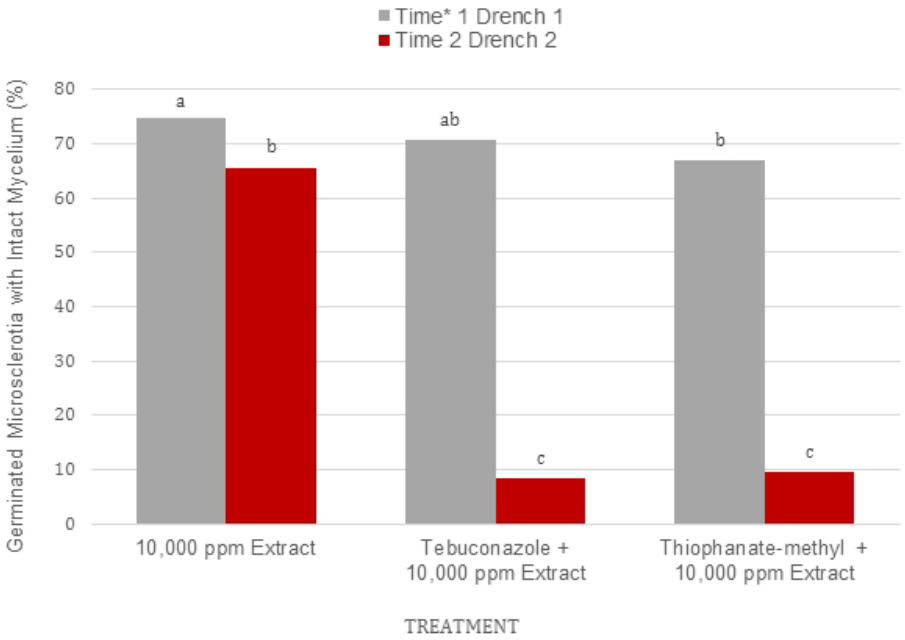
In Planta Plant Extract Studies

The number of germinating microsclerotia was significantly higher after the application of the plant extract compared to non-drench and 0 ppm controls ($p < 0.001$) (Figure 5). The use of strawberry plant extracts as germination stimulants with label rates of both fungicides lowered the number of living germinated microsclerotia significantly ($p < 0.001$) (Figure 6), 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.



*Days based on day microsclerotia were counted.

Figure 5. Percent of Macrophomina phaseolina microsclerotia that germinated after treatment drench in planta.



*Days based on day microsclerotia were counted.

Figure 6. Percent of germinated *Macrophomina phaseolina* microsclerotia with intact mycelium after drenches of plant extracts and fungicides in planta.

Discussion

Sustainable soilborne disease control alternatives to fumigation are lacking for strawberry producers. An integrated disease management approach is necessary for continued strawberry production in pathogen-infested soils. The results of this study showed that fungicides alone were ineffective at controlling *Mp* and *Fof in planta*. The use of germination stimulants to assist fungicides could be investigated further as one method for controlling these diseases. Combining the most effective fungicide treatments with other cultural methods such as anaerobic soil disinfestation (ASD), low rates of fumigants, steam, and/or germination stimulants could help to develop an integrated disease management program.

References

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